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Unlocking soil health: Are microbial functional genes effective indicators?

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ABSTRACT

Soil microbial community plays crucial roles in promoting soil functions and maintaining soil health. Microbial functional gene abundances are actively involved in soil processes which supports soil functions and wider soil health. However, their suitability as indicators to assess soil health is still debatable. In this study, we sampled soils from a 10-year long-term fertilization experiment in a wheat-maize cropping system on the North China Plain. The treatment included no fertilizer (Control), chemical fertilizers only (NPK), NPK + organic manure, NPK + straw, and NPK + manure + straw. We quantified seventeen functional genes involved in carbon (*cbbL, GH31*), nitrogen (*nifH, ureC, chiA, A-amoA, B-amoA, narG, nirK, nirS, norB* and *nosZ*), and phosphorus (*gltA, bpp, phoD, phoC, pqqC*) cycling. These genes were correlated with a suite of soil properties representing indicators of carbon (total carbon, organic carbon, and permanganate oxidizable carbon, α-1,4 glucosidase and carbon dioxide emission), nitrogen (total nitrogen, inorganic nitrogen, β-N-acetylglucosaminidase, and nitrous oxide emission), and phosphorous (available phosphorus, acid and alkaline phosphatase) pools/cycling. Soil microbial functional genes exhibited high coefficients of variation and strong sensitivity to fertilization treatments, while showing low variability among replicates within the same treatment. The abundances of functional genes, especially *GH31, cbbL, B-amoA, chiA, phoC,* and *phoD* were strongly correlated with their proxy indicators of carbon, nitrogen and phosphorus cycling. In addition, organic fertilization enhanced carbon and nutrients relevant functional gene abundances, generating positive effects on maize yield. These results indicate that microbial functional genes are sensitive to organic inputs and could provide a more detailed and mechanistic understanding of soil processes than conventional indicators by capturing the biochemical processes that govern nutrient dynamics. Our study underscores the potential of microbial functional genes as sensitive and valuable indicators for advancing soil health assessments and management practices.

1. Introduction

Healthy soils are essential for maintaining food security and agricultural sustainability (Kopittke et al., 2019) and can promote water and air quality, provide a habitat for biodiversity, facilitate the mineralization and cycling of nutrients, reduce the occurrence of pests and diseases, support the utilization and storage of carbon, and enhance crop production (Maikhuri and Rao, 2012). The capacity of soils to provide these diverse functions is commonly termed 'soil multifunctionality', which has recently been included in the foresight report on soil health (Creamer et al., 2022). However, soil multi-functionality is highly threatened by global changes and anthropogenic forces (Schloter et al., 2018). In this respect, the importance of developing robust, reliable, and resilient indicators for monitoring soil health has been emphasized, in particular when establishing an early warning system for halting soil degradation. Soil health indicators are currently focused mainly on soil physical and chemical properties (Cardoso et al., 2013), such as soil bulk density and nutrient concentrations. These physical and chemical indicators are instructive for the development of agricultural practices to increase crop productivity. However, these parameters mainly reflect

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static soil characteristics and soil fertility changes, but fail to capture the dynamic biological processes (Schoenholtz et al., 2000) which are important for understanding soil ecological functions. Additionally, they are less sensitive to short-time changes in soil quality and may not detect early signs of soil degradation or improvement (Muscolo and Attinà, 2015). In this regard, biological indicators can provide a more comprehensive understanding of soil health, as they reflect the living component of the soil and its dynamic processes (Bhaduri et al., 2022). The measurement of biological properties can capture the activities of soil microbes, enzymes, and other biota that play a vital role in nutrient cycling, carbon storage, and ecosystem services, that are important in the maintenance of soil quality and health (Creamer et al., 2022). There have been increasing efforts to incorporate soil biological measures into the monitor of soil quality and health (van Bruggen et al., 2000; Griffiths et al., 2001). However, most previous attempts to define biological indicators of soil health have focused primarily on the 'visible' players among the soil biota, such as earthworms and nematodes, while largely ignoring the 'invisible' soil microbes (Doran and Zeiss, 2000).

Soil microbial communities form the lifeblood of soil ecosystems and are involved in various soil functions, such as carbon cycling and storage, nutrient cycling, and primary production (Raza et al., 2023; Soong et al., 2020; Fan et al., 2020). However, the functional potential of these communities is affected by the diversity of the communities themselves and also other factors, such as soil properties (Jia et al., 2023), nutrient supply (Mbuthia et al., 2015), and cropping systems (Jia et al., 2022; Morugán-Coronado et al., 2022), which complicate the application of microbial communities as proxies for soil health. To date, it remain a challenge to disentangle the mechanisms underlying the relationship between microbial functional capacity and soil health (Hartmann et al., 2015; van der Bom et al., 2018). Disentangling the complex relationships between microbial functional indicators and soil health can therefore aid in developing more effective strategies for managing soil ecosystems and promoting sustainable agriculture. However, current microbial indicators of soil health rely predominantly on broad or 'black-box' parameters, such as microbial biomass and potential microbial activity, and these are insufficient to provide specific insights into soil biological processes (Schimel and Schaeffer, 2012). It has recently been proposed that the assessment of soil microbes in supporting a healthy functioning soil should be defined at the level of functional diversity rather than solely at the level of taxonomic species (Wang et al., 2022). This is partly supported by the functional redundancy of soil microbial communities, whereby the loss of one species may be compensated by others with similar functions (Bender et al., 2016). Hence, relying solely on taxonomic species composition disregards this functional redundancy. Analyzing functional genes rather than taxonomic diversity offers a glimpse into the genuine functional capacity within the whole microbial community, thereby providing a more accurate reflection of microbial contributions to soil health (Wang et al., 2022; Trivedi et al., 2016).

A number of soil microbial functional genes have been used as genetic markers to differentiate the functional activity of microbial populations (Thiele-Bruhn et al., 2020; Smith et al., 2017). For example, *nifH, A-amoA*, *B-amoA* and *nirS/nirK* genes have been used as markers to quantify nitrogen-fixing microorganisms, nitrifiers, and denitrifiers (Song et al., 2020; Ouyang et al., 2018; Ouyang et al., 2020). These functional genes are sensitive to agricultural practices, such as tillage and fertilization, and are correlated with soil properties and functions (Hayden et al., 2010; Li et al., 2023a). For instance, conservation tillage increased the abundance of *nosZ* gene abundance that is involved in the process of denitrification process, and this is associated with reduced N_2O emission (Wang et al., 2021a). On the other hand, nitrogen fertilization significantly increased the abundances of *A-amoA, B-amoA* which are associated with increased nitrification rates (Ouyang et al., 2018). The linkages between functional genes and soil processes therefore underscore their potential for evaluating soil health and the impacts of different management practices on soil function and crop productivity. However, the situation is further complicated by the fact that the relationships between soil microbial functional genes and soil processes are inherently dynamic and complex. For example, nitrification which is mediated by *A-amoA* and *B-amoA* genes increases N availability and this is favorable for soil health and crop yields (Phillips et al., 2015). On the other hand, excessive nitrification can lead to increased N_2O emissions, and this is potentially harmful to the environment and disturbs nitrogen cycling (Robertson and Vitousek, 2009). In this regard, systematic study of the abundance of microbial functional groups involved in soil C, N and P processes may provide a rapid and sensitive approach for characterizing changes in soil functions. This approach promises to provide a real-time snapshot of microbial activities and their responses to management practices, thereby enabling the development of more accurate management to maximize soil health.

This study aims to investigate the relationship between the abundance of a range of microbial functional genes, soil nutrients and carbon cycling and their relationships with crop yield. The response of the microbial functional genes and several indicators of soil functioning, focusing on C, N and P turnover, were examined in a decade long-term field experiment with different fertilization treatments (different combinations of chemical and organic fertilizers). We hypothesized that: (1) compared to conventional soil carbon and nutrient indicators, the abundances of functional genes would show greater variability in response to different fertilization treatments; (2) the abundances of specific microbial functional genes are strongly correlated with conventional measurements of soil carbon and nutrient cycling; and (3) soil amended with organic fertilizers would have higher abundances of microbial functional genes, this would contribute to crop yields compared to soils receiving the chemical fertilizer only.

2. Materials and methods

2.1. Field experiment and sample collection

Soil samples were collected from a long-term field experiment with an annual rotation of winter wheat and summer maize at the China Agricultural University Quzhou Experimental Station (36°42′ N, 114°54′ E; 40 m a.s.l.), Hebei province, north China. The silt loam soil is classed as a Cambisol. Selected initial soil properties before the start of the experiment were as follows: pH 7.24 (H_2O), soil organic matter content of 13.7 g kg^{-1}, total nitrogen content 0.90 g kg^{-1}, Olsen-P content 12.01 mg kg^{-1}, and available potassium content 176.2 mg kg^{-1} (Bei et al., 2018). The average annual temperature and mean precipitation are 13.2 °C and 494 mm, respectively.

Field plots (each 50 m^2, 5 m × 10 m) were established in 2010. There are five annual treatments with four replicate plots per treatment as follows: (1) Control, no fertilizer; (2) NPK, chemical fertilizer only; (3) NPKM, chemical fertilizer plus manure compost (6000 kg ha^{-1} yr^{-1}, dry weight); (4) NPKSW, chemical fertilizer plus straw return (wheat straw, 6.0 Mg ha^{-1} yr^{-1}; maize straw, 6.8 Mg ha^{-1} yr^{-1}); (5) MNPKSW, chemical fertilizer plus manure compost and straw return (wheat straw, 7.3 Mg ha^{-1} yr^{-1}; maize straw, 6.9 Mg ha^{-1} yr^{-1}), provided on yearly bases. The manure compost comprised 33.2% C, 2.0% N, 0.8% P and 0.7% K. Fertilizers comprised urea as the nitrogen (N) fertilizer, calcium superphosphate as the phosphorus (P) fertilizer, and potassium sulphate as the potassium (K) fertilizer, and were broadcast. All treatments were calculated to give an equivalent nitrogen application rate. Detailed information regarding fertilizer application rates is shown in Table S1. Winter wheat (cv. 'Good Star 99') was sown at a density of 225 kg ha^{-1} in mid-October following maize harvest and harvested in early June of the subsequent year. Summer maize (cv. 'Zhengdan 958') was sown with a row spacing of 60 cm and a density of 63,000 seeds ha^{-1} in mid-June, and was harvested in October. Additionally, irrigation, insecticides, and herbicides were applied according to local conventional farming practices.

