Exogenous Application of Sodium Hydrosulfide Suppresses Bacterial Wilt And Regulates The Soil Microecology

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Abstract

The role of hydrogen sulfide (H_2S) in regulating the pathogenic bacteria has been well documented. However, whether exogenous H_2S addition inhibits the pathogens in soil is not understood, and whether H_2S can suppress the plant disease caused by pathogen *R. Solanacearum* is not clear. In the present study, different concentrations of H_2S donor NaHS were applied to the tobacco field to explore the interrelation among NaHS, tobacco baterial wilt, soil physicochemical properties and microbial community. In order to decipher the disease suppression mechanism from the perspective of soil microecology. Application of NaHS significantly reduced the disease incidence and disease index of TBW, increased soil pH, alkali-hydrolyzed nitrogen (AN), available phosphorus (AP), available phosphorus (AP) and organic matter (OM). NaHS addition also changed soil microbial community composition and structure. Furthermore, NaHS addition significantly reduced the abundance of *Ralstonia* and *Fusarium*, and increas pathogenic ed beneficial microorganisms S*olirubrobacter*, *Rhodococcus*, *Rhizobium*, *Pseudomonas*, *Paenibacillus*, *Microvirga*, *Lysobacter*, *Haliangium*, *Granulicella*, *Flavobacterium*, *Bacillus*, *Trichoderma* and *Aspergillus* at the genus level. Our findings suggested that exogenous application of NaHS significantly suppressed TBW caused by *R. Solanacearum* through regulated soil microecology. This study revealed the potential of NaHS in control of bacterial wilt.

Introduction

Bacterial wilt is a bacterial soil-borne disease caused by *R. Solanacearum*[1]. The disease have become one of the potential threats to agriculture, and causing huge losses to yields of *Solanaceous* crops[2]. Lots of researches on the control of bacterial wilt focus on resistant varieties, chemical control, biological control and agricultural control. However, these traditional control methods may have limited efficacy and many problems, such as lack of resistant varieties, poor control efficacy, pathogen resistant, environmental pollution, and so on[3,4]. Therefore, more effective and environmentally friendly approaches need to be developed to control bacterial wilt.

Hydrogen sulfide (H_2S) was thought as the third newest gaseous signal molecular, which was not only applied in animal and human physiological processes (such as dermatological diseases, cell behaviors, vascular system, neuronal disease, digestive systems, COVID-19 and so on)[5–12], but also applied in agriculture[13]. A large number of studies in the field of plants have shown that H_2S can directly or indirectly involve in a wide range of plant physiological processes including stomatal movement[14], photosynthesis[15], seed germination[16], root growth[17], fruit ripening[18], as well as plant senescence[19]. H_2S can participate in enhance plant tolerance to drought, salinity, high-temperature and heavy metal stress by initiating plant redox signal, antioxidant capacity and specific components of cellular defence[20–23]. Exogenous application of H_2S induces plant cross-adaptation to multiple abiotic stresses[24]. Therefore, H_2S as a sulfur-containing defence compound, plays an important role in plant resistance to biotic and abiotic stresses[25]. Fu *et al.*[26] found that H_2S has antifungal role on the postharvest pathogens

Aspergillus niger and *Penicillium italicum*. They also demonstrated that the antifungal effect of H_2S might be associated with the increased accumulation of reactive oxygen species (ROS) in H_2S-treated fungi[26]. Previous studies showed that H_2S both positively and negatively impacts on the bacterial growth[27–29]. Some reports revealed that H_2S also regulate the pathogenic bacteria[30,31]. However, It is still unknown whether H_2S can suppress the plant disease caused by soil-born pathogen *R. Solanacearum*.

Tobacco is an important economic plants of *Solanaceous*, which is easily infected with serious soil-borne disease by *R. Solanacearum*[32]. In this study, sodium hydrosulfide (NaHS), a H_2S donor, was applied to the tobacco field. We aimed to investigate the control efficacy of NaHS application on tobacco bacterial wilt (TBW), as well as the effects on soil physicochemical properties and microbial community. The application of NaHS was proposed as a new approach to control TBW.

Results

Disease severity index of TBW. Disease incidence (I), disease index (DI) and control efficacy with five treatments were calculated at 100 d post-transplantation (Table 1). The I and DI of the control (CK) were significantly higher than those of NaHS treatments. As the concentration of NaHS increased from 200 mg/L to 800 mg/L, the I and DI continuously decreased from 44.22–15.21%, and 13.21 to 4.26, respectively. With the increase of NaHS concentration, the control efficacy increased gradually. All the results suggested that the application of NaHS reduce the disease incidence and disease index of TBW, and the control efficacy of TBW is as high as 89.49%.

Table 1
The occurrence of tobacco bacterial wilt in different concentration of NaHS.

Treatments	disease incidence (%)	disease index	Control efficacy (%)
CK	89.34 ± 3.69 a	40.56 ± 2.29 a	0 d
NaHS200	44.22 ± 5.94 b	13.21 ± 1.52 b	67.12 ± 2.82 c
NaHS400	29.35 ± 6.99 c	8.76 ± 1.10 bc	78.41 ± 2.73 b
NaHS600	20.58 ± 2.34 cd	6.93 ± 1.89 c	82.98 ± 3.32 ab
NaHS800	15.21 ± 1.63 d	4.26 ± 1.07 c	89.49 ± 1.23 a
All data are presented as the mean ± SE. The different lowcase letters in the same column indicate significant differences at $p < 0.05$ based on LSD test among different concentrations of NaHS.			

