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Glucose addition in natural forest soils has higher biological nitrogen fixation capacity than other types of soils

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Land use changes soil microbial and chemical properties, but the mechanism of biological nitrogen fixation under different land use patterns is rarely reported, so we used four types of soil: Natural forest soil (NS), healthy banana soil (HS), diseased banana soil (DS) and paddy soil (PS). Treatments included the control (CK), addition of glucose (G), addition of glucose and ammonium nitrate (GN), addition of banana straw (BS), addition of banana straw and ammonium nitrate (BSN), addition of banana root (BR), and addition of banana root and ammonium nitrate (BRN). The study found that the change of soil utilization types, glucose addition increased carbon dioxide emissions (Compared with the control, increased by 963.11%, 508.39%, 794.77% and 511.34%, respectively) and enhanced the ability of soil microbial nitrogen fixation. Importantly, natural forest soil microorganisms have a higher biological nitrogen fixation capacity compared to other types of soils. Glucose addition caused the accumulation of ammonium nitrogen (Compared with the control, increased by 426.08%, 934.21%, 420% and 1065.95%, respectively), indicating that microorganisms had higher utilization efficiency of soluble carbon and enhanced the biological nitrogen fixation capacity, and nitrogen addition caused the accumulation of ammonium nitrogen, thereby weakening the biological nitrogen fixation capacity. At the same time, glucose significantly increased the *Fimicutes* phylum (83.73%, 66.38%, 67.18% and 70.36%) and lowered the level of other bacterial phylums, thereby reducing the bacterial network structure, and the stability of the soil environment has decreased. Forest analysis showed that CO_2 was an important factor in predicting the bacterial community structure of different soil types, an increase in CO_2 content can predict drastic changes in the bacterial community. Bacteria at the *Fimicutes* phylum level preferred glucose, which may also have a negative effect on bacteria at the level of other phylums.

Keywords Nitrogen fixation, Bacteria, Soil utilization types, Tropical, Glucose addition

Soil microorganisms are an important part of the ecosystem, and they drive soil processes of function, including carbon and nitrogen fixation[1,2]. Soil microbes are play an important role in essential biological functions that improve soil development of health and inhibit plant disease[3], such as by promoting nutrient cycle, SOM conversion, plant production and contributing to the suppression of soil-borne diseases[4]. As a result of the rapid response of bacteria with changes under the soil condition, bacterial community has served as early biological indicator of soil quality[5]. Previous results have shown that fertilization impacts soil microbes and properties by addition of nutrients[6]. Appropriate N input leads to increased soil N content, reduces the restriction of N utilization by soil microorganisms, and promotes the growth of bacterial communities[7,8]. In forest ecosystems in southern China, N addition reduced soil bacterial community α-diversity because the increase of NO_3^- reduced soil pH and thus bacterial community α-diversity[9], while in grassland and farmland ecosystems, N deposition on bacterial communities was not significant influence[10,11]. Bacterial biomass generally respond to other factors such as changes in litter and plant root exudates may contribute to the response of bacterial communities to N inputs[12].

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Tropical soils are of global importance because they store large amounts of terrestrial carbon[13], and soil microbes play an important role in regulating C store by the mineralization of straw and SOM. Soil microorganisms are the major decomposers of crop residues because of their specific capability to produce enzymes that decompose both simple molecules and more complex plant-derived compounds[14]. Crop residues addition is an important way to promote soil fertility and improve crop growth, which is important sustainable agriculture development[15,16]. Microorganisms can be major drivers of nutrient biogeochemical cycles through their role in breaking down straw in soil. There is a large amount of evidence showing that different straw features and factors of environment can result in substantial changes in the diversity of soil microbial communities[17,18]. Ma et al. also found this positive and significant effect where straw addition fortifies soil microbial biomass, activity and community structure[19,20], and also augments the structure of soil particles that improve soil quality[21,22]. A previous study found that a significant increase in bacterial and actinomycete biomass occurred under short-term addition of crop straw to a field in Jiang Yan, China[5]. An easily-absorbed carbon source, glucose can be used rapidly by soil microorganisms. Different amounts of glucose were added to the soil, the greater amounts increased CO_2 flux during a 49-day experiment[23]. CO_2 flux, MBC, and dissolved organic C is influenced by the amount of glucose added to the soil. Glucose as an exogenous carbon source to study priming effects, because of glucose is a compound lacking nitrogen, and the application of glucose can even eliminate the effects of nitrogen. Glucose can directly participate in the soil carbon metabolism processes as a source of carbon and energy[24].

Biological nitrogen fixation is as the second-largest contributor to nitrogen in soil, comprising approximately 16% of the global nitrogen input[25]. Nitrogen fertilizer addition inhibited the growth of nitrogen fixation microorganisms, decreasing in the diversity of nitrogen fixation soil microorganisms, and the nitrate nitrogen emerging as the main influencing factors[26]. However, appropriate nitrogen fertilizer addition can increase soil organic carbon content and abundance of nitrogen-fixing bacteria, thereby improving nitrogen fixation capability of soil microorganisms[27]. Furthermore, other researchers found that abundance and nitrogen fixation bacteria have not significant differences following nitrogen addition[28,29]. In conclusion, we propose several hypotheses, (1) Different types of carbon (such as glucose, rice straw and roots) combined with nitrogen addition affect soil bacteria and chemical properties under types of soils; (2) The addition of organic carbon (glucose) promotes the nitrogen fixation capacity of different types of soil microorganisms, especially natural forest soils. We hope to find out the differences in the effects of different types of carbon addition on biological nitrogen fixation in different types of soils, and provide a theoretical basis for the screening of nitrogen-fixing microorganisms in the future.

2. Materials and methods

2.1 Study site description and sampling

Samples were collected from Natural forest soil (NS), Healthy banana soil (HS), Diseased banana soil (DS, high *Fusarium oxysporum* f. sp. *cubense*), and Paddy soil (PS). The sampling locations were adjacent to each other, and HS, DS, and PS all developed from NS, originally in the same location in Fig. 1. (HS, DS, and PS evolved from NS.) The soil is classified as lateritic soil that developed from basalt parent material. The four types of soil used in the experiment were gathered in Chengmai, Hainan, China (19°23', 110°15'). Soil samples were collected from the top 20 cm of soil at four randomly chosen locations. A portion of soil samples were used for soil physicochemical property analysis, and others were stored in -30 °C for DNA extraction.

2.2 Experimental design

Soil physicochemical properties were determined from the samples collected from sites representing four different land uses: NS, HS, DS, and PS (Table 1). Samples (10 g) were added to 120 mL culture flasks, along with glucose (8 mg/g soil), ammonium nitrate (1 mg/g soil), organic materials, and $^{15}N_2$ ($^{15}N_2$: O_2: Ar = 20:20:60), banana straw and roots (0.15 g), see Table 1 for all C and N content). Next, they were incubated for 42 days under 28℃ and 60% of field water capacity, and seven treatments were set up (Fig. 2): control (CK), addition of glucose (G), addition of glucose and ammonium nitrate (GN), addition of banana straw (BS), addition of banana straw and ammonium nitrate (BSN), addition of banana root (BR), addition of banana root and ammonium nitrate (BRN). Each treatment was replicated thrice. Gas is collected every seven days to calculate cumulative CO_2 emissions and then replace fresh culture gas, determine whether the environment is anaerobic conditions before collection. At the end of incubation, soil inorganic nitrogen content, soil insoluble organic carbon and nitrogen contents, bacterial gene copy number, and bacterial high-throughput sequencing were measured to quantify the carbon and nitrogen utilization and bacterial communities under different soil types.