In October 2020, soil samples were collected prior to maize harvest.

Five samples (0–20 cm depth) were collected from each plot using a 5-cm-diameter auger and mixed to give a composite sample. The samples were then divided into three parts. One part (100 g) was air-dried for the determination of soil physicochemical properties, and one part (50 g) was preserved at 4 °C for the assessment of enzyme activity, soil respiration and N_2O emission. The remaining soil was kept at −80 °C for the quantification of microbial functional genes. Maize ears were collected from designated areas ranging from 6 to 18 m^2 within each treatment. The collected grain was subsequently dried to assess maize yield.

2.2. Selection of soil microbial functional genes

Seventeen functional genes involved in the C (*cbbL, GH31*), N (*nifH, ureC, chiA, A-amoA, B-amoA, narG, nirK, nirS, norB* and *nosZ*), and P cycling (*gltA, bpp, phoD, phoC, pqqC*) were selected. Carbon cycling genes (*cbbL* and *GH31*) are involved in soil C fixation and organic matter decomposition processes, that contribute to C transformation and storage (Liao et al., 2020; Yang et al., 2021). Nitrogen cycling genes (*nifH, A-amoA, B-amoA, ureC, chiA, narG, norB, nirK, nirS* and *nosZ*) are involved in the processes of N fixation (*nifH*), ammonia oxidation (*A-amoA, B-amoA*), urea transformation (*ureC*), N mineralization (*chiA*), and denitrification (*narG, norB, nirK, nirS* and *nosZ*), respectively. These functional genes are the major players in N transformation dynamics (Ouyang et al., 2018; Colloff et al., 2008; Butterly et al., 2016). Phosphorus cycling genes (*pqqC, phoD, phoC, gltA* and *bpp*) are involved in phosphate solubilization (*pqqC*), organic P mineralization (*phoD, phoC*), and the release of bioavailable P (*gltA*, *bpp*), respectively (Shi et al., 2022; Zheng et al., 2019). These genes have been commonly used to assess P cycling and P availability (Hussain et al., 2021). These functional genes are useful indicators in environmental monitoring and ecological studies and they have been used to reflect key biogeochemical processes (Table 1; Supporting materials).

2.3. DNA extraction and quantitative PCR

Soil microbial DNA was extracted from 0.5 g soil using FastDNA® SPIN for soil kit (MP Biomedicals, Solon, OH, USA) following the manufacturer's protocol. Each sample was extracted in duplicate for DNA analysis. The quantity and quality of DNA were evaluated using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and the quality was further verified by electrophoresis on a 1.4% agarose gel stained with SYBR Safe (Life Technologies, Carlsbad, CA). Then DNA was stored at −80 °C for further processing.

Soil microbial functional genes involved in C cycling (*cbbL, GH31*), N cycling (*nifH, A-amoA, B-amoA, ureC, chiA, narG, nirK, nirS, norB, nosZ*), and P cycling (*pqqC, gltA, bpp, phoC, phoD*) were quantified using quantitative PCR. Furthermore, total bacterial and fungal abundance were quantified using the primers 515F/907R (Yusoff et al., 2013) and ITS1F/ITS2R (White et al., 1990). The specific primers used for targeting these genes are listed in Table S2. All qPCR processing was conducted using QuantStudio 6 Flex (Applied Biosystems, Waltham, MA). Each reaction was conducted in duplicate, using 10 μL of reaction mixture. The mixture consisted of 5 μL of SYBR Premix ExTaq II (2 ×) (TaKaRa Bio, Kusatsu, Shiga, Japan), 0.25 μL of each primer (10 pmol μM), 1 μL of genomic soil DNA (5 ng μL^{-1}), 3.3 μL of ddH_2O and 0.2 μL of ROX Reference Dye II. The standard curves for each gene were established through a 10-fold serial dilution series (10^8-10^2 copies) of plasmid DNA containing the target gene with known copy numbers. ddH_2O (without template DNA) served as the negative control. The amplification efficiencies ranged from 96% to 104%, and the R^2 values of the standard curves were between 0.98 and 1.00 for all genes. References to the protocols for determining the different functional genes are given in Table S2.

2.4. Selection and determination of proxy indicators of element cycling

To investigate the relationship between soil microbial functional genes and associated functional processes, we identified soil properties that are related to the process in which the microbial functional genes are active (Table 1). This resulted in selecting the following soil properties as proxies for soil functioning (henceforth termed proxy indicators): 1) the C pool/cycling (total carbon, soil organic carbon, permanganate oxidizable carbon, soil respiration and the enzymes α-1,4 glucosidase [AG, EC 3.2.1.20]); 2) the N pool/cycling (total N, ammonium and nitrate N, nitrous dioxide emission, and the enzyme β-N-acetylglucosaminidase [NAG, EC 3.2.1.14.30]); 3) the P pool/cycling

Table 1
Microbial functional genes, the soil ecological processes in which they participate, and soil properties that can be used as proxy indicators for the soil ecological processes.

Microbial functional gene		Enzyme encoding	Soil ecological process	Soil proxy indicator	Reference
Carbon	*cbbL*	Ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39)	Calvin cycle (carbon fixation)	TC, SOC, POXC	Yuan et al. (2012)
	GH31	α-glucosidases (EC 3.2.1.20)	Starch degradation	AG, SOC, soil respiration	Talbot et al. (2015)
Nitrogen	*nifH*	Nitrogenase reductase	Nitrogen-fixation	TN, NH_4^+-N, NO_3^--N	Dos Santos et al. (2012)
	ureC	Urease (EC 3.5.1.5)	Urea hydrolysis (Urea - NH_3/NH_4^+)	NH_4^+-N, NO_3^--N	Fisher et al. (2017)
	chiA	Chitinase A (EC 3.2.1.14)	Chitin degradation	NAG	Zhang et al. (2023)
	A-amoA	Ammonia monooxygenase subunit A (EC 1.14.99.39)	Nitrification (NH_4^+- NH_2OH)	NO_3^--N	Levy-Booth et al. (2014)
	B-amoA	Ammonia monooxygenase subunit A (EC 1.14.99.39)	Nitrification (NH_4^+- NH_2OH)	NO_3^--N	Levy-Booth et al. (2014)
	narG	Nitrate reductase alpha subunit (EC 1.7.99.4)	Denitrification (NO_3^--NO_2^-)	NO_3^--N, N_2O	Yang et al. (2024)
	nirK	Copper-containing nitrite reductase (EC 1.7.2.1)	Denitrification (NO_2^- –NO)	NO_3^--N, N_2O	Yang et al. (2024)
	nirS	Cytochrome cd1 nitrite reductase (EC 1.7.2.9)	Denitrification (NO_2^- –NO)	NO_3^--N, N_2O	Yang et al. (2024)
	norB	Nitric oxide reductase subunit B (EC 1.7.2.5)	Denitrification (NO–N_2O)	NO_3^--N, N_2O	Yang et al. (2024)
	nosZ	Nitrous oxide reductase (EC 1.7.2.4)	Denitrification (N_2O–N2)	NO_3^--N, N_2O	Yang et al. (2024)
Phosphorus	*gltA*	Citrate synthase (EC 2.3.3.1)	Phosphorus dissolution	AP	Li et al. (2023b)
	bpp	β-propeller phytase	Phytic acid mineralization	AP	Wang et al. (2023a)
	phoD	Alkaline phosphatase (EC 3.1.3.1)	Organic P mineralization	ALP, AP	Shi et al. (2022)
	phoC	Acid phosphatase (EC 3.1.3.2)	Organic P mineralization	ACP, AP	Shi et al. (2022)
	pqqC	Pyrroloquinoline-quinone synthase C	Inorganic P dissolution	AP	Wang et al. (2023b)

Annotation: TC: total carbon; SOC: soil organic carbon, POXC: permanganate oxidizable carbon; TN: total nitrogen; AP: available phosphorus; AG: α-1,4 glucosidase; NAG: β-N-acetylglucosaminidase. ALP: alkaline phosphatase; ACP: acid phosphatase.

(available P, the enzyme acid phosphatase [ACP, EC 3.1.3.1] and alkaline phosphatase [ALP, EC 3.1.3.2]).

Soil mineral N (NH_4^+-N, NO_3^--N) was measured by extraction with calcium chloride (Li et al., 2012). Soil organic carbon (SOC) was assessed by the $K_2Cr_2O_7$ oxidation-reduction titration method, and permanganate oxidizable carbon (POXC) was measured by using $KMnO_4$ oxidation (Weil et al., 2003). Available P (AP, Olsen-P) was determined by extraction with 0.5 mol L^{-1} $NaHCO_3$ (Olsen et al., 1954). Soil total C (TC) and total N (TN) contents were determined using dry combustion with an Elementar analyzer (Vario EL, Elementar, Germany). Soil respiration and N_2O emission were determined using a gas chromatograph (Zhang et al., 2013). A 20-g soil sample (dry weight) was placed in a 100 mL glass jar, and moistened to 60% water holding capacity. Samples were pre-incubated at 25 °C for 7 days, with periodic addition of water. After pre-incubation, jars were sealed with air-permeable paraffin film and incubated in the dark at 25 °C. After 24 h, 20-mL gas samples were collected using a gas-tight syringe and analyzed for CO_2 and N_2O concentrations using an ECD (GC 7890, Agilent Technologies, Santa Clara, CA). Soil respiration and N_2O emission rates were calculated from the increase in gas concentrations over time.