Effects of NaHS application on soil physicochemical properties. Seven physicochemical properties of the rhizosphere soil were analyzed (Table 2). The value of pH, alkali-hydrolyzed nitrogen (AN), available phosphorous (AP) and organic matter (OM) were increased as the concentration of NaHS increased from 200 mg/L to 800 mg/L. There was no significant difference in available potassium (AK) and exchangeable calcium (Ca) between NaHS treatments and CK. The results showed that the application of NaHS could increase the soil pH, AN, AP and OM. What's more, pH, AN, AP and OM showed significantly

negative ($p < 0.01$) correlation with the incidence of TBW (Table S1). These results indicated that the application of NaHS may reduce the incidence of TBW by changing soil physicochemical properties.

Table 2
Effects of different concentrations of NaHS on Soil physicochemical properties.

Treatments	pH	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	OM (%)	Ca (mg/kg)	Mg (mg/kg)
CK	5.22 ± 0.13 d	141.58 ± 7.18 d	75.30 ± 3.60 c	800.10 ± 15.90 a	2.40 ± 0.19 d	999.04 ± 251.48 a	128.47 ± 6.52 a
NaHS200	5.59 ± 0.15 c	164.39 ± 3.80 c	84.00 ± 1.24 bc	756.87 ± 25.69 a	3.18 ± 0.10 c	973.42 ± 127.30 a	123.03 ± 2.82 a
NaHS400	6.14 ± 0.07 b	170.31 ± 3.44 bc	85.92 ± 4.31 bc	744.38 ± 36.12 a	3.38 ± 0.16 bc	998.58 ± 292.25 a	120.25 ± 3.40 a
NaHS600	6.36 ± 0.09 ab	184.48 ± 3.59 ab	97.52 ± 5.88 ab	750.69 ± 41.77 a	3.69 ± 0.07 b	990.50 ± 193.05 a	112.69 ± 18.00 a
NaHS800	6.49 ± 0.06 a	188.55 ± 6.98 a	115.75 ± 11.42 a	754.51 ± 8.87 a	4.19 ± 0.13 a	987.96 ± 138.90 a	103.64 ± 2.15 b

Soil chemical properties in soils are presented as the mean ± SE. The different lowercase letters in the same column indicate significant differences at $p < 0.05$ based on LSD test among different concentrations of NaHS.

Effects of NaHS application on bacterial diversity and community. In total 679,451 high-quality raw sequences with the average length of 252 bps for bacteria were obtained from rhizospherial soil samples after quality filtering. The OTUs, Chao1 and Shannon index were used to evaluate and compare the richness and diversity of bacterial community among different treatments (Table S2). The OTUs, Chao1 and Shannon index were lower in the rhizophere soil of NaHS treatments than the CK. With the increase of NaHS concentration from 0 mg/L to 600 mg/L, the OTUs, Chao1 and Shannon index decreased gradually. Comparing with NaHS600 treatment, the OTUs, Chao1 and Shannon index in NaHS800 treatment was slightly higher (Table S2). While, the OTUs, Chao1 and Shannon index in NaHS800 treatment significantly lower than the CK. This result suggested that NaHS treatments could change the richness and diversity of soil bacterial community.

Principal coordinate analysis (PCoA) were carried out using weighted UniFrac distance in different treatments, and PC1 and PC2 explained 55.21% of total bacterial community. Bacterial community from CK and NaHS200 were clustered together, while the bacterial community of the CK, NaHS400, NaHS600 and NaHS800 were respectively separated from each other (Fig. 1A). This result indicated that the bacterial community structure of NaHS400, NaHS600 and NaHS800 were different from CK. A total of 50 bacterial phyla were identified from all soil samples. The top ten abundant bacterial phyla were selected to compare the changes of bacterial community in rhizosphere soil of five treatments, the relative abundance of the top ten predominant phyla totaled up to 94.27 ~ 97.87% (Fig. 1B). Among the top ten bacterial phyla, *Proteobacteria* included the pathogen *R. solanacearum* was the most dominant (48.01 ~ 56.91%), and followed by *Verrucomicrobia* (5.02 ~ 12.25%), *Bacteroidetes* (3.28 ~ 10.33%), *Firmicutes*

(4.24 ~ 5.92%), *Gemmatimonadetes* (3.55 ~ 6.94%), *Acidobacteria* (3.24 ~ 6.13%), *Actinobacteria* (2.24 ~ 5.28%), *Saccharibacteria* (2.24 ~ 3.84%), *Cyanobacteria* (2.74 ~ 4.89%) and *Chloroflexi* (0.92 ~ 4.23%). The relative abundance of *Proteobacteria* in NaHS800 treatment was lower than that in other treatments, while the relative abundance of *Verrucomicrobia* and *Bacteroidetes* were higher than that in other treatments (Fig. 1B). The Heatmap analysis of the top 40 genera with hierarchical clusters was used to identify the different composition of bacterial community structure. There were distinctions of bacterial community structures among different treatments in the Heatmap. The application of NaHS significantly increased the abundances of *Streptomyces*, *Microvirga*, *Rhodococcus*, *Haliangium*, *Paenibacillus*, *Chthonomonas*, *Bacillus*, *Solirubrobacter*, *Gaiella*, *Lysobacter*, *Pseudolabrys*, *Pseudomonas*, *Granulicella*, *Stenotrophomonas*, *Flavobacterium* and *Rhizobium*. In contrast, NaHS significantly decreased the abundances of *Massilia*, *Acidibacter* and *Ralstonia* (pathogen of bacterial wilt) (Fig. 1C). These results suggested that NaHS application play impact on the the structure of bacterial community.

Further analyses were carried out at the genus level, and the different distributions of the top forty abundant bacterial genera among the five treatments were illustrated in Fig. 2. Twelve varied among the five treatments were significantly different, including *Solirubrobacter*, *Rhodococcus*, *Rhizobium*, *Ralstonia*, *Pseudomonas*, *Paenibacillus*, *Microvirga*, *Lysobacter*, *Haliangium*, *Granulicella*, *Flavobacterium* and *Bacillus*. The genus *Solirubrobacter* which was dominant in NaHS treatments, and occupied low percentage in CK (Fig. 2). The trends in change in the genera *Rhodococcus*, *Rhizobium*, *Pseudomonas*, *Paenibacillus*, *Microvirga*, *Lysobacter*, *Haliangium*, *Granulicella*, *Flavobacterium* and *Bacillus* were the same as that in *Solirubrobacter*. In contrast, *Ralstonia* was dominant in CK, and decreased significantly in NaHS treatments (Fig. 2).