2.3 Determination of soil physicochemical properties

Soil chemical properties were analyzed by the method of Lu (2000)[30]. The pH was measured by a water-to-soil ratio of 4:1 (v/w). Soil volume weight and water content were determined via the drying methods. SOC was determined by $K_2Cr_2O_7$ oxidation method. Soil total nitrogen (TN) was measured by the Kjeldahl method of Lu (2000). Soil ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) were determined by a continuous flow analyzer (AA3, Germany). Soil ^{13}C and ^{15}N abundances were measured by an isotope mass spectrometer (Germany). Soil microbial respiration was measured by closed incubation and CO_2 emissions were determined by gas chromatography (Wang et al., 2003).

2.4 Microbial analyses

The complete soil microbial genome was extracted using the FastDNA® Spin Kit for Soil (MPBiomedicals). The DNA was dissolved in 100 µL of DES buffer, and the detailed procedures described in the kit instructions were followed. PCR amplification was used universal primers of bacteria (515 F/907R), and sequencing analysis was

Fig. 1. Distribution of soil sampling sites.

Parameter	pH	Wc (%)	Vw (g/cm^3)	TC (g/kg)	TN (g/kg)	NH_4^+ (mg/kg)	NO_3^- (mg/kg)	C/N
NS	5.92	44.04	1.32	20.17	1.78	15.88	8.76	11.34
HS	5.14	47.00	1.28	18.90	1.54	11.30	13.50	12.27
DS	4.91	51.63	1.20	18.93	1.49	19.16	26.11	12.67
PS	5.32	47.40	1.25	19.12	1.57	18.67	18.67	12.22
Banana straw	–	–	–	419.21	11.30	–	–	37.11
Banana roots	–	–	–	437.49	8.61	–	–	50.86

Table 1. Physical and chemical analyses of soil and organic materials. Treatments: CK: no glucose or NH_4NO_3; G: glucose; GN: glucose and NH_4NO_3; BS: banana straw; BSN: banana straw and NH_4NO_3; BR: banana root; BRN: banana root and NH_4NO_3; TC: Total C; TN: Total Nitrogen; NO_3^-: Nitrate nitrogen; NH_4^+: Ammonium nitrogen.

used by the Illumina NovaSeq platform. All raw sequences can be found at NCBI under accession numbers PRJNA906297, PRJNA906361, PRJNA906365 and PRJNA906442.

2.5 Statistical analyses

Data record and analysis was used by the Microsoft Excel 2013, and used the SPSS 26.0 to statistics and analysis the data. And all graphs were generated in Origin 2023 soft in this paper. Principal coordinate analysis (PCoA) based on Bray-Curtis distance and permutation multivariate analysis of variance were performed in the R package vegan. In the same package, redundancy analysis (RDA) was used to analysis the correlation between chemical properties and bacterial communities. Network analysis was to reduce complexity, only the OTUs with

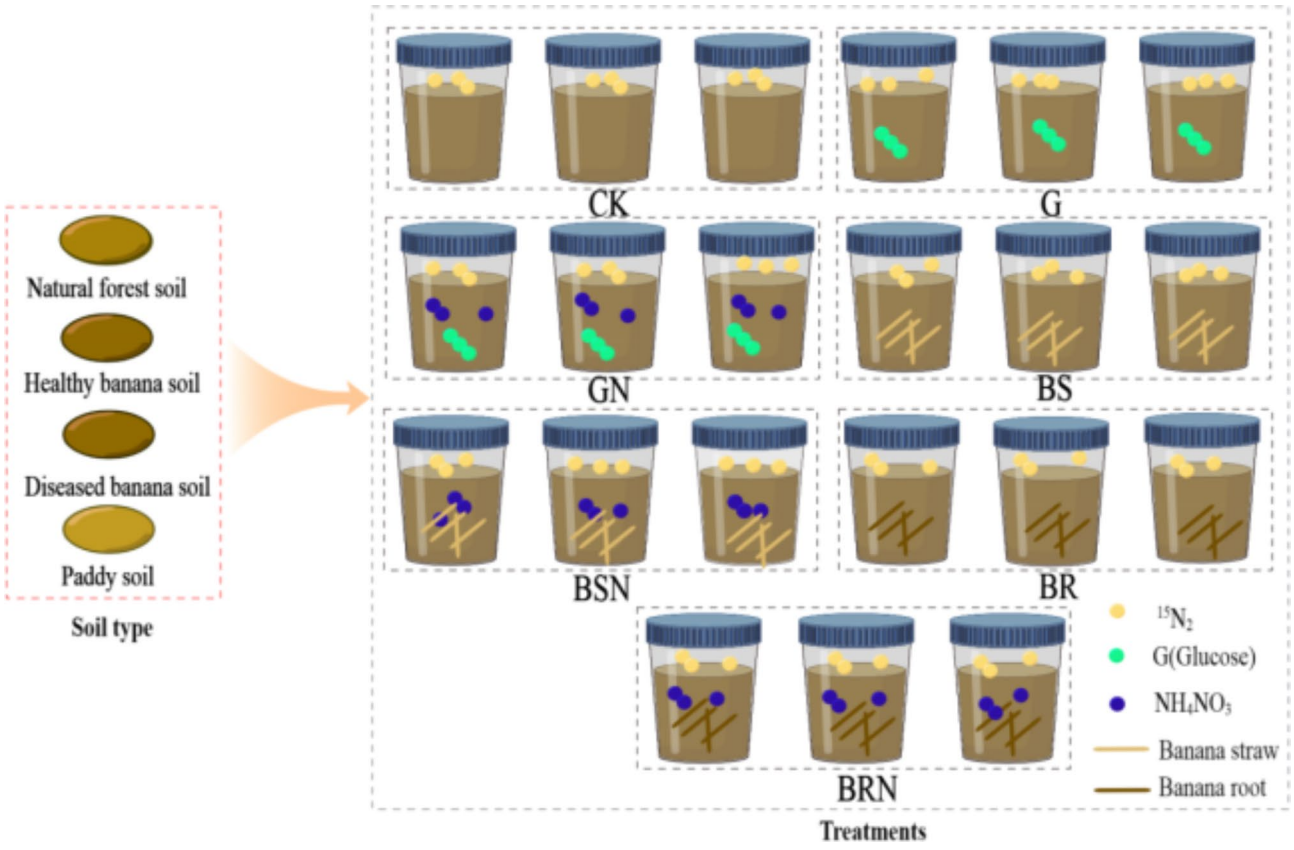

Fig. 2. Schematic diagram of experimental design.

an average relative abundance > 0.01 were retained to construct the network. The visualization of the correlation network and structural equation model was achieved using R (4.2.3).

3. Results

3.1 Microbial respiration and changes in inorganic nitrogen in the different soil types

The results showed that after 42 days of microcosm incubation, the addition of glucose and ammonium nitrate (GN) increased CO_2 emissions more than the addition of glucose alone (G), but this was not true in the banana garden soil (Fig. 3c). In contrast, the addition of banana root and ammonium nitrate (BRN) significantly reduced CO_2 emissions, more than the addition of banana root alone (BR). The G and GN treatments resulted in significantly higher CO_2 emissions than the other treatments in all four soil types. The CK treatment had the lowest emissions, and the respiration of the banana straw and root addition treatments was lower than that of the glucose addition treatments in NS. Compared with G treatment, it was reduced by 51.87% and 62.12%, which indicates that microorganisms can quickly use glucose and ammonium nitrate and emit large amounts of CO_2 in different soil types, but the conversion of organic carbon is relatively poor with banana straw and roots (Fig. 3a, b, c, d). The inorganic nitrogen content in the CK treatment was almost unchanged, and all treatments showed a greater concentration of NO_3^- than NH_4^+ at the end. The inorganic nitrogen concentration in the addition of glucose treatment (G) showed a greater concentration of NH_4^+ than NO_3^-; but at the same time, the ammonium nitrogen concentration increased significantly with the addition of glucose and ammonium nitrate (GN), and was significantly higher than the nitrate nitrogen concentration ($P < 0.05$). In all four soil types, the inorganic N contents in the banana straw (BS) and banana root (BR) treatments were significantly lower than those in the banana straw + ammonium nitrate (BSN) and banana root + ammonium nitrate (BRN) treatments. The ammonium N concentration was higher than the nitrate N concentration in the BSN treatment, decreased by 28.44%, 13.25%, 14.7% and 36.43%, respectively, but the opposite result was observed in the BRN treatment. In all four soil types, the treatments showed that nitrate-nitrogen was rapidly consumed after the addition of ammonium nitrate. The GN treatment absorbed and utilized the most nitrate-nitrogen, indicating that microbial utilization of easily-absorbed organic carbon (glucose) requires the participation of inorganic nitrogen, and nitrate-nitrogen is especially effective (Fig. 3e, f, g, h).