Fluorometric techniques were used to determine the activities of the C-acquiring enzymes α-1,4 glucosidase (AG), the N-acquiring enzyme β-N-acetylglucosaminidase (NAG), and the organic P-acquiring enzyme acid phosphatase (ACP) (Bell et al., 2013). Substrate solutions (4-MUB-α-D-glucopyranoside, 4-MUB-N-acetyl-β-D-glucosaminide, 4-MUB-phosphate) were used for the enzyme assays. Soil samples (1 g, dry weight equivalent) were homogenized in 100 mL of 50 mM sodium acetate buffer (pH 6.0) and shaken for 30 min at 200 rpm. Then, 200 μL of suspension and 50 μL of 200 μM MUB-labeled substrate were transferred into a 96-well plate and incubated in the dark at 25 °C for 4 h. The fluorescence was measured using an automated spectrophotometer (FLx 800 microplate, Bio-Tek Instruments, Winooski, VT), with emission at 450 nm and excitation at 345 nm. Enzyme activities are expressed as MUB release in nmol per gram of soil per hour (nmol h^{-1} g^{-1}). Alkaline phosphatase activity was assessed at 37 °C using *p*-nitrophenyl phosphate (*p*-NPP) as substrate (Fraser et al., 2017). Fresh soil samples (1 g, dry weight equivalent) were homogenized with 1 mL of modified universal buffer (pH 11), *p*-NPP, and incubated at 37 °C for 1 h. The reaction was terminated by adding 0.5 M NaOH, and the absorbance of the resulting *p*-nitrophenol (p-NP) was measured at 420 nm. Enzyme activity was expressed as micromoles of p-NP produced per gram of soil per hour (μmol pNP g^{-1} h^{-1}).

2.5. Statistical analysis

All statistical analysis was conducted using R (version 4.2.2), unless otherwise stated. One-way analysis of variance was used to assess the effects of the different fertilization treatments on soil microbial functional gene abundances and the proxy indicators for element cycling. Levene's test was performed to assess the homogeneity of variances across groups. Additionally, the Shapiro-Wilk test was used to determine whether the functional gene abundances and proxy indicators were normally distributed. Data were transformed to achieve normal distribution prior to further analysis if the functional gene abundances and proxy indicators were not normally distributed. Coefficients of variation (CV) were calculated at the experimental field level, i.e., combining all treatments, to determine the whole experimental field variability in functional gene abundances and proxy indicators. Additionally, CV values were calculated within each treatment to determine the variation specific to individual treatment conditions. Furthermore, partition variances among treatments and among field replicates within treatments were quantified using a nested model based on the *lme4* package. Ordinary least squares (OLS) linear regressions were used to evaluate the associations between functional gene abundances and proxy indicators. The Shapiro-Wilk test was conducted to test whether residuals were normally distributed in the regression analysis. Partial least squares path modeling (PLS-PM) was employed to determine the impacts of soil carbon and nutrient inputs on the relationship between microbial functional gene abundances, the proxy indicators for element cycling, and crop yield using the R package *plspm*. The initial PLS-PM is presented in Fig. S1. The prediction performance of models was estimated by using the goodness of fit index. To assess the contribution of microbial functional genes and proxy indicators to variations in crop yield as initiated by the fertilization treatments, we conducted the all-subsets procedure model selection process using the R package *glmulti*. A range of candidate models was generated, and the Akaike Information Criterion (AICc) was used to select the best model. In the case of nonsignificant differences between models ($\leq$2) with the lowest AIC, model averaging was applied to effectively capture the relative importance of parameters across all candidate models (Anderson et al., 2007). To determine the significance of predictors, parameter weights were esimated by summing up the Akaike weights assigned to each individual model to calculate their relative contribution (Calcagno and de Mazancourt, 2010). These weights served as a metric of the overall support for each predictor.

3. Results

3.1. Effects of fertilization on abundance of microbial functional genes and proxy indicators for element cycling

In general, the abundance of soil microbial functional genes and most proxy indicators were significantly affected by the fertilization treatments (Table 2; Figs. S2–S4; Table S3).The C cycling proxy indicators (SOC, TC, POXC, soil respiration, AG) and the abundance of C associated functional genes *cbbL* and *GH31* were significantly affected by the fertilization treatments. Maximum values occurred in the MNPKSW treatment, and minimum values were in the Control (Table 2; Fig. S2; Table S3). Compared with the NPK only treatment, manure application and straw return significantly increased SOC by 20.3% and 22.4%, TC by 8.1% and 14.54%, POXC by 9.8% and 19.5%, soil respiration by 8.6% and 20.7%, the activity of the enzymes AG by 1.1% and 10.9%, and the gene abundance of *cbbL* by 3.7% and 5.9% and *GH31* by 4.4% and 4.4%, respectively (Table 2; Fig. S5).

The fertilization treatments, especially manure application and straw return, significantly increased total soil N content and the abundances of the functional genes of *narG, nirK*, *nirS, norB, nosZ, ureC, nifH* and *chiA* (Table 2; Figs. S3 and S5; Table S3). NO_3^--N content and the abundances of functional genes *A-amoA* and *B-amoA* were highest in the NPK and NPKSW treatments. NH_4^+-N content and N_2O emission were not significantly affected by fertilization. Compared with NPK only, manure application significantly increased the activity of NAG by 4.9% (Table 2; Fig. S5). Manure application significantly increased available P content by 46.1% compared to the NPK treatment (Table 2; Fig. S5). Manure application and straw return increased the abundance of the functional genes of *bpp* by 3.1% and 2.1%, *phoC* by 3.3% and 20.3%, *phoD* by 1.3% and 3.5%, *pqqC* by 1.9% and 1.3% and *gltA* by 7.3% and 8.4%, respectively (Table 2; Fig. S4). The bacterial, fungal abundance and ratio of fungi to bacteria increased significantly in the fertilization treatment, especially in the manure application and straw return treatments (Table S4). However, DNA concentration did not show significant differences among different fertilization treatments (Table S3), with the minimum values in the Control.

The CV values of the proxy indicators related to the C cycle ranged from 8% to 36% and the corresponding values were 35–52% for the related functional genes of *cbbL* and *GH31* (Table 2; Figs. S2 and S5). The CVs of the proxy indicators related to the N cycle, combining all treatments, ranged from 10% to 54%, strongly overlapped with the CV values (23–54%) of the functional microbial genes (Table 2; Figs. S3 and S5). Moreover, the CV values of the proxy indicators related to the P cycle, combined for all treatments (11–62%), overlapped with those of the microbial functional genes (23–62%) (Table 2; Figs. S4 and S5). In

Table 2
Descriptive statistics of soil proxy indicators and soil microbial functional gene abundances across different fertilizer treatments. Control, no fertilizer; NPK, chemical fertilizer; MNPK, manure with chemical fertilizer; NPKSW, chemical fertilizer with straw return; MNPK, manure with chemical fertilizer and straw return. Coefficients of variation (CV) were calculated as the ratio of the mean value of each indicator across all samples and their standard deviation at the experimental field level. F- and *p*-values are based on analysis of variance of different treatments.