Effects of NaHS application on fungal diversity and community. The difference of the OTUs, Chao1 and Shannon index of fungal community among different treatments were also analyzed (Table S2). There was no significant difference in OTUs, Shannon and Chao1 indexes between CK and NaHS 200. With increase of NaHS concentration from 400 mg/L to 800 mg/L, the OTUs, Chao1 and Shannon index reduced significantly. The results showed that NaHS application could impact the diversity and richness of soil fungi.

According to PCoA analysis, PC1 and PC2 explained 23.47% and 13.75% of the total fungal community variations respectively (Fig. 3A). The distribution of fungi among different treatments was relatively discrete, indicating that there were obvious differences in the fungal community structure among different treatments. 6 main known fungal phyla were identified from all soil samples, including *Ascomycota* (43.56 ~ 73.45%), followed by *Basidiomycota* (7.98 ~ 28.21%), *Chytridiomycota* (6.84 ~ 30.15%), *Glomeromycota* (1.00 ~ 8.16%), *Neocallimastigomycota* (0.05 ~ 6.19%) and *Zygomycota* (0.66 ~ 9.31%) (Fig. 3B). The relative abundance of *Ascomycota* decreased as the concentration of NaHS increased from 200 mg/L to 600 mg/L, and slightly increased when the concentration of NaHS was 800 mg/L. However, the relative abundance of *Ascomycota* was lower in NaHS800 than in CK. The relative abundance of *Zygomycota* increased as the concentration of NaHS increased from 200 mg/L to 600 mg/L, and significantly decreased when the concentration of NaHS was 800 mg/L. The relative

abundance of *Basidiomycota*, *Glomeromycota*, *Chytridiomycota* and *Zygomycota* also varies with the concentration of NaHS. These results indicated that NaHS altered the fungal community composition, which was associated with NaHS concentration. (Fig. 3B). In the Heatmap for fungal community, The relative abundance of *Xanthoria*, *Monographella*, *Candida*, *Paludomyces*, *Microidium* and *Sakaguchia* in CK were significantly higher than in NaHS treatment. NaHS800 significantly enriched the relative abundance of *Batrachochytrium*, *Gorgonomyces*, *Populocrescentia*, *Cladosporium*, *Rhodosporidium*, *Aspergillus*, *Tomentella*, *Lycogalopsis*, *Trichoderma*, *Pseudocamarosporium*, *Russula*, *Byssochlamys* and *Paecilomyces* (Fig. 3C).

The different distributions of the top forty abundant fungal at genus level among the five treatments were analyzed (Fig. 4). Three were significantly different among the five treatments, including *Trichoderma*, *Fusarium* and *Aspergillus*. The genus *Trichoderma* and *Aspergillus* were dominant in NaHS, and occupied low percentage in CK (Fig. 4). In contrast, *Fusarium* was dominant in CK, and decreased significantly in NaHS treatments (Fig. 4).

The relationship between rhizosphere soil physicochemical properties and microbial community. The relationship between rhizosphere soil physicochemical properties and microbial community structure were analysed by redundancy analysis (RDA). The results showed that 64.27% and 59.47% of bacterial and fungal community variation, respectively (Fig. 5). The bacterial *Rhodococcus*, *Solirubrobacter*, *Paenibacillus*, *Haliangium*, *Bacillus*, *Lysobacter*, *Pseudomonas*, *Flavobacterium* and *Granulicella* were positively correlated with pH, AN, AP and OM. While, *Ralstonia* presented contrasting behavior that was negatively correlated with pH, AN, AP and OM (Fig. 5A). The fungal *Trichoderma* and *Aspergillus* were positively correlated with AN, Ca, AK, AP and pH. While, *Fusarium* showed negatively correlated with AN, Ca, AK, AP and pH (Fig. 5B). The redundancy analysis revealed that rhizosphere soil AN, Ca, AK, AP and pH had great influence on microbial community.

Discussion

H_2S has long been considered as a phytotoxin. But in recent years, it has been found that it play an important role in plant physiological process as gas signal molecule[14–19]. Application of exogenous H_2S to plants can provide additional protection against stresses, such as drought, salinity and heavy metals are mainly induced antioxidant system to reduce oxidative cell damage[13]. H_2S has also been found to inhibited the growth of pathogens *Aspergillus niger*, *Penicillium italicum*, *Rhizopus oryzae*, *Candida albicans*, and so on[26–30]. However, all above evidence focused on the interaction between plant and H_2S, whether exogenous H_2S addition directly inhibits the pathogens in soil is not clear, and whether H_2S can suppress the plant disease caused by pathogen *R. Solanacearum* is still unknown. In the present study, different concentrations of H_2S donor NaHS were applied to the tobacco field, we found that the exogenous NaHS significantly suppressed TBW caused by *R. Solanacearum* through changing the soil physicochemical properties and microbial community.

Since researchers indicated that the exogenous application of H_2S can affect plant growth by altering soil nutrient content[33,34]. In our study, the exogenous application of NaHS increased the the soil pH, AN, AP and OM (Table 2). Increase pH is important for inhibited the survival of *R. Solanacearum*, and increase OM, N and P meet the need of plant growth[35,36]. In addition, the higher soil carbon and phosphorus could increase activity of beneficial microorganisms against pathogen[36]. This study demonstrated that the application of NaHS significantly reduce the disease incidence and disease index of TBW, and pH, AN, AP and OM were significantly negative correlation with the incidence of TBW. It was speculated that application of NaHS changed soil physicochemical properties that indirectly suppressed *R. Solanacearum* growth by promoting antagonistic microorganisms.