3.2 Insoluble organic carbon and nitrogen content in different soil types

The results showed that after 42 days of incubation, the content of insoluble organic carbon in the CK treatment measured from lowest to highest in PS > DS > HS > NS. In different soil types, the content of insoluble organic carbon in each treatment showed an increasing trend with the addition of organic carbon and inorganic nitrogen.

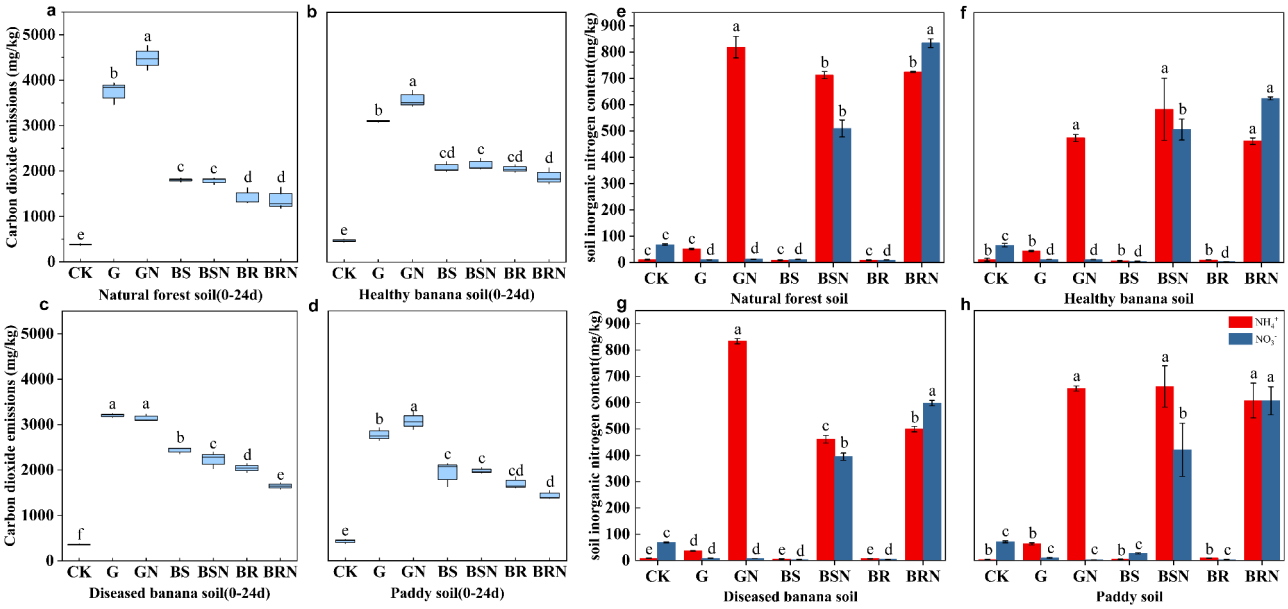

Fig. 3. Microbial respiration (Carbon dioxide emissions) under the NS (**a**), HS (**b**), DS (**c**) and PS (**d**); Inorganic N (NH_4^+, NO_3^-) content under the NS (**e**), HS (**f**), DS (**g**) and PS (**h**) after 42 days of cultivation. Different letters represent significant correlations in treatments ($P < 0.05$). Treatments: CK: no glucose or NH_4NO_3; G: glucose; GN: glucose and NH_4NO_3; BS: banana straw; BSN: banana straw and NH_4NO_3; BR: banana root; BRN: banana root and NH_4NO_3.

Thus the content of insoluble organic carbon in the BR and BRN treatments was higher than in other treatments in different soil types, and increased by 30.16% and 26.91%, respectively (Fig. 4a, b, c, d). Compared with the control, the addition of glucose (G) reduced the insoluble organic nitrogen in NS, HS and PS soils, and decreased by 9.67%, 3.08% and 2.22%, respectively (Fig. 4e, f, g, h), while the addition of banana straw or root increased the insoluble organic nitrogen in all soils except NS. The insoluble organic nitrogen content was higher in paddy soils than in other soil types, with the GN treatment having the lowest insoluble organic nitrogen content (Fig. 4h). At the same time, the C/N ratio also showed a significant upward trend in all different soil types. The C/N ratio was significantly higher in the BR and BRN treatments than in the others, which indicates that the organic carbon provided by banana roots was more easily converted into soil organic carbon, so it significantly improved the soil C/N ratio, increased soil disease resistance, and fostered soil health (Fig. 4i, j, k, l).

3.3 ^{15}N abundance in different soil types

The study found that by adding $^{15}N_2$, the ^{15}N abundance in NS, HS, DS and PS was significantly higher in G treatments than in other treatments (Fig. 5a, b, c, d), with the greatest ^{15}N in NS, which increased by 436.38%, 303.22%, 244.92% and 316.21%, respectively. The addition of other types of carbon (banana straw or root) and nitrogen did not have a significant effect on the conversion of $^{15}N_2$, which indicates that the addition of glucose can increase the uptake and transformation of N_2 by soil microorganisms and can improve the transformation efficiency of N.

3.4 Copy number of 16 S gene in different soil types

In general, the bacterial copy number and nifH copy number of BR were significantly higher than those of other treatments in natural forest soil and increased by 74.04%, 82.64%, 69.66%, 94.36%, 48.76% and 56.23%, respectively compared with the control (Fig. 6a, e). In healthy banana soil and rice soil, the bacterial copy number and nifH copy number of BS were significantly higher than those of other treatments (Fig. 6b, d, f, h). In the diseased banana soil, the bacterial copy number and nifH copy number of BSN were significantly higher than those of other treatments (Fig. 6c, g).

3.5 Diversity of bacterial communities

In natural forest soils, the bacterial diversity of BR and BRN treatments was significantly higher than that of other treatments. In healthy banana soil and paddy soil, each treatment was significantly lower than that of CK treatment. However, in the diseased banana soil, Chao 1 and ACE were significantly lower than CK treatments, but the BR treatments Shannon and Simpson were significantly higher than those of other treatments (Table 2).