	Control	NPK	MNPK	NPKSW	MNPKSW	CV	F	*p*
Soil proxy indicators								
Carbon cycling								
SOC (g kg^{-1})	7.64 ± 0.39d	8.80 ± 0.28c	10.59 ± 0.63b	10.77 ± 0.59b	13.62 ± 0.63a	0.20	75.48	<0.001
TC (g kg^{-1})	19.59 ± 0.64c	20.28 ± 0.45c	21.92 ± 1.52b	23.23 ± 1.08b	26.63 ± 0.81a	0.12	32.90	<0.001
POXC (g kg^{-1})	0.33 ± 0.02c	0.41 ± 0.03b	0.45 ± 0.02 ab	0.49 ± 0.06 ab	0.48 ± 0.04a	0.15	12.78	<0.001
Soil respiration (g C kg^{-1} d)	14.97 ± 1.67b	17.04 ± 2.95b	18.51 ± 3.14b	20.57 ± 6.21 ab	24.95 ± 1.67a	0.23	4.62	0.01
AG (nmol h^{-1} g^{-1})	9.56 ± 0.11b	9.95 ± 0.68b	10.06 ± 0.24b	11.03 ± 0.7a	11.47 ± 0.21a	0.08	38.20	<0.001
Nitrogen cycling								
TN (g kg^{-1})	1.55 ± 0.07c	1.66 ± 0.11bc	1.70 ± 0.07b	1.79 ± 0.09b	1.95 ± 0.12a	0.16	10.22	<0.001
NO_3^--N (mg kg^{-1})	3.22 ± 0.89c	20.75 ± 8.44 ab	10.45 ± 3.3bc	25.42 ± 13.9a	17.51 ± 7.66 ab	0.54	8.23	0.001
NH_4^+-N (mg kg^{-1})	1.23 ± 0.76 ab	0.87 ± 0.2b	1.05 ± 0.33 ab	1.56 ± 0.07a	1.32 ± 0.36 ab	0.36	1.62	0.22
N_2O (ug N kg^{-1} d)	0.34 ± 0.01a	0.35 ± 0.02a	0.36 ± 0.03a	0.4 ± 0.06a	0.39 ± 0.04a	0.10	1.80	0.18
NAG (nmol h^{-1} g^{-1})	4.04 ± 0.54c	4.89 ± 0.71bc	5.13 ± 0.79a	4.24 ± 0.39bc	6.47 ± 0.63a	0.21	9.37	<0.001
Phosphorous cycling								
AP (mg/kg)	1.98 ± 0.55bc	6.14 ± 1.31c	8.97 ± 2.71b	5.86 ± 1.45c	15.24 ± 2.77a	0.62	25.32	<0.001
ACP (nmol h^{-1} g^{-1})	15.29 ± 2.14c	17.97 ± 0.61b	19.17 ± 0.53 ab	19.50 ± 0.19 ab	20.40 ± 1.33a	0.11	11.06	<0.001
ALP (mg PNP h^{-1} g^{-1})	1.68 ± 0.54c	2.57 ± 0.09b	2.74 ± 0.08b	2.79 ± 0.03b	3.41 ± 0.42a	0.23	16.15	<0.00
Microbial functional genes								
Carbon cycling								
cbbL (log_{10} copies ng^{-1} DNA)	3.51 ± 0.1c	3.56 ± 0.07c	3.69 ± 0.09b	3.77 ± 0.03b	3.9 ± 0.04a	0.35	18.63	<0.001
GH31(log_{10} copies ng^{-1} DNA)	3.53 ± 0.1c	3.65 ± 0.09c	3.81 ± 0.04b	3.81 ± 0.15b	4.1 ± 0.08a	0.52	19.52	<0.00
Nitrogen cycling								
A-amoA (log_{10} copies ng^{-1} DNA)	3.18 ± 0.07c	3.43 ± 0.03a	3.36 ± 0.03 ab	3.37 ± 0.04 ab	3.28 ± 0.15bc	0.23	6.46	0.003
B-amoA (log_{10} copies ng^{-1} DNA)	3.28 ± 0.07d	4.26 ± 0.05a	3.88 ± 0.03c	4.20 ± 0.06a	4.09 ± 0.05b	0.54	209.79	<0.001
ureC (log_{10} copies ng^{-1} DNA)	4.35 ± 0.09d	4.61 ± 0.05bc	4.67 ± 0.05 ab	4.56 ± 0.05c	4.76 ± 0.06a	0.30	25.04	<0.001
nifH (log_{10} copies ng^{-1} DNA)	3.69 ± 0.10b	3.71 ± 0.05b	3.87 ± 0.04a	3.83 ± 0.04a	3.93 ± 0.08a	0.25	8.86	<0.001
chiA (log_{10} copies ng^{-1} DNA)	4.10 ± 0.14c	4.14 ± 0.07c	4.29 ± 0.05b	4.29 ± 0.05b	4.51 ± 0.07a	0.38	15.92	<0.001
narG (log_{10} copies ng^{-1} DNA)	3.26 ± 0.05b	3.3 ± 0.05b	3.33 ± 0.06b	3.30 ± 0.03b	3.51 ± 0.08a	0.27	12.50	<0.001
nirK (log_{10} copies ng^{-1} DNA)	3.53 ± 0.14c	3.73 ± 0.07b	3.85 ± 0.06 ab	3.79 ± 0.1 ab	3.89 ± 0.05a	0.29	9.53	<0.001
nirS (log_{10} copies ng^{-1} DNA)	3.55 ± 0.04d	3.71 ± 0.07c	3.83 ± 0.04b	3.81 ± 0.09b	3.94 ± 0.06a	0.31	23.62	<0.001
norB (log_{10} copies ng^{-1} DNA)	1.24 ± 0.09c	1.33 ± 0.11c	1.53 ± 0.04 ab	1.51 ± 0.05b	1.64 ± 0.09a	0.35	16.05	<0.001
nosZ (log_{10} copies ng^{-1} DNA)	3.41 ± 0.07d	3.55 ± 0.06c	3.84 ± 0.02b	3.84 ± 0.05b	3.97 ± 0.05a	0.43	80.37	<0.001
Phosphorous cycling								
bpp (log_{10} copies ng^{-1} DNA)	4.16 ± 0.07c	4.21 ± 0.04c	4.34 ± 0.03 ab	4.30 ± 0.02b	4.40 ± 0.07a	0.23	14.63	<0.001
phoC (log_{10} copies ng^{-1} DNA)	1.70 ± 0.07d	1.82 ± 0.11c	1.88 ± 0.04c	2.19 ± 0.05b	2.39 ± 0.04a	0.62	73.41	<0.001
phoD (log_{10} copies ng^{-1} DNA)	3.63 ± 0.09d	3.97 ± 0.07c	4.02 ± 0.04bc	4.11 ± 0.07b	4.40 ± 0.06a	0.58	66.41	<0.001
pqqC (log_{10} copies ng^{-1} DNA)	3.56 ± 0.13c	3.72 ± 0.05b	3.79 ± 0.04b	3.77 ± 0.05b	3.93 ± 0.07a	0.30	12.36	<0.001
gltA (log_{10} copies ng^{-1} DNA)	2.70 ± 0.14c	2.74 ± 0.08c	2.94 ± 0.13b	2.97 ± 0.05b	3.15 ± 0.08a	0.43	13.35	<0.001

Annotation: TC: total carbon; SOC: soil organic carbon, POXC: permanganate oxidizable carbon; TN: total nitrogen; AP: available phosphorus; AG: α-1,4 glucosidase; NAG: β-N-acetylglucosaminidase; ALP: alkaline phosphatase; ACP: acid phosphatase.

general, all CVs of the functional genes across all treatments were >23%, and 62% of the soil proxy indicators had a CV ≤ 20%. Among the proxy indicators, soil respiration emission (23%), NO_3^-N content (54%), NH_4^+-N content (36%), and AP (62%) had relatively high CV values. The value of their corresponding functional genes were *GH31* 51%, *B-amoA* 54%, and *phoC* 63%, respectively. Within treatments the CV values of all C, N and P cycling gene were lower than those of their corresponding proxy indicators (Table S5). Furthermore, the standard deviations among treatments were greater than the standard deviations within field replicates for all of the microbial functional genes and proxy indicators (Table S5).

3.2. Correlations between the abundance of microbial functional genes and proxy indicators for element cycling

The Ordinary Least Squares (OLS) regression results showed positive relationships between the abundance of the carbon cycling gene *cbbL* and TC, SOC as well as POXC across all soil samples (Table 3). Furthermore, the abundance of the carbon cycling gene *GH31* was positively related to the activities of the enzymes AG and soil respiration (Table 3) across all soil samples.

Regarding the functional genes in the N cycle, there were positive relationships between *ureC, nifH*, *A-amoA*, and *B-amoA* abundance and soil NO_3^--N content across all soil samples (Table 3). The *nifH* and *chiA* gene abundances were positively associated with TN content and NAG enzyme activity, respectively (Table 3). However, there was no significant correlation between gene *ureC, nifH* abundance and NH_4^+-N content. The abundances of *narG*, *nirK*, *nirS* and *norB* were only positively correlated with NO_3^--N content, but not with N_2O emission (Table 3). The *nosZ* abundance was positively correlated with NO_3^--N content and N_2O emission. The abundances of all P functional genes (*bpp, phoC, phoD, qppC* and *gltA*) were positively correlated with AP content. Moreover, *phoC* and *phoD* were positively correlated with the activities of ACP and ALP across all soil samples.

Most C and P cycling gene abundances remained positively correlated with soil proxy indicators across fertilization treatments after excluding the Control. The positive correlations between the C cycling gene *cbbL* and POXC were removed. Moreover, the positive correlations between the abundances of N genes (*ureC, nifH, A-amoA, narG, nirK, nirS, norB, nosZ*) and their proxy indicators (nitrate content and N_2O emission) diminished after excluding the Control, while the positive correlations between *nifH* and total N, *chiA* and NAG, *B-amoA* and NO3–N content remained (Table S6).

3.3. Relationships among nutrient input, functional gene abundances, proxy indicators and maize yield

The carbon PLS-PM showed that straw carbon input increased the abundance of gene *GH31* which was positively related to α-glucosidase activity (Fig. 1A). The increased α-glucosidase activity promoted CO_2

Table 3
Relationship between microbial functional gene abundances and their corresponding proxy indicators associated with carbon, nitrogen, and phosphorus pools/cycling in fields with different fertilizer treatments. * indicates $p < 0.05$; ** indicates $p < 0.01$, *** indicates $p < 0.001$, respectively.

Carbon		TC	SOC	POXC	SR	AG
	cbbL	0.85***	0.89***	0.65***	NA	NA
	GH31	NA	NA	NA	0.71***	0.85***
Nitrogen		NH_4^+-N	NO_3^--N	TN	NAG	N_2O emission
	ureC	−0.13	0.6**	NA	NA	NA
	nifH	0	0.52*	0.63**	NA	NA
	chiA	NA	NA	NA	0.63**	NA
	A-amoA	NA	0.65**	NA	NA	NA
	B-amoA	NA	0.79***	NA	NA	NA
	narG	NA	0.45*	NA	NA	0.18
	nirK	NA	0.52*	NA	NA	0.39
	nirS	NA	0.66**	NA	NA	0.38
	norB	NA	0.55*	NA	NA	0.4
	nosZ	NA	0.61**	NA	NA	0.44
Phosphorus		AP	ACP	ALP		
	bpp	0.81***	NA	NA		
	phoC	0.74***	0.72***	NA		
	phoD	0.85***	NA	0.87***		
	pqqC	0.79***	NA	NA		
	gltA	0.73***	NA	NA		

Annotation: TC: total carbon; SOC: soil organic carbon, POXC: permanganate oxidizable carbon; SR: soil respiration; AG: α-1,4 glucosidase; TN: total nitrogen; NAG: β-N-acetylglucosaminidase; AP: available phosphorus; ACP: acid phosphatase; ALP: alkaline phosphatase.

emissions. Manure and straw carbon input both increased POXC content which was positively correlated with the abundance of the *cbbL* gene. Furthermore, the abundance of the *cbbL* gene was positively linked to SOC content. The nitrogen PLS-PM indicated that organic fertilization increased SOC content which was positively correlated with the abundances of *nifH, chiA* and *ureC* genes (Fig. 1B). The abundance of the *nifH* gene was positively correlated with the *B-amoA* gene abundance. In contrast, the *chiA* gene abundance was negatively correlated with the *B-amoA* gene abundance. Inorganic N content was positively correlated with the *ureC* gene abundance which was positively correlated with the abundances of *B-amoA* and *A-aomA* genes. The abundance of *B-amoA* was significantly related to NO3–N content which positively affected yield (Fig. 1B). The nitrogen PLS-PM suggested that no direct relationship was found between inorganic N input and NO_3^--N or maize yield, but indirect effects through changes in the microbial community was observed on maize yield. The phosphorus PLS-PM indicated that organic inputs increased SOC content which showed significantly positive relationships with the abundances of *gltA, phoC, phoD, pqqC* and *bpp*. The abundances of *phoD* and *phoC* genes was positively correlated with the activities of ACP and ALP. ALP was positively correlated with maize yield but not with AP content (Fig. 1C). In contrast, there was a significant relationship between ACP and AP content but not with maize yield. In contrast to the nitrogen PLS-PM, the phosphorous PLS-PM suggested a direct relationship between inorganic fertilizer input and yield.