The rhizosphere soil microbial community structure influences the plant immunity and quality, and the microbial community are considered to be a key mechanism that can suppress soil-born pathogens[37,38]. Our findings explored that application of NaHS altered the diversity and richness of soil microbial (Table S2), which was consistent with the findings of Fang *et al*.[34] In this study, microbial analysis revealed a different pattern among treatments (Fig. 1A; Fig. 3A). NaHS application significantly influenced the composition of the soil microbial community (Fig. 1;Fig. 3). NaHS treatments reduced the relative abundance of *Proteobacteria* (Fig. 1B), which was similar to the results by Li *et al*.[38] The phylum *Proteobacteria* included the pathogen *R. solanacearum* is less abundant in healthy soils than in the bacterial wilt soil[38]. NaHS treatments also reduced the relative abundance of *Acidobacteria* (Fig. 1B). *Acidobacteria* was mainly driven by soil pH, and the low pH was more suitable for the survival of *Acidobacteria*[39]. Our results also showed that the relative abundance of *Bacteroidetes* were increased in NaHS treatments (Fig. 1B), which could promote plant growth, and improve the resistance of plants to environmental stress[40]. The Heatmap based on significant changes indicated that NaHS application significantly increased the abundances of some bacteria and fungi (such as *Paenibacillus*, *Bacillus*, *Lysobacter*, *Aspergillus* and *Trichoderma* etc.) (Fig. 1C; Fig. 3C). The function of these increased genera may be related to soil physicochemical properties and pants, which can accelerate the cycling of elements and promote plant growth and environment adaption.

In the present investigation, certain genera of microorganisms of S*olirubrobacter*, *Rhodococcus*, *Rhizobium*, *Pseudomonas*, *Paenibacillus*, *Microvirga*, *Lysobacter*, *Haliangium*, *Granulicella*, *Flavobacterium*, *Bacillus*, *Trichoderma* and *Aspergillus* were significantly high in NaHS treatments. However, *Ralstonia* and *Fusarium* were significantly low in NaHS treatments (Fig. 2; Fig. 4). The genus *Ralstonia* includes many soil-borne pathogens, which only infects root via wounds caused by microbe and insect[41]. Pathogenic *Fusarium* can infect the root by penetration hyphae, causing more wounds to the root, and thus increasing the infection of the root by pathogenic *Ralstonia*[42]. The application of NaHS may inhibited soil-borne diseases caused by pathogenic *Ralstonia and Fusarium.* Some species in *Solirubrobacter* and *Granulicella* have positive effects on the transition of the organic carbon in the soil[43]. *Rhodococcus* and *Bacillus* were reported as phosphate-mobilizing bacteria, which have the ability to solubilize organic and inorganic phosphate[44]. Furthermore, some species of the genus *Bacillus* can

affect the growth and virulence traits of *Ralstonia* by producing volatile organic compounds[45]. The genus *Pseudomonas* is known for its ability to promote plant growth, inhibit pathogens, and induce the systemic resistance to diseases in many plants[46]. *Microvirga* is nitrogen fixing bacteria, which can involved in nitrogen cycling[44]. Previous studies have documented that *Pseudomonas*, *Rhizobium*, *Paenibacillus*, *Lysobacter*, *Haliangium*, *Flavobacterium* and *Bacillus* as antagonistic bacteria can mitigate many soil-borne diseases and promote plant growth and health[45, 47–49]. *Trichoderma* and *Aspergillus* have been reported as antagonistic fungal. They directly interact with roots to produce bioactive substances, which can improve plant growth, and resist abiotic and biotic stress[50,51]. The application of NaHS may provide a suitable environment for promoting the growth of these beneficial microorganisms, increasing the relative abundance of these beneficial microorganisms, and reducing the incidence of TBW. In this study, the redundancy analysis (RDA) revealed that these beneficial microorganisms were positively correlated with pH, AN and AP, whereas The genus *Ralstonia* and *Fusarium* were negatively correlated with pH, AN and AP (Fig. 5). In sum, the soil microecology changes induced by NaHS are strong related to the suppression of soil-borne diseases.

Conclusion

Our study demonstrated that exogenous NaHS significantly reduced the disease incidence and disease index of TBW. The application of NaHS increased soil pH and improved soil nutrient status, and some soil physicochemical properties had a positive relationship with the abundance of the soil microbial community. NaHS addition changed soil microbial community composition and structure at the phylum and genus levels. Taken together, exogenous application of NaHS shifting the soil microecology to suppresses TBW, which may provide a new perspective to control TBW.

Materials And Methods

Field experiment. The field experiment were performed in a continuous cropping tobacco field at a tobacco plantation in Xuan'en County (109°26' E, 29°59' N), Enshi City, Hubei province, China, from April to September in 2019. The field experimental followed rules for cultivation of flue-cured tobacco (GB/T 23221 – 2008). Tobacco seedlings were cultivated in greenhouse and sterile tobacco plants with 4–5 leaves were transplanted into field. The experimental design consisted of three blocks, each 200 m^2 in size, each block was divided into five plots of 40 m^2, 60 plants in each plots. Five treatments with three replicates in completely randomized blocks were established. The treatments were: (1) control, 0 mg/L NaHS (CK); (2) 200 mg/L NaHS (NaHS200); (3) 400 mg/L NaHS (NaHS400); (4) 600 mg/L NaHS (NaHS600); (5) 800 mg/L NaHS (NaHS800). 50 mL NaHS of different concentrations was applied to each tobacco root when transplantation. The planting density of all treatments were the same.