3.6 Community structure of bacteria in different soil types

Firmicutes, *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, and *Chloroflexi* were the dominant microbial phylum (relative abundance > 1%) in the different soil types. The composition of bacteria varied in each treatment and soil, but in general, the relative abundance of *Firmicutes* was significantly higher in the G and GN treatments

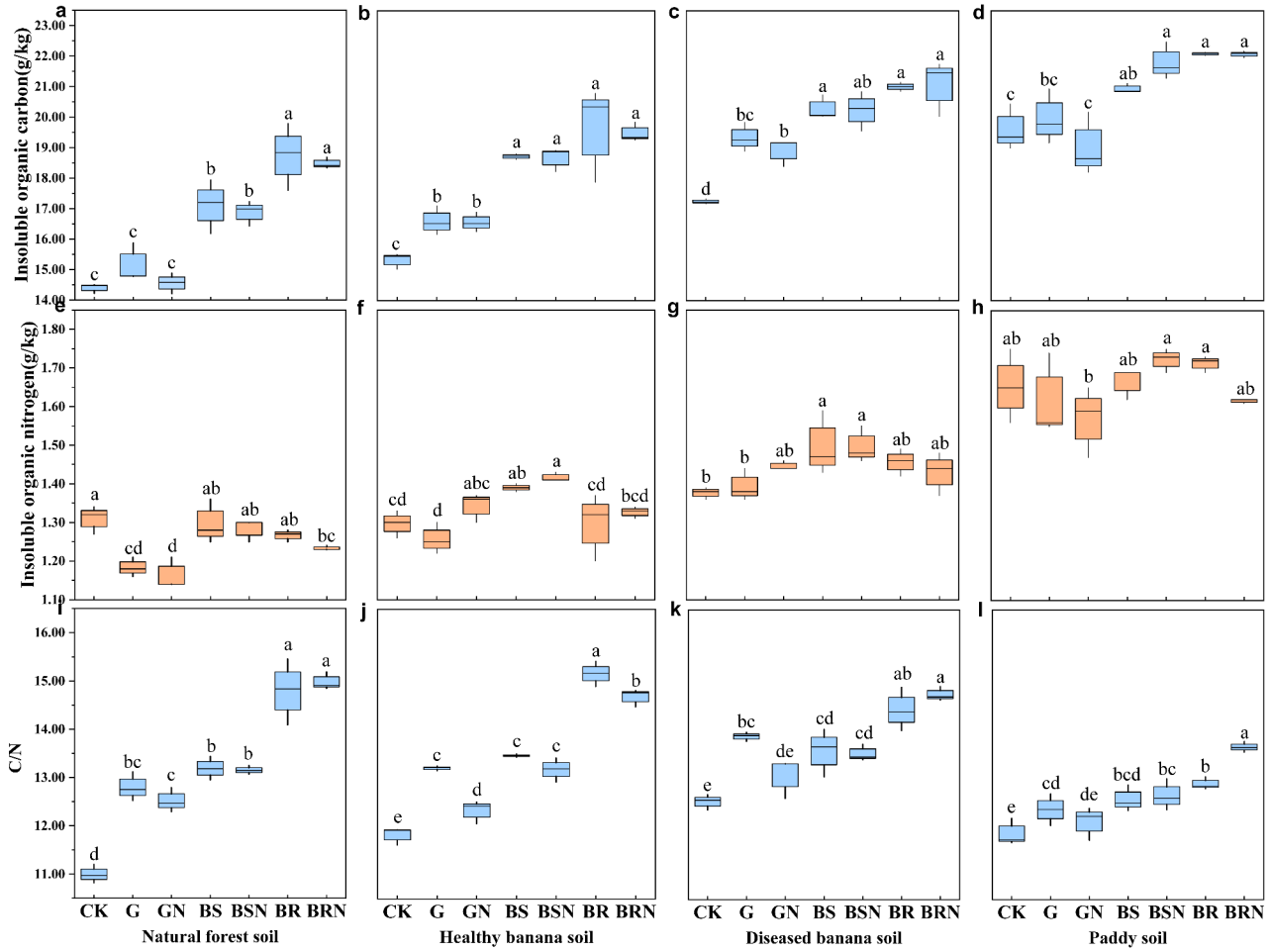

Fig. 4. Insoluble organic carbon in NS (**a**), HS (**b**), DS (**c**) and PS (**d**); Nitrogen content in NS (**e**), HS (**f**), DS (**g**) and PS (**h**); C/N Ratio in NS (**i**), HS (**j**), DS (**k**) and PS (**l**). Different letters represent significant correlations in treatments ($P < 0.05$). Treatments: CK: no glucose or NH_4NO_3; G: glucose; GN: glucose and NH_4NO_3; BS: banana straw; BSN: banana straw and NH_4NO_3; BR: banana root; BRN: banana root and NH_4NO_3.

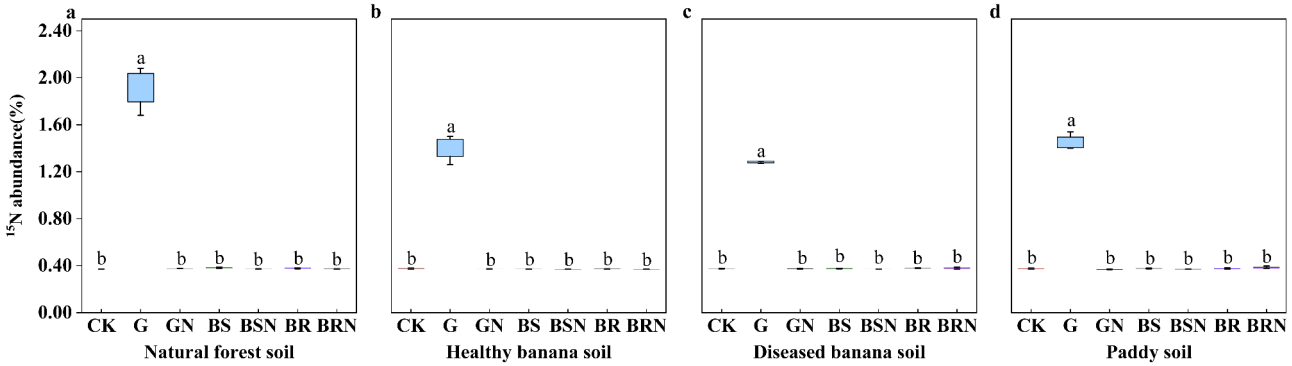

Fig. 5. ^{15}N abundance in NS (**a**), HS (**b**), DS (**c**) and PS (**d**). Different letters represent significant correlations in treatments ($P < 0.05$). Treatments: CK: no glucose or NH_4NO_3; G: glucose; GN: glucose and NH_4NO_3; BS: banana straw; BSN: banana straw and NH_4NO_3; BR: banana root; BRN: banana root and NH_4NO_3.

than in the others, with respective values of 83.73% and 76.56% in NS, 66.39% and 54.87% in HS, 67.19% and 60.13% in DS and 70.36% and 65.67% in PS. In HS, DS and PS, the relative abundance of *Actinobacteria* was higher in the BS, BSN, BR, and BRN treatments than in the others, but it was lower in NS. This indicates that the addition of glucose and ammonium nitrate increased the relative abundance of *Firmicutes*, independent of