Multiple regression and automated model selection showed that soil microbial functional genes explained 84.5% of the variation in maize yield (Table 4). The gene abundances of *A-amoA, B-amoA, nosZ, phoD, qppC* and *GH31* were identified as important factors in explaining variation in maize yield. The model based on soil proxy indicators explained 69.3% of the variation in maize yield (Table S7). ALP, NO_3^--N and α-1,4 glucosidase activity were the main significant indicators explaining variations in maize yield. There were positive relationships between the abundances of *A-amoA, B-amoA, nosZ, phoD, pqqC, GH31,* the alkaline phosphatase activity, NO_3^--N content and α-1,4 glucosidase activity and maize yield (Table 5).

4. Discussion

4.1. Microbial functional genes are sensitive to fertilization

In line with our first hypothesis, higher variations were observed among treatments than within replicates for functional gene abundances, in particular the genes *phoC, phoD, B-amoA, chiA*, *GH31* and *cbbL*, compared with their corresponding proxy indicators (Table 2). These results indicate that soil microbial functional genes tend to exhibit a greater degree of variability than proxy indicators in response to agricultural managements. Consistent with our results, Chinnadurai et al. (2014) observed that organic manure and chemical fertilizer affected the abundance of microbial functional genes (e.g., *nifH* and

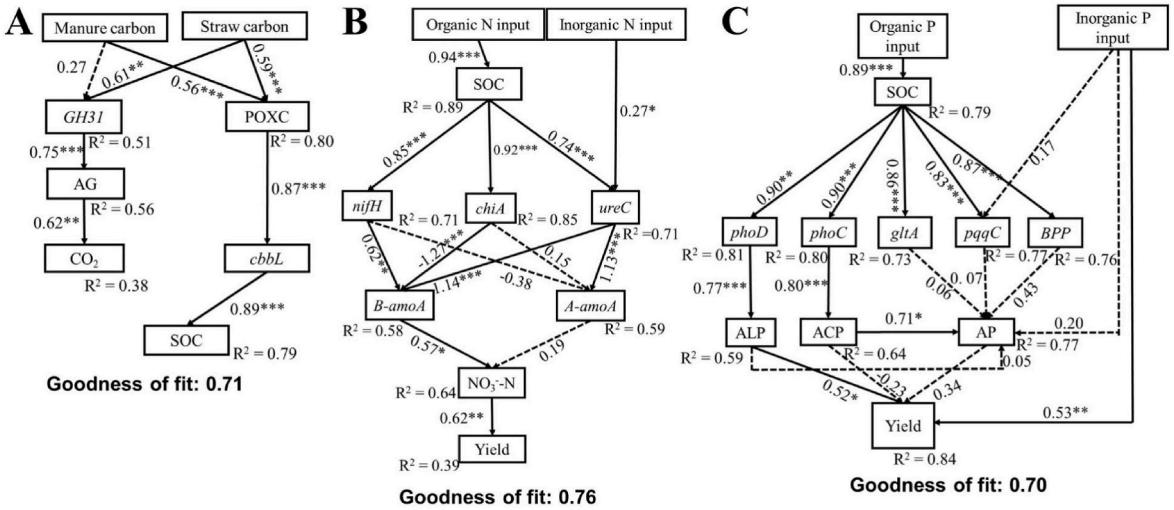

Fig. 1. Partial least squares path analysis for the effects of manure and straw carbon input on the carbon cycling process (A); and the effects of organic and inorganic nitrogen (B) and phosphorus (C) input on the nitrogen and phosphorus cycling process and crop yield, respectively. * indicates $p < 0.05$; ** indicates $p < 0.01$, *** indicates $p < 0.001$, respectively. Continuous and dashed lines indicate significant and nonsignificant relationships, respectively. R^2 denotes the proportion of variance explained. SOC: soil organic carbon, POXC: permanganate oxidizable carbon; AG: α-1,4 glucosidase; CO_2: soil respiration; AP: available phosphorus; ALP: alkaline phosphatase; ACP: acid phosphatase.

Table 4
Predicted model parameters for different soil microbial functional genes based on all-subsets procedure model selection process. All functional genes were divided by 10^3. The statistical test used is the F-test based on a one-sided test, and significant effects ($p \leq 0.05$) denote the significance of the model parameter and are given in bold font. Model: $R^2 = 84.52\%$, $p < 0.001$***.

	Estimate	Adjusted SE	*p*	weight
(Intercept)	5.96	1.96		
A-amoA	**−2.78**	**1.24**	**0.02**	**0.67**
A-aomB	**0.32**	**0.13**	**0.02**	**0.90**
nosZ	**0.98**	**0.33**	**0.003**	**0.81**
bpp	−0.27	0.23	0.24	0.46
phoD	**−39.17**	**16.88**	**0.02**	**0.76**
pqqC	**0.94**	**0.44**	**0.03**	**0.56**
GH31	**0.35**	**0.13**	**0.04**	**0.53**
phoC	−0.07	0.21	0.72	0.15
gltA	1.60	2.09	0.44	0.09
narG	−2.95	1.61	0.07	0.30
norB	−110.00	166.90	0.51	0.14
ureC	0.13	0.08	0.09	0.24
chiA	0.06	0.16	0.69	0.08
nifH	0.16	0.55	0.77	0.07
nirS	0.65	0.57	0.25	0.12
nirK	0.13	0.40	0.75	0.06

Table 5
Relationship between soil microbial functional gene abundances and proxy indicators (selected based on glmulti analysis, Table 4 and S6) and crop yield.

	Soil indicator	R	*p*
Microbial functional gene	*A-amoA*	0.54	0.01
	B-amoA	0.83	<0.001
	nosZ	0.72	<0.001
	phoD	0.73	<0.001
	pqqC	0.69	<0.001
	GH31	0.59	0.006
Proxy indicator	Alkaline phosphatase	0.71	<0.001
	NO_3^--N	0.62	0.003
	α-1,4 glucosidase	0.52	0.02

A-amoA), but not the metabolic quotient. Xue et al. (2013) also found that the abundance of the *narG* gene involved in the denitrification process differed significantly between conventional and organic agricultural land, but the proxy indicator N_2O emissions was not significantly affected. These results support the notion that the use of microbial functional genes has considerable potential for soil health assessment, as they show higher sensitivity to agricultural management practices than conventional measurements.

In the present study, fertilization significantly influenced microbial functional gene abundances (Table 2), which could be associated with changes in soil properties resulting from fertilization management practices. All C cycling genes (*cbbL* and *GH31*) in the organic fertilization and straw return treatment increased significantly compared with those in the NPK treatment (Table 2). SOC content and pH were important drivers of changes in the *cbbL* gene abundance (Qin et al., 2021; Liao et al., 2020). Organic fertilization could provide essential nutrients and carbon to autotrophic bacterial communities, and these resources s would promote bacterial growth (Wang et al., 2021b). Soil pH in the current study ranged from alkaline to neutral, and was therefore favorable for microbial growth (Table S4). The increased abundance of *GH31* in the NPK treatment may be ascribed to the unbalanced soil stoichiometry which leads to microbial carbon mining (Wei et al., 2020). The lower C/N ratio in the NPK treatment compared with that in the organic fertilization treatment indicates that carbon supply was the factor limiting microbial growth (Table S4). Consequentially, soil microbes may accelerate the breakdown of existing soil organic C to meet their metabolic needs (Chen et al., 2018).

Most N-cycling functional genes, especially the *chiA* gene, increased significantly in the organic fertilizer treatments and showed high variations (Table 2). Soil pH and N content in the fertilization treatment were reported to be important factors affecting *chiA* abundance and community (Zhang et al., 2022). By contrast, *B-amoA* responded strongly to the NPK treatment (Fig. 1; Table S3), and this may be attributed to the preference of *B-amoA* for N-rich and high NH_4^+ environments (Bei et al., 2018; Li et al., 2021; Kong et al., 2019). In addition, all P cycling genes increased in the fertilization treatment relative to the Control (Table 2). Genes *phoC and phoD,* which regulate soil organic P mineralization, showed higher variation than other functional genes and the proxy indicators. Genes *phoC* and *phoD* are sensitive to fertilization and are related to changes in soil parameters such as pH and C/N ratio (Fraser et al., 2017; Zheng et al., 2019), and this is supported by the present study (Fig. S6). The current results suggest that microbial functional genes offer a promising tool for the early detection of changes in microbial activities, which may not be easily detected by the use of conventional soil properties. For instance, genes involved in N cycling, such as *chiA* and *B-amoA*, showed strong responses to the fertilization treatments (Table 2). In contrast, soil N content which reflects the combined outcome of these processes cannot be used to distinguish different N cycling pathways, or to estimate the contributions of specific microbial guilds to nutrient cycling (He et al., 2018). It is proposed that the direct linkages between functional genes and key ecosystem processes allow them to be used as functionally based indicators that may reflect soil ecosystem health more accurately than the proxy indicators (Trivedi et al., 2013; Levy-Booth et al., 2014; Wilhelm et al., 2023). Overall, our findings underline the potential of targeting specific functional genes as reliable indicators for monitoring and managing soil health, offering a more sensitive and process-oriented approach to soil management in agricultural systems.

Notably, quantifying gene copy numbers per gram of soil is a straightforward and intuitive method. Soil DNA concentration was a powerful indicator for precise estimation of microbial biomass content in arid and semi-arid regions of northern China (Gong et al., 2021). However, total soil microbial DNA concentrations in different treatments may be potentially misleading, as they may overestimate the treatment effects on soil health (Carini et al., 2016). Here, soil microbial functions (but not total DNA concentration) changed significantly among the different fertilization treatments (Tables S3 and S4). These findings are consistent with those of Lennon et al. (2018), suggesting that total DNA concentration may not always be a sensitive indicator of shifts in microbial activities. Nevertheless, it serves as a useful normalization factor for gene copy number estimation, ensuring accurate comparisons of microbial functional dynamics across treatments (Gong et al., 2021). Thus, while total DNA concentration alone may not fully capture soil microbial functionality, its integration with functional gene quantification provides a more comprehensive assessment of soil health and microbial processes.