Soil sample collection and physicochemical properties analysis. Rhizosphere soil were collected by five-spot-sampling method at 100 d post-transplantation when recording the disease occurrence. Then the soil samples from the five separate sites were mixed to one soil sample, partitioned into two subsamples,

ones were immediately transported on ice to the laboratory and stored at − 80°C for genomic DNA extraction, and the other subsamples were air-dried for physicochemical properties analysis. The analysis of soil pH, alkali-hydrolyzed nitrogen (AN), available phosphorus (AP), available potassium (AK), organic matter (OM), exchangeable calcium (Ca) and exchangeable magnesium (Mg) was performed according to Hu *et al.*[52]

Bacterial wilt recording. Symptoms of TBW were monitored at five different sites in each plot at 100 d post-transplantation. The TBW disease index (DI) based on severity scale of 0–9 was described in a previous study[52]. Briefly, “0” represents the plants without visible symptoms; “1” represents the presence of occasional chlorotic spots on stems, or less than half of the leaves wilted on unilateral stems; “3” represents the presence of a black streak less than half the height of the stem, or between half to two-thirds of the leaves wilted on unilateral stems; “5” represents the presence of a black streak over half the length of the stem, but not reaching the top of the stem, or more than two-thirds of the leaves wilted on unilateral stems; “7” represents the presence of a black streak reaching the top of the stem, or all leaves wilted; and “9” represents the dead plant. Based on the number of plants in each rating scale, disease incidence (I) and disease index (DI) of TBW were calculated as $I = n'/ N \times 100\%$ and $DI = \sum(r \times n)/(N \times 9) \times 100$, where n' is the total number of infected tobacco plants, r is the rating scale of disease severity, n is the number of infected tobacco plants with a rating of r, and N is the total number of plants. Control efficacy = [(I of control − I of treatment)/I of control] × 100%.

DNA extraction and gene amplification. DNA was extracted from 0.5 g rhizosphere soil using the FastDNA Spin Kit (MP Biomedicals, USA) in accordance with the protocol of the manufacturer. The integrity of DNA samples were determined by 1% agarose gel electrophoresis. Then the concentration and purity of the DNA were determined using a Nanodrop 1000 spectrophotmeter (Nanodrop Techenologies, Wilmington, DE, USA)[52].

The extracted DNA from each soil sample was used as a template for amplification. The V4 region of the bacterial 16S rRNA genes and the ITS1 regions of the fungal rRNA genes were amplified. Each the DNA sample was amplified separately using the primers 515F (5’-GTGCCAGCMGCCGCGGTAA-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’)[53] for bacterial, ITS5-1737F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2-2043R (5'-GCTGCGTTCTTCATCGATGC-3')[54] for fungi.

Sequences processing and analysis. All PCR reactions were performed on Illumina HiSeq platforms (Illumina Inc., USA) at Novogene Bioinformatics Technology Co., Ltd (Beijing, China). The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. The sequence quality was statistically analyzed by CASAVA1.8. The raw sequence data was preliminarily filtrate using the FASTX Toolkit 0.0.13 software package, removing the low mass base at the tail of the sequence (Q value less than 20) and the sequences with lengths less than 35 bp. finally, the length of the valid reads was approximately 250 bp. All effective tags of all samples were clustered using Uparse software (V7.0.1001, http://drive5.com/uparse/). Sequences with ≥ 99.5% identity for 16S rDNA and sequences with ≥ 97% identity for ITS were assigned to the same OTUs (operational taxonomic units).

The OTUs, Chao1 and Shannon index were calculated with QIIME (Version 1.7.0) to evaluate richness and diversity of soil microbial community[52].

Statistical analysis. The data were analyzed with Microsoft Excel 2007 and SPSS version 18.0 (IBM, USA). Differences between treatments were assessed by one-way analysis of variance (ANOVA) and least significant difference (LSD) test ($p < 0.05$). Correlation between disease incidence and soil physicochemical properties was analyzed by Pearson correlation analysis. Principal coordinate analysis (PCoA) with the weighted Unifrac distance and redundancy analysis (RDA) were carried out using R (Version 2.15.3). Correlation analysis was conducted by Pearson (2- tailed).