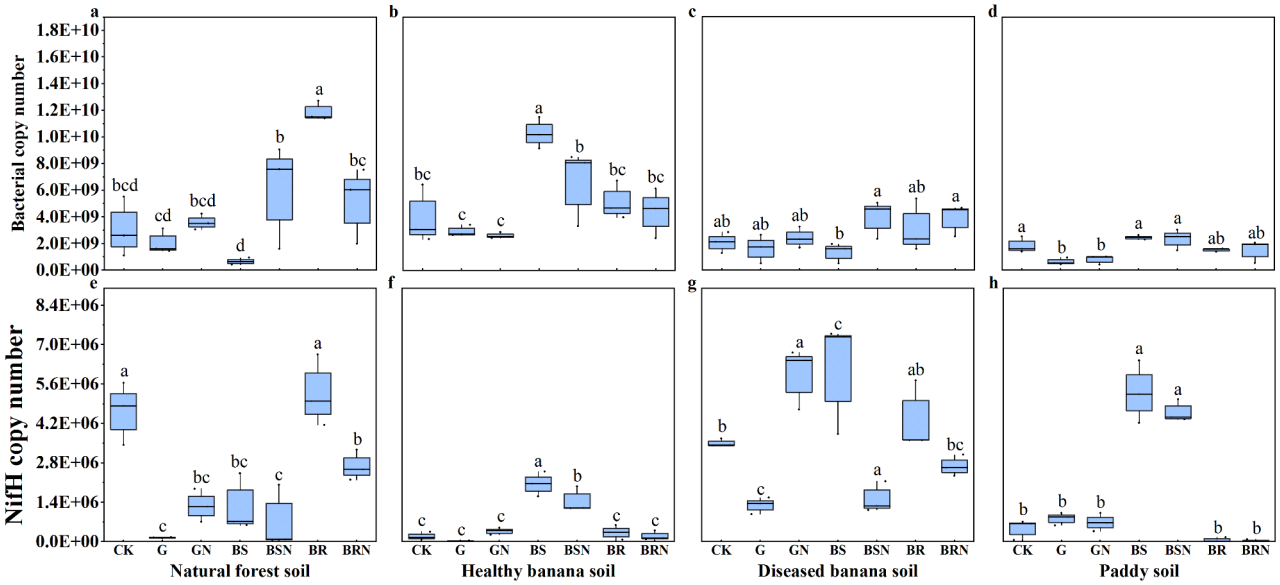

Fig. 6. Copy number of 16 S gene in NS (**a**), HS (**b**), DS (**c**) and PS (**d**) and Copy number of nifH gene in NS (**e**), HS (**f**), DS (**g**) and PS (**h**); Different letters represent significant correlations in treatments ($P < 0.05$). Treatments: CK: no glucose or NH_4NO_3; G: glucose; GN: glucose and NH_4NO_3; BS: banana straw; BSN: banana straw and NH_4NO_3; BR: banana root; BRN: banana root and NH_4NO_3.

soil type, but the addition of organic carbon (banana straw or roots), which is difficult to absorb, increased the relative abundance of *Actinobacteria* in all treatments except NS. The rise in relative abundance of *Actinobacteria* in the treatments adding banana straw or roots in NS is more appropriately attributed to soil type (Fig. 7a, b, c, d). The PCoA of bacterial community structure in the different soil types revealed that the first two axes explained 99.39, 97.31, 96.26, and 99.34 of the total variances, respectively (Fig. 7e, f, g, h). In addition, the community structure of bacteria in the CK treatment differed significantly from the other treatments (G, GN, BS, BSN, BR, BRN). In particular, the G and GN treatments also were distinct from other treatments. These results indicate that differences in organic C and/or inorganic N across different soil types can change the bacterial community structure.

3.7 Relationships between bacterial community composition and soil properties

The correlations between soil properties and bacterial community structure were assessed using RDA. Overall, the first two axes explained 83.40%, 84.56%, 78.07%, and 81.82% of the total variability in the community structures of bacteria in NS, HS, DS and PS, respectively. Among the multiple soil variables, nutrient parameters primarily influenced bacterial community structure (Fig. 8). Nitrate nitrogen was strongly correlated with bacterial community composition in natural forest soils (Fig. 8a), soil insoluble organic carbon and inorganic nitrogen were strongly correlated with bacterial community composition in healthy plantain and paddy soils (Fig. 8b, d), and insoluble organic nitrogen was least correlated with bacterial community composition in diseased plantain soils (Fig. 8c). In NS, NH_4^+ significantly affected bacterial colony structure and correlated negatively with bacterial colonies, but soil ION content correlated positively with bacterial colonies, and IOC was significantly and positively correlated with both *Actinobacteria* and *Cyanobacteria* (Fig. 8e). In HS and DS, IOC content was significantly and positively correlated with both *Actinobacteria* and *Cyanobacteria* (Fig. 8f, g), and in PS, IOC content was significantly and positively correlated with *Actinobacteria* (Fig. 8h). This indicates that soil IOC content could positively regulate bacterial *Actinobacteria* and Cyanobacteria in different soil types.

3.8 Network analysis between species

We found that the complexity of species networks showed obvious differences across different treatments. Taking each treatment in natural forest soil as an example, the network was most complex in the CK treatment, and the complexity decreased significantly in the G treatment. With the addition of ammonium nitrate concurrently with glucose (GN treatment), the network complexity of bacteria increased significantly. This indicates that bacterial growth requires carbon and nitrogen sources at the same time, and a single carbon source will not increase species richness, but may instead reduce it. At the same time, the banana straw and root addition treatments (BS, BR) showed a decrease in species network complexity compared to the CK treatment, but the supplementation of nitrogen sources improved this phenomenon (Fig. 9).

3.9 Correlation analysis of different types of soil properties

We used the structural equation model to analyze the effects of carbon and nitrogen addition on the physicochemical properties and bacterial community characteristics of different soil types, and we found that the effects of carbon and nitrogen addition on soil physical and chemical properties and on bacterial community

Soil types	Treatments	Chao 1	Shannon	ACE	Simpson
NS	CK	6974.86 ± 105.31b	6.50 ± 0.03bc	7170.50 ± 79.86b	0.98 ± 0.00a
	G	4917.84 ± 36.16c	3.94 ± 0.02e	5161.95 ± 63.78c	0.85 ± 0.01c
	GN	4694.00 ± 224.67c	4.85 ± 0.32d	4940.01 ± 243.99c	0.94 ± 0.02b
	BS	6625.42 ± 209.43b	6.47 ± 0.03bc	6771.56 ± 211.31b	0.98 ± 0.00a
	BSN	6450.81 ± 115.67b	6.23 ± 0.01c	6641.38 ± 120.11b	0.98 ± 0.00a
	BR	8815.39 ± 652.20a	6.98 ± 0.02a	9067.51 ± 647.94a	0.99 ± 0.00a
	BRN	8567.98 ± 121.28a	6.70 ± 0.02ab	8772.50 ± 152.40a	0.99 ± 0.00a
HS	CK	7170.07 ± 171.25a	6.69 ± 0.00a	7299.45 ± 133.16a	0.99 ± 0.00a
	G	5363.76 ± 94.04d	4.89 ± 0.11d	5598.04 ± 95.63c	0.92 ± 0.01c
	GN	6207.26 ± 238.02bc	5.64 ± 0.11c	6405.37 ± 209.97b	0.96 ± 0.00b
	BS	6392.27 ± 39.24b	6.17 ± 0.02b	6585.46 ± 47.85b	0.98 ± 0.00a
	BSN	5403.85 ± 106.13d	5.64 ± 0.02c	5609.01 ± 53.66c	0.98 ± 0.00a
	BR	6894.09 ± 169.40a	6.51 ± 0.01a	7052.47 ± 187.88a	0.99 ± 0.00a
	BRN	5803.00 ± 72.33 cd	5.86 ± 0.09c	5898.59 ± 69.51c	0.98 ± 0.00a
DS	CK	6713.96 ± 85.96a	6.46 ± 0.05b	6860.84 ± 108.03a	0.99 ± 0.00ab
	G	5188.67 ± 68.31c	4.87 ± 0.06e	5350.19 ± 48.85b	0.93 ± 0.00e
	GN	5149.45 ± 226.09c	5.49 ± 0.05d	5336.86 ± 231.54b	0.97 ± 0.00c
	BS	6279.95 ± 118.07b	6.31 ± 0.03b	6490.15 ± 114.17a	0.99 ± 0.00ab
	BSN	5385.10 ± 139.98c	5.47 ± 0.01d	5531.88 ± 122.93b	0.96 ± 0.00d
	BR	6655.15 ± 122.47ab	6.63 ± 0.01a	6783.28 ± 136.74a	0.99 ± 0.00a
	BRN	6433.03 ± 48.61ab	6.15 ± 0.08c	6628.44 ± 91.62a	0.98 ± 0.00bc
PS	CK	7113.07 ± 79.11a	6.55 ± 0.01a	7370.68 ± 57.60a	0.99 ± 0.00a
	G	5731.03 ± 198.91b	5.14 ± 0.05e	5939.02 ± 190.33b	0.94 ± 0.00b
	GN	6113.55 ± 64.86b	5.29 ± 0.13e	6280.69 ± 83.47b	0.94 ± 0.01b
	BS	5978.42 ± 160.89b	6.32 ± 0.01ab	6217.76 ± 182.57b	0.99 ± 0.00a
	BSN	5624.20 ± 105.79b	6.00 ± 0.05c	5789.58 ± 171.03b	0.98 ± 0.00a
	BR	5361.00 ± 568.42b	6.14 ± 0.04bc	5584.34 ± 569.15b	0.99 ± 0.00a
	BRN	5532.29 ± 84.29b	5.60 ± 0.12d	5788.34 ± 84.53b	0.97 ± 0.00a

Table 2. Diversity of bacterial communities. Treatments: CK: no glucose or NH_4NO_3; G: glucose; GN: glucose and NH_4NO_3; BS: banana straw; BSN: banana straw and NH4NO3; BR: banana root; BRN: banana root and NH_4NO_3.

