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Comparative effects of two humic substances on microbial dysbiosis in the rhizosphere soil where cucumber (*Cucumis sativus* L.) is grown

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Abstract

The extraction of humic substances from weathered coal and peat has been regarded as an effective way to utilize these wastes. However, the effects of humic acid (HA) and sodium humate (HN) (derived from weathered coal and peat, respectively) on soil microbial dysbiosis remain elusive. By virtue of Illumina sequencing and FUNGuild, we investigated the interrelationships between soil microbial communities and potential phytopathogenic fungi, found that merely HA surprisingly ($p < 0.05$) reduced the relative richness of potential phytopathogenic fungi in contrast to the controls. The relative abundances of potential pathogenic fungi, fungal community structures, network degrees, and the average degrees of potential pathogenic fungi were recognized as the crucial factors for the determination of cucumbers' aboveground biomasses via Random Forest classification. In addition, the aboveground biomasses were significantly ($p < 0.05$) and negatively related to the relative richness of potential pathogenic fungi. Among the 28 operational taxonomic units (OTU) that played the most important roles in determining the relative abundance of potential phytopathogenic fungi, 3 OTU belonging to the *Penicillium* and *Nocardioides* genera were significantly ($p < 0.05$) more abundant in the HA treatment. Therefore, it was obvious that HA facilitated the growth of beneficial microbes and increased the microbial network complexity, subsequently decreasing the relative abundance of phytopathogenic fungi and consequently increasing cucumbers' aboveground biomasses. The present research not only highlighted the differences between humic substances originating from weathered coal and peat but also indicated the significance of humic substances in regulating microbial communities, suppressing potential phytopathogenic fungi and facilitating cucumber growth.

KEYWORDS
co-occurrence networks, humic substances, microbial communities, potential phytopathogenic fungi, rhizosphere soil

1 | INTRODUCTION

Weathered coal (rich in humic substances) is a kind of low-rank coal widely distributed in China (Zhou et al., 2019). The direct utilization efficiency of weathered coal is very limited because of its low activity, and difficulty is encountered when employing it in industry and agriculture (He et al., 2003). Similarly, low efficiency is also shown for peats utilization (peats containing 20%–40% wt of humic substances)

(Botero et al., 2010). With the purpose of efficiently using weathered coal and peat resources, increasing emphasis has been placed on the extraction of humic substances from them (Saito & Seckler, 2014; Zhou et al., 2019).

As important organic amendments, humic substances are widely adopted in agriculture and environmental protection (Nichols & Wright, 2005). For instance, some studies have well documented the stimulatory effects imposed by humic substances on crop growth (Ciarkowska et al., 2017; Jindo et al., 2012). Some studies have shown that humic substances could impose positive effects on the accumulation of aboveground biomass, increase root activity, and promote the synthesis of proteins and nucleic acids in many crops (Daur & Bakhashwain, 2013; Xu et al., 2012). The combined application of mineral fertilizer and humic substances can not only maintain high crop productivity but also improve soil fertility and enhance the utilization efficiency of chemical fertilizers (Mamedov et al., 2014). Additionally, humic substances that have various kinds of functional groups with large specific surfaces could act as adsorbents of heavy metals and antibiotic contaminants from water or soil environments (Botero et al., 2010). In general, previous studies mostly emphasized the effects of humic substances on stimulating crop growth, improving soil fertility, and reducing pollution in the environment. However, studies focusing on the biological activity of humic substances and their effects on soil microbes are rare.

Soil microbes are crucial to crop growth and plant health (Berendsen et al., 2012; Jeffries et al., 2003). Soil dysbiosis induced by the disturbance of soil microbial communities is one of the most significant factors affecting the occurrence and expansion of crop-related diseases (Gattinger et al., 2008; Wei et al., 2015; Zhao et al., 2019), thus, soil-borne diseases can actually be referred as 'microbiome diseases.' Soil micro-organisms are important for maintaining soil functions and significantly affect the health of plants. Substantial research studies have revealed that microbe diversities can indicate illness susceptibility in massive plants, and microbiota are vital for sustaining the health of plants (Wei, Wu, et al., 2019; Wei, Friman, et al., 2020).

With the growing demands for the production of greenhouse cucumbers and the increasing consciousness for environmental protection, it is imperative to develop environmentally friendly agrochemicals with the intention of controlling soil-borne diseases. To this end, the control of rhizosphere phytopathogenic fungi through organic amendments (such as acids) has been considered an effective way to decrease the levels of crop diseases (Li, Fang, et al., 2019; Li, Liu, et al., 2019). Nevertheless, most of the previous studies mainly emphasized the effects imposed by humic substances on several specific phytopathogenic fungi (Wei, Wu, et al., 2019). These studies contrasted the role of humic substances in specific micro-organisms in vitro and provided insufficient enlightenment with regard to the impacts imposed by humic substances on microbiota. In addition, there are many microbes in rhizosphere soils, and it is impossible to artificially study every potential phytopathogenic fungus in vitro. Currently, with the emergence of FUNGuild, an annotation tool, it is possible to analyze soil potential phytopathogenic fungi more comprehensively (Nguyen et al., 2016).