4.2. Microbial functional gene abundances are closely related to soil functions and maize yield

The soil functional potential mediated by microbes can be reflected by the abundance of microbial functional genes (Hu et al., 2021). Although bacterial and fungal abundances have been shown to correlate with various soil functions, such as C decomposition and sequestration (Bailey et al., 2002; Xu et al., 2015), these correlations remain relatively indirect compared to the direct role of functional genes encoding enzymes involved in specific soil processes. They can serve as a bridge connecting functional microbial abundances to ecosystem functioning (Wang et al., 2022). In line with our second hypothesis, most microbial functional gene abundances were strongly correlated with their corresponding proxy indicators (C, N and P cycling/pool), even after excluding the Control (Table 3; Table S6). Consistent with our result, Hayden et al. (2010) and Hu et al. (2021) also found that microbial functional genes, such as such as *nifH* and *B-amoA* were positively

correlated with their proxy indicators (total N and NO_3^--N content) in both managed and unmanaged land. The consistency of these findings across diverse environments suggests that microbial functional genes are robust biomarkers for assessing soil functional potential. Furthermore, the strong correlations between functional genes and proxy indicators, along with the results of PLS-PM and linear regression analyses, suggested mechanistic links between enhanced element cycling under fertilization treatments and maize crop yields (Fig. 1). These results further suggest that process-based selections of certain microbial functional genes are likely to drive changes in proximal indicators of soil functions, highlighting their potential as valuable indicators of soil functioning processes. Notably, microbial functional genes exhibited higher explanations for maize yield than those of the proxy indicators (Table 4; Table S7). The increase in maize yield in the fertilization treatments was associated with increased microbial activities and nutrient cycling (Fig. 1). This reinforces the interconnectedness of soil health and crop productivity. In fact, soil health has been found to be positively correlated with high crop productivity (Romero et al., 2024). These results underscore the potential of microbial functional genes for guiding soil health management strategies to increase agricultural productivity.

Aligned with our third hypothesis, organic inputs increased the abundance of microbial functional genes related to carbon and nutrient cycling, thereby enhancing soil functioning and contributing to high maize yields (Fig. 1; Table 4). Manure and straw contain active C sources for microbial growth, increasing the *cbbL* gene abundance involved in the carbon-fixing process. This increase was positively correlated with the increase in total organic C content (Fig. 1A). In addition, The *GH31* gene, which is responsible for the decomposition of xyloglucan and xylan (Yuan et al., 2008), showed increased abundance in the straw return treatment (Fig. 1A). The results indicate that organic amendments can enhance microbial activities and carbon utilization. The positive relationships between *GH31* and α-glucosidases, the enzyme that catalyzes the degradation of organic matter (Fig. 1A), further reinforce the role of these genes in carbon cycling and CO_2 evolution.

Compared to the NPK only treatment, organic fertilization significantly increased the abundances of *nifH, chiA* and *ureC* genes that are part of the N cycles. The *nifH* gene is involved in biological nitrogen fixation and converts atmospheric nitrogen into ammonia (Ladha et al., 2022). This aligns with previous studies suggesting that organic amendments can promote N-fixing bacteria, thereby increasing N availability in the soil (Ghadimi et al., 2021). In adition, the increase in *chiA*, a gene associated with N mineralization, indicates that organic fertilization may enhance the breakdown of organic N compounds (Lindsay et al., 2010). The positive correlation between *chiA* abundance and β-N-acetylglucosaminidase enzyme activity (Table 3) indicate that organic fertilizers may stimulate specific microbial processes involved in N cycling. The *ureC* gene, which encodes for urease and is responsible for converting urea into ammonia (Zhang et al., 2023), showed increased abundance in both organic and NPK only treatments. In contrast, the abundance of *B-amoA* but not *A-amoA* was significantly associated with a higher soil NO_3^--N content (Fig. 1B). These results indicate that change in *B-amoA* is a key microbial response to inorganic N inputs while *ureC* appears to be a universal microbial response to N input regardless of fertilization type.

All P functional genes were positively related to proxy indicators. However, PLS-PM showed that only *phoD* encoding alkaline phosphatase was positively correlated with maize yield. ACP, which was positively related to the gene *phoC*, was positively related to available P content but not with maize yield (Fig. 1C). These results emphasize the complementary roles of *phoC* and *phoD* in the mineralization of organic P under different fertilization treatments (Karl and Björkman, 2015; Liu et al., 2023). Notably, *pqqC*, a key biomarker of microbial inorganic P solubilization (Wang et al., 2023b), also exhibited positive correlations with available P and maize yield (Tables 3 and 5). These results provide evidence that functional genes can reflect the underlying microbial processes driving soil health and functions, enhancing the predictive power of conventional soil indicators.

Functional genes such as *ureC* and *B-amoA* play important roles in enhancing N availability and soil fertility, their activities can also connected with potential environmental disservices. The increased abundance of *B-amoA* may increase the risk of nitrate leaching and groundwater contamination. In addition, although no significant correlations were found between the abundances of *A-amoA* and *B-amoA* with N_2O emission (Table S8), their impacts on soil health is context-dependent (Robertson and Vitousek, 2009; Norton and Ouyang, 2019). While the outcome of nitrification is to provide available N to plants, attention should also be paid to its environmental impact. Practices that balance the nitrification rates to optimize nutrient availability while minimizing greenhouse gas emissions are critical for sustainable soil management. Furthermore, the abundances of the functional genes *narG*, *nirK*, *nirS*, *norB* and *nosZ* which are involved in the denitrification process, showed significant positive correlations with NO_3^--N content (Fig. S2). Denitrification is a process in which NO_3^--N serves as an alternative electron acceptor for microorganisms, resulting in the reduction of NO_3^--N to N_2 gas and the provision of energy to microbes (Burgin et al., 2007). A higher NO_3^--N concentration is considered to be a strong inducer of transcription of *nir* and *nor*, leading to an increased abundance of denitrification genes (Wallenstein et al., 2006). However, inconsistent with previous results, a strong positive correlation between *nosZ* and N_2O emission were found, whereas a negative relationship is usually reported (Itakura et al., 2013; Shaaban et al., 2018). This discrepancy suggests that the activity of nitrous oxide reductase, encoded by *nosZ,* plays a critical role in N_2O emissions, rather than merely the presence or abundance of the *nosZ* gene itself (Liu et al., 2014; Wertz et al., 2016). Such insights highlight the importance of incorporating RNA-based methods to measure transcriptional activity, providing a more comprehensive mechanistic understanding of N_2O emissions (Butterly et al., 2016; Wertz et al., 2016). Moreover, we only examined the transformation of NO_3^--N pools at maturity. However, NO_3^--N concentrations can vary substantially over time as a result of environmental factors, microbial activity and plant uptake (Laverman et al., 2000). A one-time measurement may have drawbacks for the accurate reflection of the temporal dynamics of soil N supply levels. Additionally, the denitrification process requires the participation of multiple functional genes which are interconnected, making it difficult to use only one gene as a proxy for denitrification (Philippot et al., 2007). Future research should explore the dynamic changes in order to improve predictions of N availability and crop yield, thus supporting better soil management decisions.

5. Conclusion

Our results showed that fertilization significantly affected the abundance of soil microbial functional genes involved in C, N and P cycling. Most functional genes, in particular *phoC, phoD, B-amoA, chiA, GH31* and *cbbL* showed higher variability among treatments and lower variability among replicates within treatments than their corresponding proxy indicators, indicating that functional genes were more responsive to fertilization than the selected proxy indicators for soil functioning. Furthermore, regression analysis showed that microbial functional gene abundances and the corresponding proxy indicators were strongly correlated. Partial least squares path analysis showed that the organic fertilization increased soil microbial functional gene abundances, especially *GH31, cbbL, chiA, B-amoA, phoC*, and *phoD*, which promoted the C sequestration and decomposition, N mineralization, ammonia oxidation and P cycling process, producing positive effects on maize yield. These microbial functional genes offer a more detailed understanding of soil functions than conventional proxy indicators due to their more direct and specific relationship with the underlying biochemical processes. The results strongly endorse that the use of functional genes that can serve as crucial biomarkers for understanding

the complex dynamics of soil processes and as indispensable biological indicators for assessing soil health.

CRediT authorship contribution statement

Jiyu Jia: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Ron de Goede:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Yizan Li:** Writing – review & editing, Formal analysis. **Jiangzhou Zhang:** Investigation, Conceptualization. **Guangzhou Wang:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Junling Zhang:** Writing – review & editing, Writing – original draft, Supervision, Data curation, Conceptualization. **Rachel Creamer:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Formal analysis, Data curation, Conceptualization.