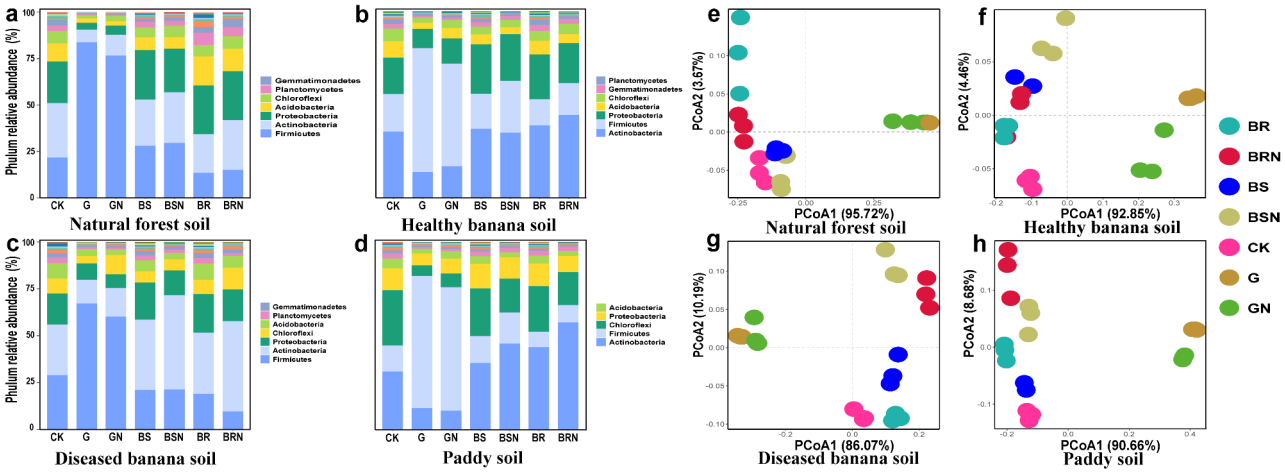

Fig. 7. Community structure of bacteria in NS (**a**), HS (**b**), DS (**c**) and PS (**d**); The PCoA of bacterial community structure in NS (**e**), HS (**f**), DS (**g**) and PS (**h**). Different letters represent significant correlations in treatments ($P < 0.05$). Treatments: CK: no glucose or NH_4NO_3; G: glucose; GN: glucose and NH_4NO_3; BS: banana straw; BSN: banana straw and NH_4NO_3; BR: banana root; BRN: banana root and NH_4NO_3.

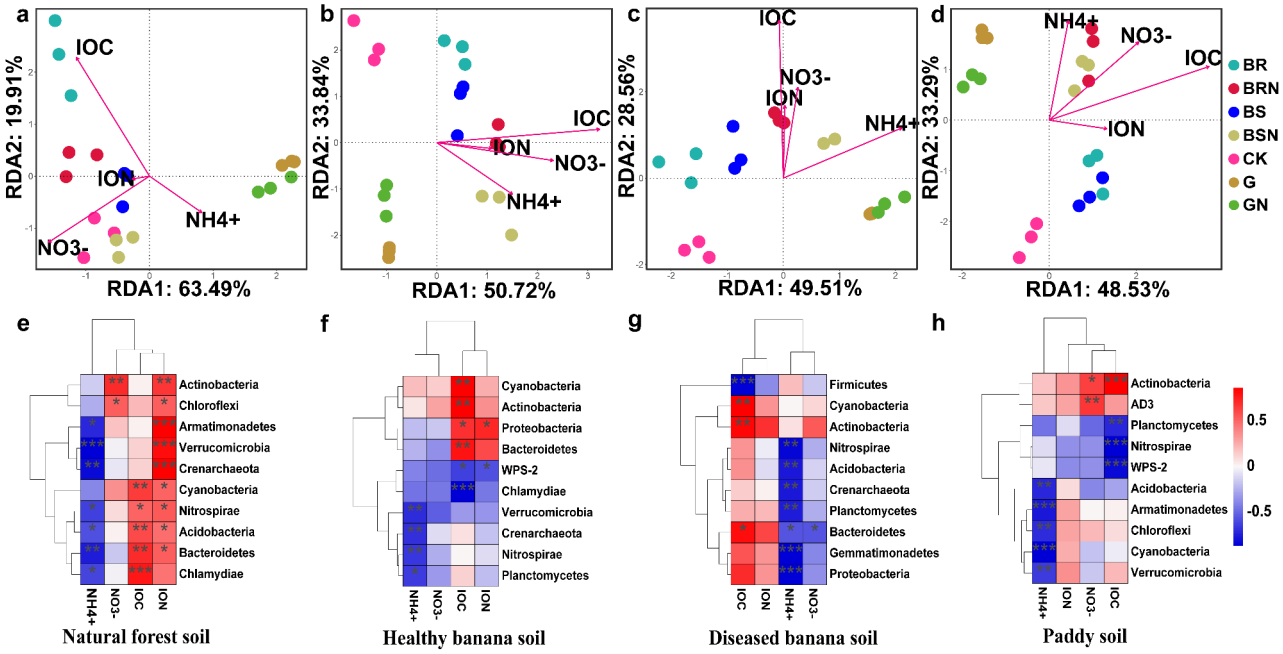

Fig. 8. RDA analysis in NS (**a**), HS (**b**), DS (**c**) and PS (**d**). Relationships between bacterial community composition, and soil properties in NS (**e**), HS (**f**), DS (**g**) and PS (**h**). D Different letters represent significant correlations in treatments ($P < 0.05$). Treatments: CK: no glucose or NH_4NO_3; G: glucose; GN: glucose and NH_4NO_3; BS: banana straw; BSN: banana straw and NH_4NO_3; BR: banana root; BRN: banana root and NH_4NO_3.