Declarations

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Figures

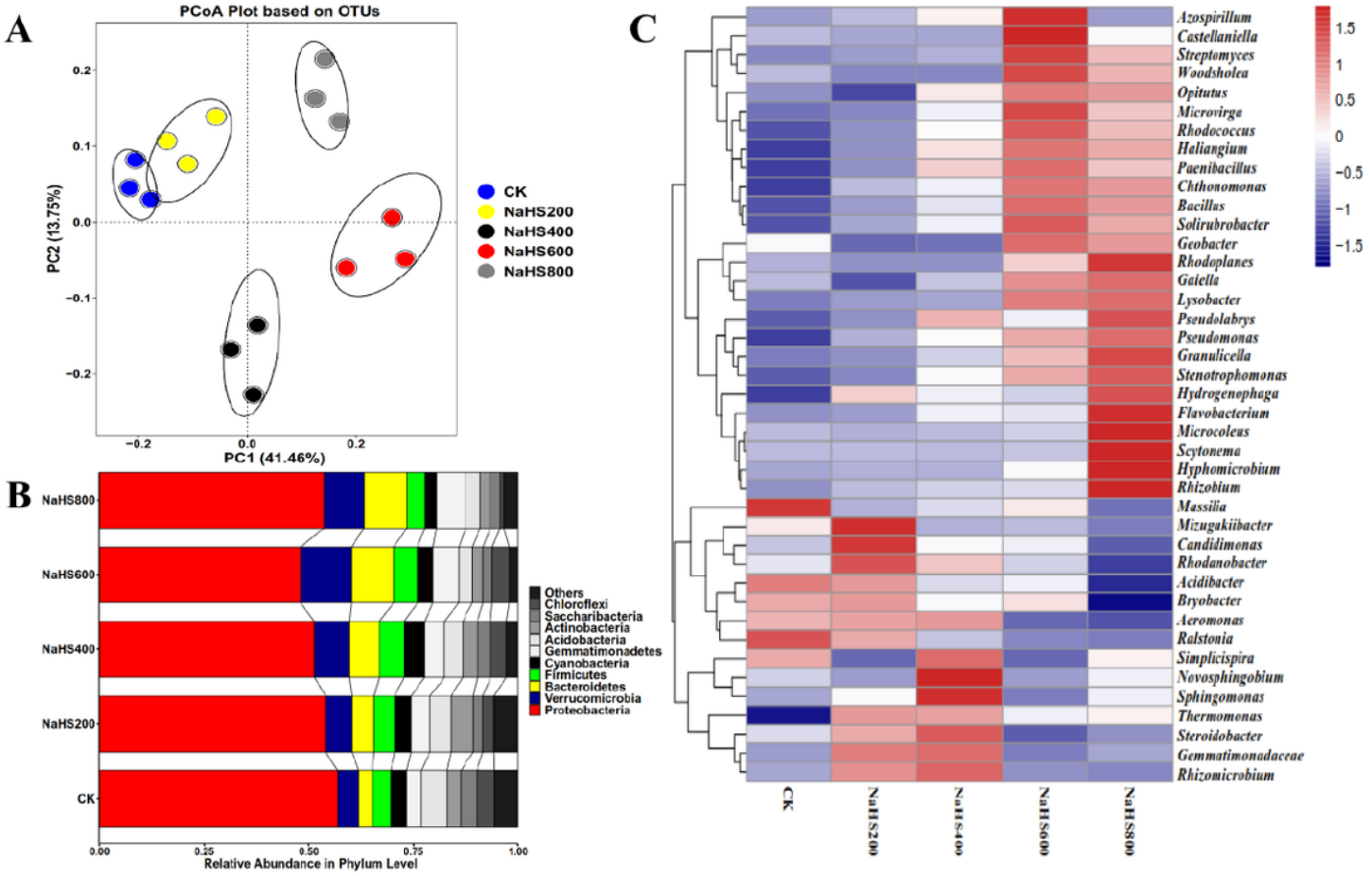

Figure 1

Soil bacterial community in five treatments. (A) The Principal coordinate analysis (PCoA) of soil bacterial community; (B) The relative abundance of bacterial phyla in soil samples; (C) Hierarchical cluster analysis of predominant bacterial genera. (CK: 0 mg/L NaHS, NaHS200: 200 mg/L NaHS, NaHS400: 400 mg/L NaHS, NaHS600: 600 mg/L NaHS, NaHS800: 800 mg/L NaHS.)

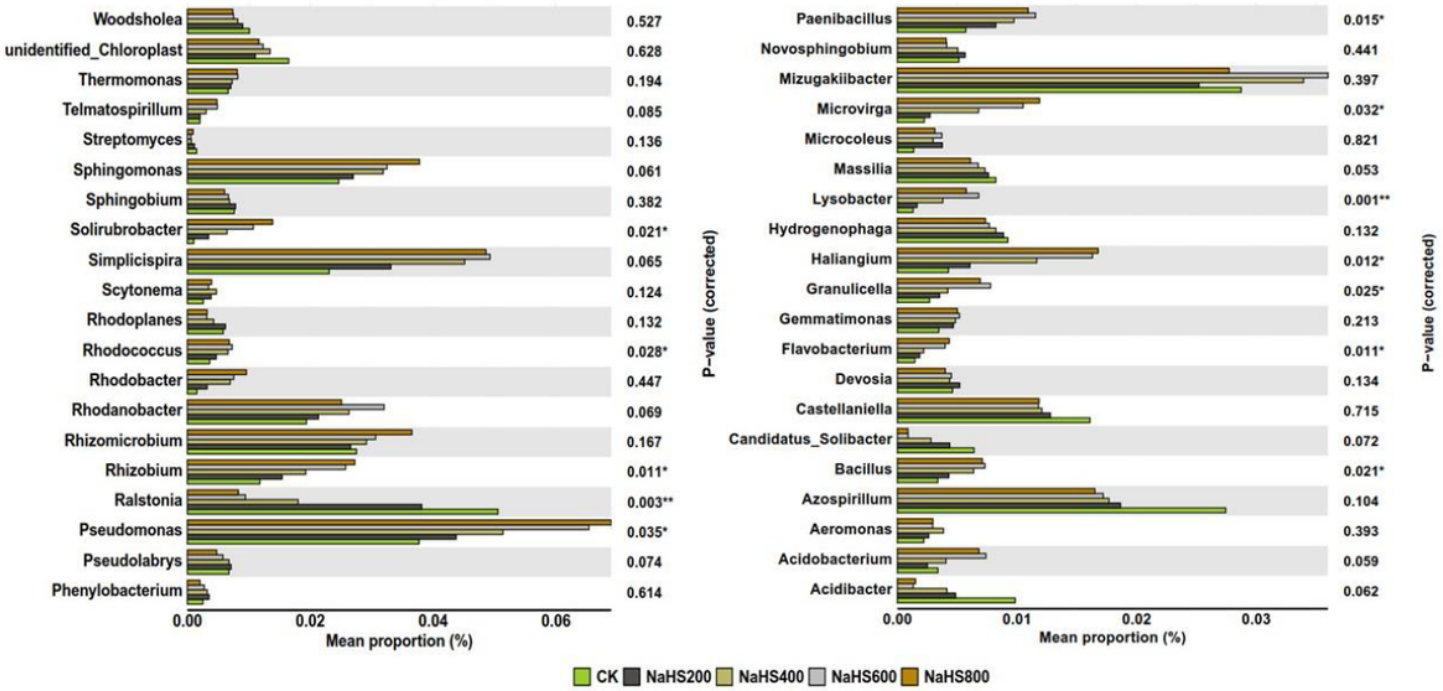

Figure 2

The relative abundances of the top 40 classified bacterial genera among different treatments. The abundances of different genera were analyzed by one-way ANOVA. * $p < 0.05$, **$p < 0.01$. (CK: 0 mg/L NaHS, NaHS200: 200 mg/L NaHS, NaHS400: 400 mg/L NaHS, NaHS600: 600 mg/L NaHS, NaHS800: 800 mg/L NaHS.)