In addition, as different types of humic substances contain different functional groups or display different chemical constitution proportions, different humic substances may inhibit phytopathogenic fungi and facilitate plant growth to different extent (Wei, Wu, et al., 2019; Wei et al., 2021). According to Loffredo et al. (2008), one type of humic substance is capable of inhibiting several specific phytopathogenic fungi in vitro. Nevertheless, the soil environment is intricate, and the roles of diverse humic substances in soil phytopathogenic fungi remain elusive. Therefore, the role of humic substances in potential phytopathogenic fungi in cucumber rhizosphere soil may be different from that in culturing systems. The interrelationships among humic substances, soil microbiota, and the inhibition of potential phytopathogenic fungi ought to be clarified.

In this study, the major assumption was that diverse types of humic substances could change soil microbiota in the rhizosphere soil of cucumbers and suppress potential phytopathogenic fungi to different extent. Accordingly, 2 kinds of humic substances were selected: humic acid (HA) and sodium humate (HN) (from weathered coal and peat, respectively). Our study aimed to provide data support for the utilization of humic substances to suppress potential phytopathogenic fungi in rhizosphere soil and to offer enlightenment pertaining to the resource utilization of weathered coal and peat.

2 | MATERIALS AND METHODS

2.1 | Pot experiment

The cucumber cultivar Zhongnong 6 was used in this study. Seeds were superficially sterilized with NaClO (2%, for 180 s) and ethyl alcohol (75%, for 120 s) and rinsed three times with sterile water before germination. The trialled soil utilized in our study was a clay loam, collected from a greenhouse at the 'Nanjing Vegetables (and Flowers) Scientific Institute' in Jiangsu, PR China (32°13′N, 119°04′E). This greenhouse has a history of cultivating cucumbers for over 3 years. Before experimentation, the soil used in the study was air-dried, thoroughly mixed, and sieved via a 5 mm sieve to clear clods and plant debris. The physicochemical attributes of the soil samples were determined before cucumber transplantation in May 2020 (Table S1).

The detailed information about the HA and HN extraction procedures was delineated in the report finished by Wu et al. (2016). The element (carbon, nitrogen, and sulfur) amount and the chemical structures of the humic substances (HA and HN) were determined by organic analyses and Fourier transform infrared spectroscopy (FT-IR) analyses.

Before cucumber transplantation, 0.3 g N kg^{-1} (based on urea), 0.69 g P_2O_5 kg^{-1} (based on ordinary superphosphate) and 0.36 g K_2O kg^{-1} (based on potassium sulfate) were applied as basal fertilizer; humic substances (HA and HN) were utilized with chemical fertilizers at 4.4 g kg^{-1} soil. In this study, the treatment with chemical fertilizers only was considered as control (CK), while the treatment with chemical fertilizers and HA was denoted as the HA treatment, and the treatment with chemical fertilizers and HN was denoted as the HN

treatment. Hence, our study contained a total of three treatments (CK, HA, and HN). Each treatment was conducted using 24 pots with one cucumber seedling per pot. Cucumber seedlings were transplanted at the two cotyledons stage. At the flowering stage, 8 pots were randomly selected from the 24 pots of the 3 treatments to collect bulk soil and rhizosphere soil. The bulk soil was collected from each of the 8 pots using a soil auger (0.19 cm in diametre) with three randomized soil cores. After the removal of the loosely attached soil on roots, the soil brushed down from roots was rhizosphere soil (Veneklaas et al., 2003). The bulk soil specimens were sieved via a 2 mm sieve and separated into two parts for air-drying and stored below 4°C. The pH (soil: water = 1:2.5, wt/vol), nutrient contents [including soil organic carbon (SOC), total nitrogen (TN), available nitrogen (AN), available phosphorus (AP) and available potassium (AK), and cation exchange capacity (CEC)] of the soil specimens were determined as per the approaches delineated by Lu (2000). Briefly, the SOC content was determined by using potassium dichromate oxidation; the TN level was identified via the Kjeldahl method; the AN was measured by alkali hydrolysis diffusion; the available P was identified via the 0.5 mol L^{-1} NaHCO$_3$ approach; the available K was identified via the 1 mol L^{-1} NH$_4$Ac abstraction approach; the CEC was extracted by cobalt hexa ammonia trichloride and determined by using a spectrophotometric method.