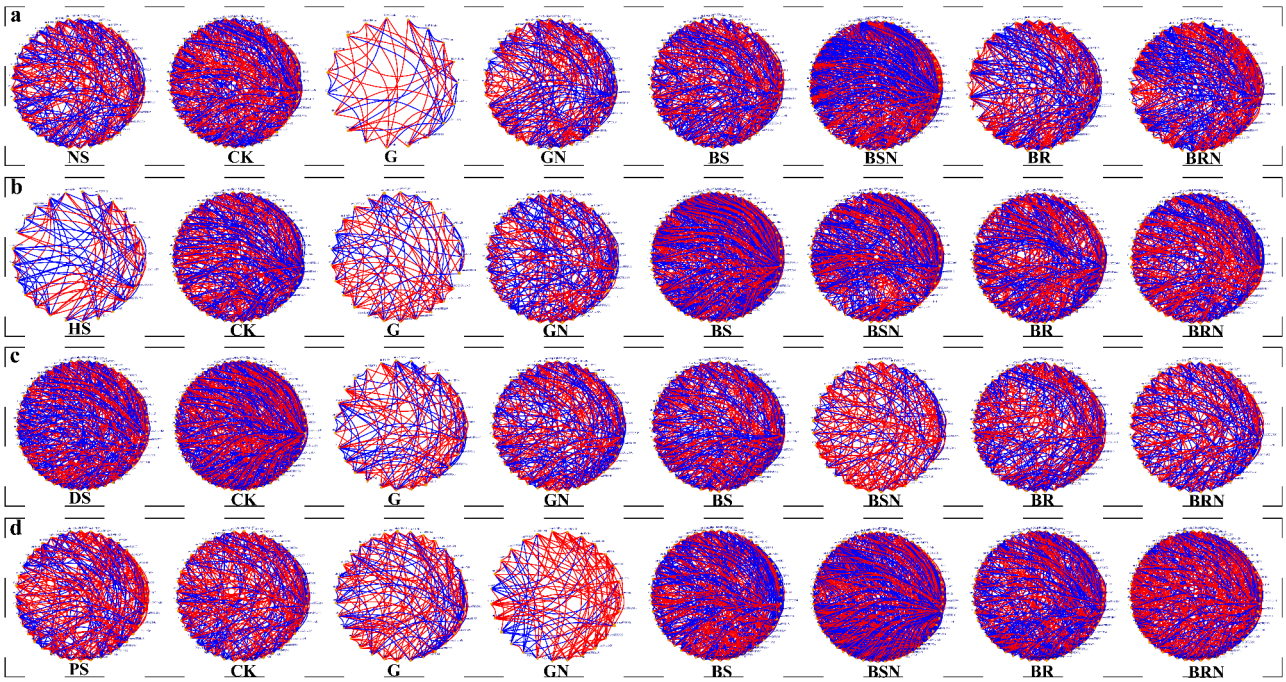

Fig. 9. Network analysis between species in NS (**a**), HS (**b**), DS (**c**) and PS (**d**). Treatments: CK: no glucose or NH_4NO_3; G: glucose; GN: glucose and NH_4NO_3; BS: banana straw; BSN: banana straw and NH_4NO_3; BR: banana root; BRN: banana root and NH_4NO_3.

characteristics differed greatly across different soil types, though the effects were not significant in forest soil and banana soil (Fig. 10a, b, c). In paddy soil, however, carbon and nitrogen addition was significantly negatively correlated with bacterial community structure (Fig. 10d). In all four types of soil, nitrogen addition correlated positively with NH_4^+ soil content, and carbon addition was significantly positively correlated with poorly-soluble

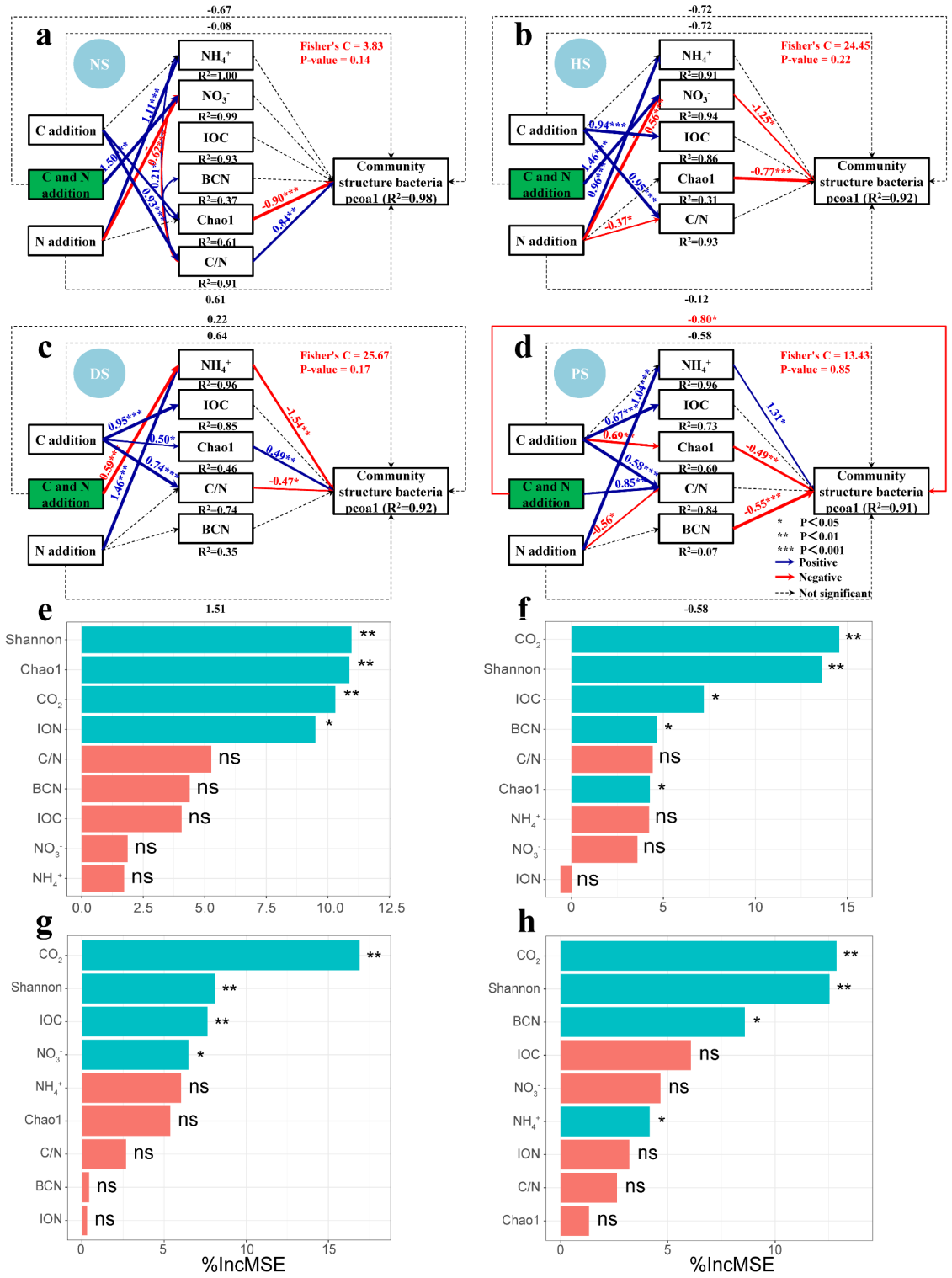

Fig. 10. Relationship between bacterial community composition and soil properties under different soil types. Structural equation model in NS (**a**), HS (**b**), DS (**c**) and PS (**d**). Random forest analysis in NS (**e**), HS (**f**), DS (**g**) and PS (**h**). CO_2: carbon dioxide emissions; IOC: Insoluble organic carbon; ION: Insoluble organic nitrogen; NO_3^-: nitrate nitrogen; NH_4^+: ammonium nitrogen; C/N: IOC/ION; BCN: Bacterial copy number.

organic carbon content and C/N in all soils except forest soil (Fig. 10). At the same time, the C/N of forest soil was significantly positively correlated with bacterial community structure, and the Chao 1 index was significantly negatively correlated with bacterial community structure (Fig. 10a). NO_3^- and the Chao 1 index of healthy banana soil were significantly negatively correlated with bacterial community structure (Fig. 10b). The NH_4^+ and C/N of diseased banana soil were significantly negatively correlated with bacterial community structure (Fig. 10c). NH_4^+ in paddy soil was significantly positively correlated with bacterial community structure, but the Chao 1 index and BCN were significantly negatively correlated with bacterial community structure (Fig. 10d). Random forest analysis showed that Shannon, Chao1 and CO_2 were important factors for predicting bacterial community structure in natural forest soil (Fig. 10e), CO_2 and Shannon were important factors for predicting bacterial number in healthy banana and rice soil (Fig. 10f, h), and IOC, CO_2 and Shannon were important factors for predicting bacterial number in diseased banana soil (Fig. 10g).