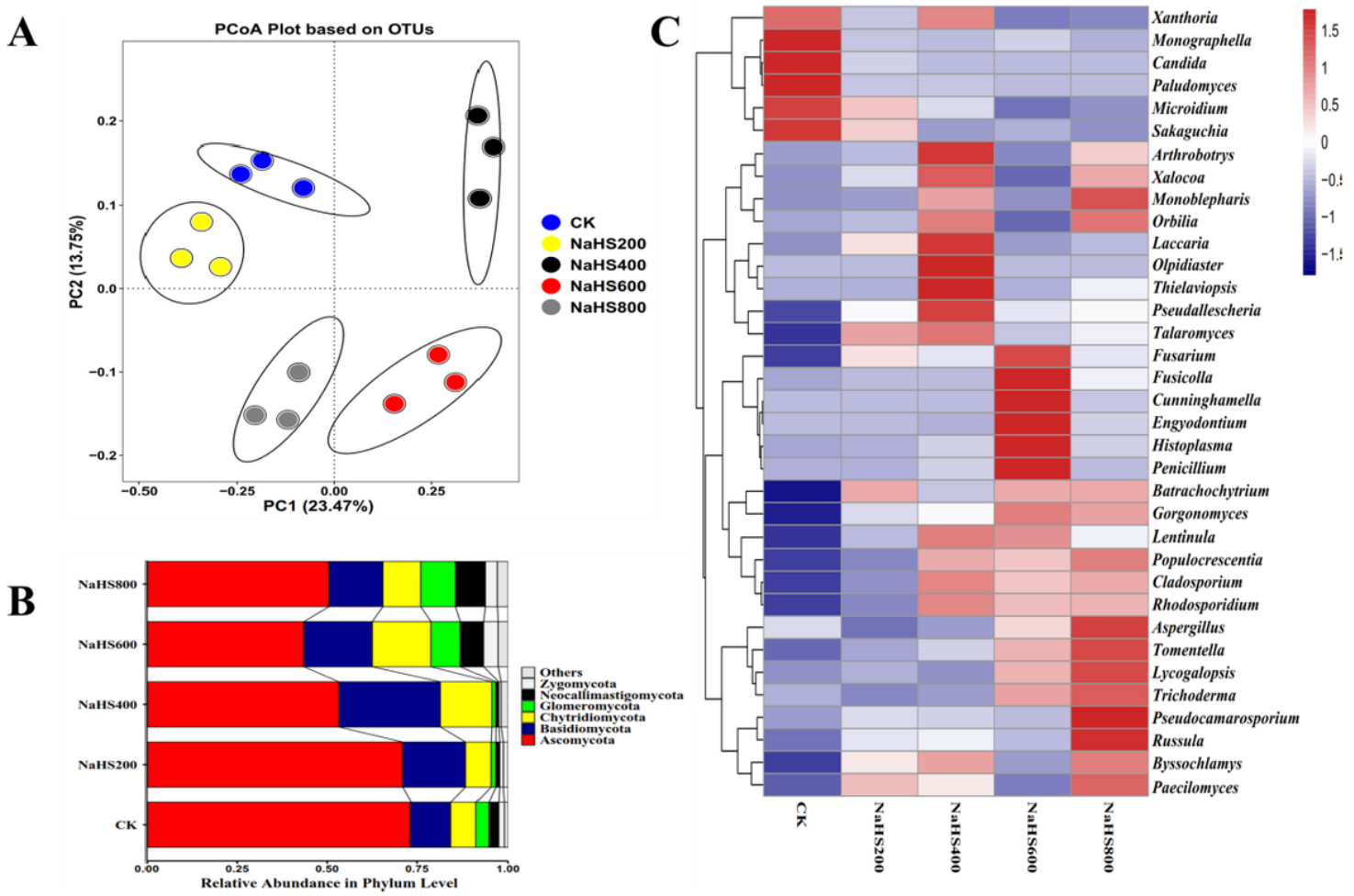

Figure 3

Soil fungal community in five treatments. (A) The Principal coordinate analysis (PCoA) of soil fungal community; (B) The relative abundance of bacterial phyla in soil samples; (C) Hierarchical cluster analysis of predominant fungal genera. (CK: 0 mg/L NaHS, NaHS200: 200 mg/L NaHS, NaHS400: 400 mg/L NaHS, NaHS600: 600 mg/L NaHS, NaHS800: 800 mg/L NaHS.)