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

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References

Anderson, D.R., 2007. Model Based Inference in the Life Sciences: a Primer on Evidence. Springer Science and Business Media.
Bailey, V.L., Smith, J.L., Bolton Jr, H., 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biol. Biochem. 34, 997–1007.
Bei, S., Zhang, Y., Li, T., Christie, P., Li, X., Zhang, J., 2018. Response of the soil microbial community to different fertilizer inputs in a wheat-maize rotation on a calcareous soil. Agric., Ecosyst. Environ. 260, 58–69.
Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. J. Visualized Exp. 81, e50961.
Bender, S.F., Wagg, C., van der Heijden, M.G., 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends in Ecology & Evolution 31, 440–452.
Bhaduri, D., Sihi, D., Bhowmik, A., Verma, B.C., Munda, S., Dari, B., 2022. A review on effective soil health bio-indicators for ecosystem restoration and sustainability. Frontiers in Microbiology 13, 938481.
Burgin, A.J., Hamilton, S.K., 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Frontiers in Ecology and the Environment 5, 89–96.
Butterly, C.R., Phillips, L.A., Wiltshire, J.L., Franks, A.E., Armstrong, R.D., Chen, D., Mele, P.M., Tang, C., 2016. Long-term effects of elevated CO_2 on carbon and nitrogen functional capacity of microbial communities in three contrasting soils. Soil Biol. Biochem. 97, 157–167.
Calcagno, V., de Mazancourt, C., 2010. glmulti: an R package for easy automated model selection with (generalized) linear models. J. Stat. Softw 34, 1–29.
Cardoso, E.J.B.N., Vasconcellos, R.L.F., Bini, D., Miyauchi, M.Y.H., Santos, C.A.D., Alves, P.R.L., Paula, A.M., Nakatani, A.S., Pereira, J.M., Nogueira, M.A., 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? Sci. agric. 70, 274–289.
Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N., 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat. Microbiol. 2, 1–6.
Chen, L., Liu, L., Mao, C., Qin, S., Wang, J., Liu, F., Blagodatsky, S., Yang, G., Zhang, Q., Zhang, D., Yu, J., Yang, Y., 2018. Nitrogen availability regulates topsoil carbon dynamics after permafrost thaw by altering microbial metabolic efficiency. Nature Communications 9, 3951.
Chinnadurai, C., Gopalaswamy, G., Balachandar, D., 2014. Long term effects of nutrient management regimes on abundance of bacterial genes and soil biochemical processes for fertility sustainability in a semi-arid tropical Alfisol. Geoderma 232, 563–572.
Colloff, M.J., Wakelin, S.A., Gomez, D., Rogers, S.L., 2008. Detection of nitrogen cycle genes in soils for measuring the effects of changes in land use and management. Soil Biol. Biochem. 40, 1637–1645.
Creamer, R.E., Barel, J.M., Bongiorno, G., Zwetsloot, M.J., 2022. The life of soils: integrating the who and how of multifunctionality. Soil Biol. Biochem. 166, 108561.
Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: managing the biotic component of soil quality. Applied Soil Ecology 15, 3–11.
Dos Santos, P.C., Fang, Z., Mason, S.W., Setubal, J.C., Dixon, R., 2012. Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. BMC Genomics 13, 1–12.
Fan, K., Delgado-Baquerizo, M., Zhu, Y.G., Chu, H., 2020. Crop production correlates with soil multitrophic communities at the large spatial scale. Soil Biol. Biochem. 151, 108047.
Fisher, K.A., Yarwood, S.A., James, B.R., 2017. Soil urease activity and bacterial ureC gene copy numbers: effect of pH. Geoderma 285, 1–8.
Fraser, T.D., Lynch, D.H., Gaiero, J., Khosla, K., Dunfield, K.E., 2017. Quantification of bacterial non-specific acid (phoC) and alkaline (phoD) phosphatase genes in bulk and rhizosphere soil from organically managed soybean fields. Applied Soil Ecology 111, 48–56.
Ghadimi, M., Sirousmehr, A., Ansari, M.H., Ghanbari, A., 2021. Organic soil amendments using vermicomposts under inoculation of N2-fixing bacteria for sustainable rice production. PeerJ 9, e10833.
Gong, H., Du, Q., Xie, S., Hu, W., Akram, M.A., Hou, Q., Dong, L., Sun, Y., Manan, A., Deng, Y., Ran, J., Deng, J., 2021. Soil microbial DNA concentration is a powerful indicator for estimating soil microbial biomass C and N across arid and semi-arid regions in northern China. Applied Soil Ecology 160, 103869.
Griffiths, B.S., Bonkowski, M., Roy, J., Ritz, K., 2001. Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. Applied Soil Ecology 16, 49–61.
Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J 9, 1177–1194.
Hayden, H.L., Drake, J., Imhof, M., Oxley, A.P., Norng, S., Mele, P.M., 2010. The abundance of nitrogen cycle genes amoA and nifH depends on land-uses and soil types in South-Eastern Australia. Soil Biol. Biochem. 42, 1774–1783.
He, L., Bi, Y., Zhao, J., Pittelkow, C.M., Zhao, X., Wang, S., Xing, G., 2018. Population and community structure shifts of ammonia oxidizers after four-year successive biochar application to agricultural acidic and alkaline soils. Sci. Total Environ. 619, 1105–1115.
Hu, Y., Jiang, H., Chen, Y., Wang, Z., Yan, Y., Sun, P., Lu, X., 2021. Nitrogen addition altered the microbial functional potentials of carbon and nitrogen transformation in alpine steppe soils on the Tibetan Plateau. Glob. Ecol. Conserv. 32, e01937.
Hussain, S.I., Phillips, L.A., Hu, Y., Frey, S.K., Geuder, D.S., Edwards, M., Lapen, D.R., Ptacek, C.J., Blowes, D.W., 2021. Differences in phosphorus biogeochemistry and mediating microorganisms in the matrix and macropores of an agricultural clay loam soil. Soil Biol. Biochem. 161, 108365.
Itakura, M., Uchida, Y., Akiyama, H., Hoshino, Y.T., Shimomura, Y., Morimoto, S., Minamisawa, K., 2013. Mitigation of nitrous oxide emissions from soils by Bradyrhizobium japonicum inoculation. Nature Climate Change 3, 208–212.
Jia, J., Zhang, J., Li, Y., Koziol, L., Podzikowski, L., Delgado-Baquerizo, M., Zhang, J., 2023. Relationships between soil biodiversity and multifunctionality in croplands depend on salinity and organic matter. Geoderma 429, 116273.
Jia, J., Zhang, J., Li, Y., Xie, M., Wang, G., Zhang, J., 2022. Land use intensity constrains the positive relationship between soil microbial diversity and multifunctionality. Plant and Soil 1–14.
Karl, D.M., Björkman, K.M., 2015. Dynamics of dissolved organic phosphorus. In: Biogeochemistry of Marine Dissolved Organic Matter. Academic Press, pp. 233–334.
Kong, Y., Ling, N., Xue, C., Chen, H., Ruan, Y., Guo, J., Guo, S., 2019. Long-term fertilization regimes change soil nitrification potential by impacting active autotrophic ammonia oxidizers and nitrite oxidizers as assessed by DNA stable isotope probing. Environmental Microbiology 21, 1224–1240.
Kopittke, P.M., Menzies, N.W., Wang, P., McKenna, B.A., Lombi, E., 2019. Soil and the intensification of agriculture for global food security. Environment International 132, 105078.
Ladha, J.K., Peoples, M.B., Reddy, P.M., Biswas, J.C., Bennett, A., Jat, M.L., Krupnik, T.J., 2022. Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. Field Crop Res 283, 108541.
Laverman, A.M., Zoomer, H.R., Van Verseveld, H.W., Verhoef, H.A., 2000. Temporal and spatial variation of nitrogen transformations in a coniferous forest soil. Soil Biol. Biochem. 32, 1661–1670.
Lennon, J.T., Muscarella, M.E., Placella, S.A., Lehmkuhl, B.K., 2018. How, when, and where relic DNA affects microbial diversity. MBio 9, 10–1128.
Levy-Booth, D.J., Prescott, C.E., Grayston, S.J., 2014. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biol. Biochem. 75, 11–25.
Li, K.Y., Zhao, Y.Y., Yuan, X.L., Zhao, H.B., Wang, Z.H., Li, S.X., Malhi, S.S., 2012. Comparison of factors affecting soil nitrate nitrogen and ammonium nitrogen extraction. Communications in Soil Science and Plant Analysis 43, 571–588.
Li, H.P., Han, Q.Q., Liu, Q.M., Gan, Y.N., Rensing, C., Rivera, W.L., Zhang, J.L., 2023b. Roles of phosphate-solubilizing bacteria in mediating soil legacy phosphorus availability. Microbiol. Res., 127375
Li, R., Ren, C., Wu, L., Zhang, X., Mao, X., Fan, Z., Cui, W., Zhang, W., Wei, G., Shu, D., 2023a. Fertilizing-induced alterations of microbial functional profiles in soil nitrogen cycling closely associate with crop yield. Environmental Research 231, 116194.