4. Discussion

4.1 Underground plant residues contributed more to soil organic carbon than the above-ground

Land management was determined by different land utilization types, which thereby impacted soil physical and chemical properties[31,32]. Soil basic chemical properties indicate the levels of soil nutrients, and the analysis of variance for soil characteristics found that there are significantly different under different land utilization patterns. There are also differences in the use of fertilizers under different soil utilization patterns. Bio-fertilizers, chemical fertilizers and crop residues are well-known additives for preserving and enhancing soil quality[33] by preserving or enhancing soil organic carbon[34]. Thus, these additives can improve microbial activity and function in the field and improve biomass and C sequestration capacity of a plant[35]. SOM plays a significant role in ecosystem function and soil fertility, and it determines the nutrient sequestration capacity of the soil[36]. This study showed that the addition of organic carbon (glucose or banana straw) and inorganic nitrogen together increased the soil insoluble organic carbon content and improved the soil organic carbon content. It is speculated that the main reason for this is that the input of carbon and nitrogen nutrients promotes the reproduction of soil microorganisms, which leads to the increase of soil insoluble carbon and nitrogen (mainly microbial biomass carbon and nitrogen), which is generally consistent with results of previous research. Many studies have found that below-ground carbon inputs (root and root exudation) form stable carbon more efficiently than above-ground litter carbon inputs[37–39]. In this study, we found that insoluble organic carbon was higher in the banana root treatment than in the banana straw treatment. This indicates that the underground plant residues contributed more to soil organic carbon than did the above-ground part. This is mainly closely related to the ability of microorganisms to decompose, and it may be that the ratio of carbon to nitrogen in plant roots is more suitable for decomposition by microorganisms, which was also basically consistent with previous studies.

4.2 Efforts of carbon and nitrogen addition on microbial respiration and nitrogen fixation

The average CO_2 production rate is typically used as an effective indicator of soil microbial activity, and it also reveals the ability of carbon sources to be utilized by soil microbial communities[40], and the higher residue carbon inputs may lead to high rates of carbon sequestration[41]. It is well known that soil respiration demonstrate biological activity and decomposition of organic residues[42,43]. Higher respiration rate of microbes in soil may show great microbial transformation efficiency in the environment[44]. The present study showed that organic carbon and inorganic nitrogen addition increased microbial respiration and improved the conversion efficiency of nutrients, which is consistent with results of previous research. We also found that the same phenomenon occurred in different types of soil microorganisms, indicating that soil types did not affect the assimilation effect of microorganisms on carbon and nitrogen. And the nitrogen fixation capacity of different soil types had a significant decrease after the nitrogen addition. It can be evidenced by a decrease in the abundance of ^{15}N. The reason may be that excessive soluble nitrogen addition has a toxic effect on nitrogen-fixing microorganisms, resulting in a decrease in the number of nitrogen-fixing microorganisms, which in turn reduces the overall biological nitrogen fixation capacity[45].

4.3 Efforts of carbon and nitrogen addition on bacterial community composition

Microbial diversity is considered to be an important component in global carbon cycle, but bacterial communities composition are often overlooked in stoichiometric nutrient cycling study[46,47]. Some studies have demonstrated that bacteria in soil could increase growth of plant, nutrient allocation by means of nutrient element turnover in soil, and changes in bacterial community structure and diversity also alter soil quality, pH and other environmental indicators[48,49]. The composition of soil microbial communities in systems where fertilizer is applied is largely affected by soil chemical properties, which has been reported by many investigators[50,51], and factors in environment such as pH, SOM and AP have the potential to affect soil microbial communities in different ecological system[52,53], and the bacterial community structure was remarkably correlated with soil organic carbon and total nitrogen[54]. In this study, nitrate nitrogen was strongly correlated with bacterial community composition in natural forest soils, insoluble organic carbon and inorganic nitrogen in soil were strongly correlated with bacterial community composition in healthy plantain and paddy soils, and insoluble organic nitrogen was least correlated with bacterial community composition in diseased plantain soils, indicating that there were obvious differences in the responses of different types of soil microorganisms to carbon and nitrogen, and the bacterial communities in forest soil were more sensitive to nitrogen. Additionally, the *Actinomycetales* order is believed a main contributor to carbohydrate (CHO) and amino acid (AA) metabolism under various conditions[55,56]. We found that the addition of glucose and ammonium nitrate increased the relative abundance of *Firmicutes*, independent of soil type, but the addition of difficult-to-absorb organic carbon (banana straw or roots) increased the relative abundance of *Actinomycetes* in all soils except NS. In all ecosystems, soil microbes

play an increasingly greater role in decomposition of SOM, N cycle and providing sufficient nutrients for plants. Thus microbes act as a sensitive indicator for forecasting soil biological conditions and recommended agricultural practices in soil ecosystem[57]. The soil microbial community is closely linked with agricultural production, including plant straw, organic material, and crop management, etc[58]. Soil organic carbon and soil eco-environment can be significantly improved through the application of crop residues[59]. These provide carbon and make the environment hospitable for the proliferation of soil microbes. In our study, it was found that the addition of banana straw or root increased the soil organic carbon content, which is consistent with the results of previous research. Frasier et al. found that soil microbial communities were larger in areas where crop residue treatments were applied, compared to those without[60]. Soil microbes were better able to uptake C sources in zero-tillage conditions than under conventional tillage[61]. Diversity and stability of soil microbe communities increased under conditions of long-term no-tillage and organic input practice[62]. Abundance of soil fungi increased under a management plan of no-tillage and straw mulching[63]. PCoA differed significantly ($p < 0.05$) in bacterial communities under different treatments, this is in accordance with the results that documented clear separation of bacterial and fungal community composition between those using straw and those not using it[64]. One study demonstrated no significant difference among varying regimes of long-term maize straw utilization and fertilization on the bacterial community composition under the phylum level[65]. According to the PCoA results in our study, the addition of banana straw or root resulted in a bacterial community structure that was significantly distinct from the control. These results indicated that the addition of materials with different C/N ratios would have a great impact on the soil bacterial community, which might cause the formation of a large number of carbon-sequestration microorganisms.

5. Conclusion

With the change of different soil types utilization, glucose addition increased carbon dioxide emissions and enhanced the ability of soil microorganisms nitrogen fixation, but caused the accumulation of ammonium nitrogen, indicating that microorganisms had higher utilization efficiency of soluble carbon and enhanced the biological nitrogen fixation capacity, and nitrogen addition caused the accumulation of ammonium nitrogen, thereby weakening the biological nitrogen fixation capacity. At the same time, glucose significantly increased the *Fimicutes* phylum and lowered the level of other bacterial phylums, thereby reducing the bacterial network structure, and forest analysis showed that CO_2 was an important factor in predicting the bacterial community structure of different soil types, indicating that bacteria at the *Fimicutes* phylum level preferred glucose, which may also have a negative effect on bacteria at the level of other phylums. In summary, in the agricultural production process, the application of organic carbon should be increased, and at the same time attention should be paid to the management of nitrogen, especially to reduce the application of ammonium nitrogen, to ensure the healthy development of soil microbial community.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due but are available from the corresponding author on reasonable request.

Received: 30 March 2024; Accepted: 10 September 2024
Published online: 13 October 2024

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Acknowledgements

We would like to thank Dr. Daniel Petticord for helping English language of the manuscript. The research was funded by Project of Sanya Yazhou Bay Science and Technology City (SCKJ-JYRC-2022-94), Strategic Priority Research Program of Chinese Academy of Sciences (XDA28020203), Postgraduate Innovation Research Project of Hainan Province (Qhyb2022-67), and PhD Scientific Research and Innovation Foundation of Sanya Yazhou Bay Science and Technology City (HSPHDSRF-2023-12-008).

Author contributions

ZJ, YR and LM contributed to conceive and design the experiment. CH, KL contributed to perform the experiment and writes this paper. The authors have confirmed the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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