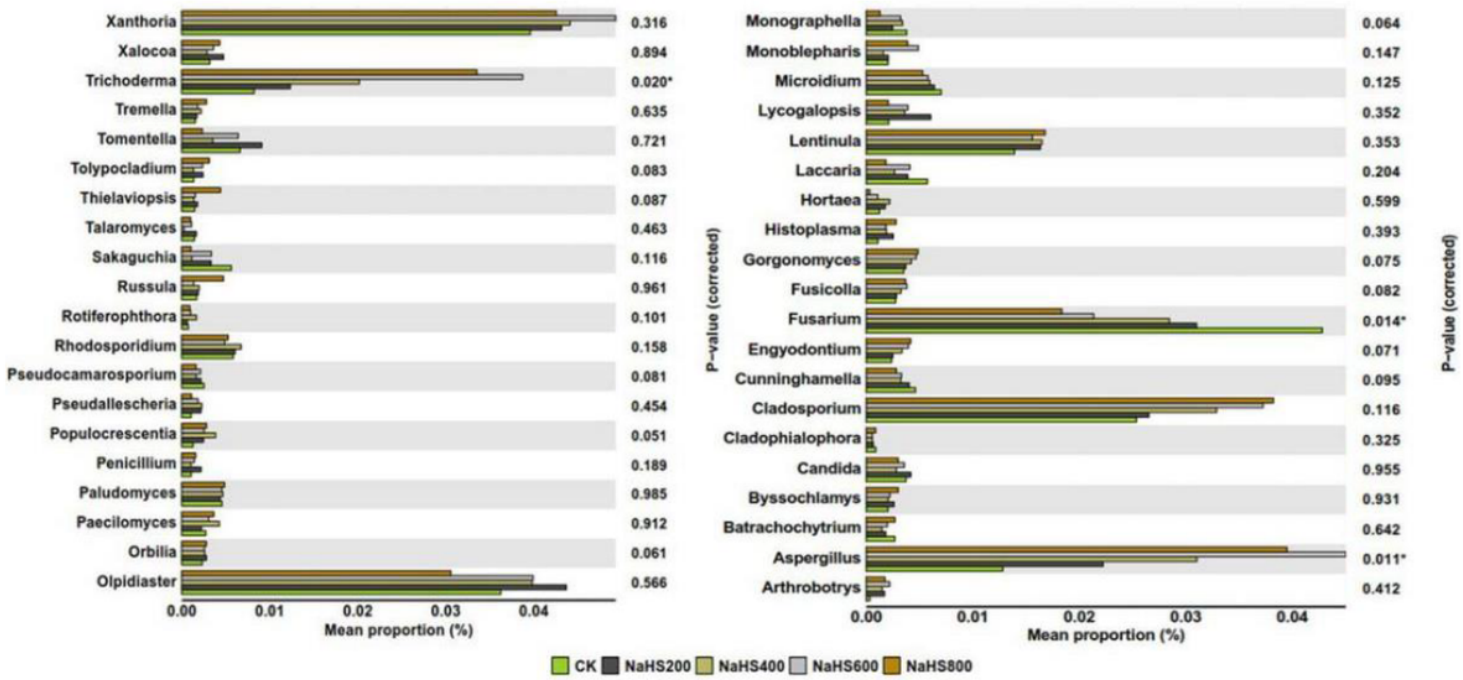

Figure 4

The relative abundances of the top 40 classified fungal genera among different treatments. The abundances of different genera were analyzed by one-way ANOVA. * $p < 0.05$, **$p < 0.01$. (CK: 0 mg/L NaHS, NaHS200: 200 mg/L NaHS, NaHS400: 400 mg/L NaHS, NaHS600: 600 mg/L NaHS, NaHS800: 800 mg/L NaHS.)

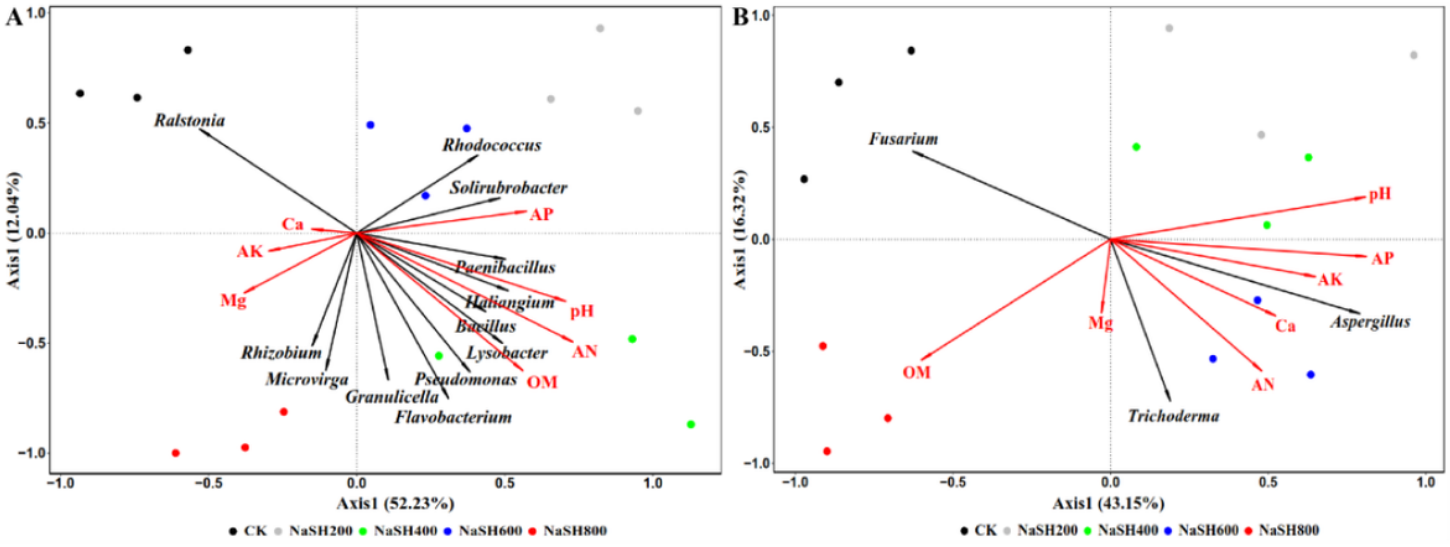

Figure 5

Redundancy analysis between soil physicochemical properties and rhizosphere microbial community. (A) bacterial community; (B) fungal community. (CK: 0 mg/L NaHS, NaHS200: 200 mg/L NaHS, NaHS400: 400 mg/L NaHS, NaHS600: 600 mg/L NaHS, NaHS800: 800 mg/L NaHS.)

Supplementary Files

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