Li, X., Wang, Y., Zhang, Y., Lang, M., Christie, P., Bei, S., Zhang, J., 2021. Dynamics of ammonia oxidizers in response to different fertilization inputs in intensively managed agricultural soils. Applied Soil Ecology 157, 103729.
Liu, B., Frostegård, Å., Bakken, L.R., 2014. Impaired reduction of N_2O to N_2 in acid soils is due to a posttranscriptional interference with the expression of nosZ. mBio 5, 10–1128.
Liao, H., Qin, F., Wang, K., Zhang, Y., Hao, X., Chen, W., Huang, Q., 2020. Long-term chemical fertilization-driving changes in soil autotrophic microbial community depresses soil CO_2 fixation in a Mollisol. Sci. Total Environ. 748, 141317.
Lindsay, E.A., Colloff, M.J., Gibb, N.L., Wakelin, S.A., 2010. The abundance of microbial functional genes in grassy woodlands is influenced more by soil nutrient enrichment than by recent weed invasion or livestock exclusion. Applied and Environmental Microbiology 76, 5547–5555.
Liu, L., Gao, Z., Yang, Y., Gao, Y., Mahmood, M., Jiao, H., Wang, Z., Liu, J., 2023. Long-term high-P fertilizer input shifts soil P cycle genes and microorganism communities in dryland wheat production systems. Agric., Ecosyst. Environ. 342, 108226.
Maikhuri, R.K., Rao, K.S., 2012. Soil quality and soil health: a review. International Journal of Ecology & Environmental Sciences 38, 19–37.
Mbuthia, L.W., Acosta-Martínez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Eash, N., 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: implications for soil quality. Soil Biol. Biochem. 89, 24–34.
Morugán-Coronado, A., Pérez-Rodríguez, P., Insolia, E., Soto-Gómez, D., Fernández-Calvino, D., Zornoza, R., 2022. The impact of crop diversification, tillage and fertilization type on soil total microbial, fungal and bacterial abundance: a worldwide meta-analysis of agricultural sites. Agriculture, Ecosystems & Environment 329, 107867.
Muscolo, A., Settineri, G., Attinà, E., 2015. Early warning indicators of changes in soil ecosystem functioning. Ecological Indicators 48, 542–549.
Norton, J., Ouyang, Y., 2019. Controls and adaptive management of nitrification in agricultural soils. Frontiers in Microbiology 10, 1931.
Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. USDA Circular, Washington, DC, p. 18.
Ouyang, Y., Norton, J.M., 2020. Short-term nitrogen fertilization affects microbial community composition and nitrogen mineralization functions in an agricultural soil. Applied and Environmental Microbiology 86, e02278.
Ouyang, Y., Evans, S.E., Friesen, M.L., Tiemann, L.K., 2018. Effect of nitrogen fertilization on the abundance of nitrogen cycling genes in agricultural soils: a meta-analysis of field studies. Soil Biol. Biochem. 127, 71–78.
Philippot, L., Hallin, S., Schloter, M., 2007. Ecology of denitrifying prokaryotes in agricultural soil. Advances in Agronomy 96, 249–305.
Phillips, L.A., Schefe, C.R., Fridman, M., O'Halloran, N., Armstrong, R.D., Mele, P.M., 2015. Organic nitrogen cycling microbial communities are abundant in a dry Australian agricultural soil. Soil Biol. Biochem. 86, 201–211.
Qin, J., Li, M., Zhang, H., Liu, H., Zhao, J., Yang, D., 2021. Nitrogen deposition reduces the diversity and abundance of *cbbL* gene-containing CO_2-fixing microorganisms in the soil of the Stipa baicalensis steppe. Frontiers in Microbiology 12, 570908.
Raza, T., Qadir, M.F., Khan, K.S., Eash, N.S., Yousuf, M., Chatterjee, S., Oetting, J.N., 2023. Unrevealing the potential of microbes in decomposition of organic matter and release of carbon in the ecosystem. J. Environ. Manage. 344, 118529.
Robertson, G.P., Vitousek, P.M., 2009. Nitrogen in agriculture: balancing the cost of an essential resource. Annual Review of Environment and Resources 34, 97–125.
Romero, F., Labouyrie, M., Orgiazzi, A., Ballabio, C., Panagos, P., Jones, A., Tedersoo, L., Bahram, M., Guerra, C.A., Eisenhauer, N., Tao, D., 2024. Soil health is associated with higher primary productivity across Europe. Nat. Ecol. Evol. 8, 1847–1855.
Schimel, J., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3, 31913.
Schoenholtz, S.H., Van Miegroet, H., Burger, J.A., 2000. A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. FOREST ECOL MANAG. 138, 335–356.
Shaaban, M., Wu, Y., Khalid, M.S., Peng, Q.A., Xu, X., Wu, L., Hu, R., 2018. Reduction in soil N_2O emissions by pH manipulation and enhanced *nosZ* gene transcription under different water regimes. Environ. Pollut. 235, 625–631.
Shi, W., Xing, Y., Zhu, Y., Gao, N., Ying, Y., 2022. Diverse responses of *pqqC*-and *phoD*-harbouring bacterial communities to variation in soil properties of Moso bamboo forests. Microbial Biotechnology 15, 2097–2111.
Smith, C.J., McKew, B.A., Coggan, A., Whitby, C., 2017. Primers: functional genes for nitrogen-cycling microbes in oil reservoirs. Hydrocarbon and Lipid Microbiology Protocols: Primers 207–241.
Song, Y., Liu, C., Wang, X., Ma, X., Jiang, L., Zhu, J., Song, C., 2020. Microbial abundance as an indicator of soil carbon and nitrogen nutrient in permafrost peatlands. Ecological Indicators 115, 106362.
Soong, J.L., Fuchslueger, L., Marañon-Jimenez, S., Torn, M.S., Janssens, I.A., Penuelas, J., Richter, A., 2020. Microbial carbon limitation: the need for integrating microorganisms into our understanding of ecosystem carbon cycling. Global Change Biology 26, 1953–1961.
Talbot, J.M., Martin, F., Kohler, A., Henrissat, B., Peay, K.G., 2015. Functional guild classification predicts the enzymatic role of fungi in litter and soil biogeochemistry. Soil Biol. Biochem. 88, 441–456.
Thiele-Bruhn, S., Schloter, M., Wilke, B.M., Beaudette, L.A., Martin-Laurent, F., Cheviron, N., Mougin, C., Römbke, J., 2020. Identification of new microbial functional standards for soil quality assessment. Soil 6, 17–34.
Trivedi, P., Anderson, I.C., Singh, B.K., 2013. Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. Trends in Microbiology 21 (12), 641–651.
Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C., Singh, B.K., 2016. Microbial regulation of the soil carbon cycle: evidence from gene–enzyme relationships. ISME J 10, 2593–2604.
van Bruggen, A.H., Semenov, A.M., 2000. In search of biological indicators for soil health and disease suppression. Applied Soil Ecology 15, 13–24.
van der Bom, F., Nunes, I., Raymond, N.S., Hansen, V., Bonnichsen, L., Magid, J., Jensen, L.S., 2018. Long-term fertilisation form, level and duration affect the diversity, structure and functioning of soil microbial communities in the field. Soil Biol. Biochem. 122, 91–103.
Wallenstein, M.D., Myrold, D.D., Firestone, M., Voytek, M., 2006. Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. Eco. Appl 16, 2143–2152.
Wang, J., Xie, J., Li, L., Luo, Z., Wang, L., Jiang, Y., 2021b. The impact of fertilizer amendments on soil autotrophic bacteria and carbon emissions in maize field on the semiarid loess plateau. Frontiers in Microbiology 12, 664120.
Wang, G., Jin, Z., George, T.S., Feng, G., Zhang, L., 2023b. Arbuscular mycorrhizal fungi enhance plant phosphorus uptake through stimulating hyphosphere soil microbiome functional profiles for phosphorus turnover. New Phytologist 238 (6), 2578–2593.
Wang, L., Wang, J., Yuan, J., 2023a. Long-term organic fertilization strengthens the soil phosphorus cycle and phosphorus availability by regulating the pqqC- and phoD-harboring bacterial communities. Microbial Ecology 86, 2716–2732.
Wang, T., Duan, Y., Liu, G., Shang, X., Liu, L., Zhang, K., Fang, W., 2022. Tea plantation intercropping green manure enhances soil functional microbial abundance and multifunctionality resistance to drying-rewetting cycles. Sci. Total Environ. 810, 151282.
Wang, W., Hou, Y., Pan, W., Vinay, N., Mo, F., Liao, Y., Wen, X., 2021a. Continuous application of conservation tillage affects in situ N_2O emissions and nitrogen cycling gene abundances following nitrogen fertilization. Soil Biol. Biochem. 157, 108239.
Wei, X., Zhu, Z., Liu, Y., Luo, Y., Deng, Y., Xu, X., Ge, T., 2020. C: N: P stoichiometry regulates soil organic carbon mineralization and concomitant shifts in microbial community composition in paddy soil. Biology and Fertility of Soils 56, 1093–1107.
Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. Am. J. Altern. Agric. 18, 3–17.
Wertz, S., Goyer, C., Zebarth, B.J., Tatti, E., Burton, D.L., Chantigny, M.H., Filion, M., 2016. The amplitude of soil freeze-thaw cycles influences temporal dynamics of N_2O emissions and denitrifier transcriptional activity and community composition. Biology and Fertility of Soils 52, 1149–1162.
White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18, 315–322.
Wilhelm, R.C., Amsili, J.P., Kurtz, K.S., van Es, H.M., Buckley, D.H., 2023. Ecological insights into soil health according to the genomic traits and environment-wide associations of bacteria in agricultural soils. ISME Commun 3, 1.
Xu, Z., Yu, G., Zhang, X., Ge, J., He, N., Wang, Q., Wang, D., 2015. The variations in soil microbial communities, enzyme activities and their relationships with soil organic matter decomposition along the northern slope of Changbai Mountain. Applied Soil Ecology 86, 19–29.
Xue, K., Wu, L., Deng, Y., He, Z., Van Nostrand, J., Robertson, P.G., Schmidt, T.M., Zhou, J., 2013. Functional gene differences in soil microbial communities from conventional, low-input, and organic farmlands. Applied and Environmental Microbiology 79, 1284–1292.
Yang, S., Liebner, S., Walz, J., Knoblauch, C., Bornemann, T.L., Probst, A.J., in t Zandt, M.H., 2021. Effects of a long-term anoxic warming scenario on microbial community structure and functional potential of permafrost-affected soil. Permafrost Periglac 32, 641–656.
Yang, Y., Liu, H., Chen, Y., Wu, L., Huang, G., Lv, J., 2024. Soil nitrogen cycling gene abundances in response to organic amendments: a meta-analysis. Sci. Total Environ., 171048
Yuan, H., Ge, T., Wu, X., Liu, S., Tong, C., Qin, H., Wu, M., Wei, W., Wu, J., 2012. Long-term field fertilization alters the diversity of autotrophic bacteria based on the ribulose-1, 5-biphosphate carboxylase/oxygenase (RubisCO) large-subunit genes in paddy soil. Applied Microbiology and Biotechnology 95, 1061–1071.
Yuan, X.L., van der Kaaij, R.M., van den Hondel, C.A., Punt, P.J., van der Maarel, M.J., Dijkhuizen, L., Ram, A.F., 2008. Aspergillus Niger genome-wide analysis reveals a large number of novel alpha-glucan acting enzymes with unexpected expression profiles. Mol. Genet. Genomics. 279, 545–561.
Yusoff, M.Z.M., Hu, A., Feng, C., Maeda, T., Shirai, Y., Hassan, M.A., Yu, C.P., 2013. Influence of pretreated activated sludge for electricity generation in microbial fuel cell application. Bioresource Technol 145, 90–96.
Zhang, L., Gao, J., Wang, L., Sun, Y., Dong, X., Pei, J., Shi, Y., 2023. Poly-γ-glutamic acid differentially alters the abundances and communities of N functional genes involved in urea hydrolysis, nitrification and denitrification when applied with different nitrogen fertilizers. Applied Soil Ecology 190, 105015.
Zhang, Y., Mu, Y., Fang, S., Liu, J., 2013. An improved GC-ECD method for measuring atmospheric N_2O. Journal of Environmental Sciences 25, 547–553.
Zhang, Y., Sun, C., Wang, S., Xie, H., Jiang, N., Chen, Z., Wei, K., Bao, X., Song, X., Bai, Z., 2022. Stover and biochar can improve soil microbial necromass carbon, and enzymatic transformation at the genetic level. GCB Bioenergy 14, 1082–1096.
Zheng, M.M., Wang, C., Li, W.X., Song, W.F., Shen, R.F., 2019. Soil nutrients drive function and composition of phoC-harboring bacterial community in acidic soils of Southern China. Frontiers in Microbiology 10, 2654.