Approximately, 0.5 g rhizosphere soil (fresh weight) was collected from every treatment for DNA abstraction via the Fast$^{®}$ DNA SPIN Tool as per the supplier's specification (MP Biomedicals, Santa Ana, CA). The abstracted DNA was subjected to quantification via a Nano Drop ND-1000 spectral photometer (NanoDrop Technologies, DE, USA).

For the 16S rRNA-seq assay, each of the 24 DNA specimens was magnified individually via microbial PCR primers 515F (5′GTGCC AGCMGCCGCGGTAA-3′)/907R (5′-CCGTCAATTCCTTTGAGTTT-3′), which targeted the V4-V5 hyper-variant region of the 16S rRNA gene utilized to magnify the fragments of the 16S rRNA genes (Biddle et al., 2008). For ITS sequencing, DNA specimens were magnified individually via the fungus PCR primers ITS1F (5′-CTTGGTCA TTTAGAGGAAGTAA-3′) (Gardes & Bruns, 1993) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) (White et al., 1990), which targeted the internal transcribed spacer 1 (ITS1) region. The PCR results were afterwards subjected to sequencing via an Illumina MiSeq PE250 equipment. The original sequencing data were studied via the Quantitative Insight into Microbe Ecology (QIIME) pipeline (http://qiime.sourceforge.net/) (Caporaso et al., 2010). Reads with a length <200 bp or with average quality scoring <25 were discarded, which generated 379,550 and 107,909 high quality bacteria and fungus sequences, which were afterward integrated via the MOTHUR program package (Schloss et al., 2009). Clustering the sequencing results into OTU at a 97% nucleotide resemblance was completed via UCLUST (Edgar, 2010). The taxonomy identity of every OTU was afterward identified via comparing with the UNITE data base (https://unite.ut.ee/). Furthermore, all 24 plants from each pot were collected to identify the above ground biomasses in late June 2020. Plant specimens were desiccated under 105°C for 0.5 hr and afterward under 70°C to an unchanged weight, which represented the aboveground biomass of cucumbers.

2.2 | Statistics

Analysis of variance (ANOVA) was employed to identify the diversities between the aboveground biomass of cucumbers, relative richness of potential phytopathogenic fungi, microbe diversities, and topological properties of the microbe (bacteria and fungi) co-occurrence networks among the three treatments (IBM SPSS 22.0). The sequence information was normally distributed through Hellinger transformation, and afterward, principal coordinates analyses (PCoA) were leveraged to investigate the variations in the microbe and fungus community architectures on the foundation of Bray-Curtis dissimilarity among diverse treatments through the cmdscale function in the vegan package in R language (Kuang et al., 2016).

By calculating Spearman's correlation, co-occurrence networks of rhizosphere microbes (bacteria and fungi) were established. The correlative liminal value was >0.7, and p was <0.01. Subsequently, the Brown's approach (Brown, 1975) and the Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995) were utilized to decrease the possibility of acquiring false-positive outcomes. For a meaningful contrast of the network performances of every treatment, 24 sub-networks were produced from the 3 microbe (bacteria and fungus) networks using the subgraph function in the igraph package in R language (Ma et al., 2015). The network analysis and visualization based on the OTU were completed via CYTOSCAPE 3.6.1 and GEPHI 0.9.2 (Bastian et al., 2009; Faust & Raes, 2016). A random forest classifier was used to recognize the most important factors (or/and OTU) that determined the cucumbers' aboveground biomass and the relative abundance of potential pathogenic fungi.

3 | RESULTS

3.1 | The effects of humic substances on the soil physicochemical properties and cucumber growth

The chemical properties of the bulk soils under different treatments are shown in Table S3. The soil pH, CEC, and soil nutrient (SOC, TN, AN, AP, and AK) contents were not significantly affected by the HA and HN application. The aboveground biomass of cucumbers under different treatments is shown in Figure 1a. HA and HN increased the aboveground biomass of cucumbers by 57.8% and 29.6% compared with the CK, respectively.

3.2 | Fungistatic activities of the humic substances

In comparison with CK, the abundance of potential phytopathogenic fungi categorized by FUNGuild in cucumber rhizosphere soils

significantly ($p < 0.05$) reduced by HA application (Figure 1b). In contrast to HA, HN failed to significantly reduce the number of potential pathogenic fungi in the rhizosphere soils of cucumber. In our study, HA was significantly ($p < 0.05$) higher in N and S contents and C/N ratios than HN (Table S2). HA also showed significantly ($p < 0.05$) higher abundances of –OH groups and –COOH groups than HN (Figure S1). As humic substances with more active functional groups could inhibit pathogenic fungi more effectively, the relative abundance of potentially phytopathogenic fungi was significantly ($p < 0.05$) lower in the HA soil than in the CK and HN soils (Figure 1b). The HA treatment decreased the relative abundance of potential plant pathogens over CK by 38.4%.
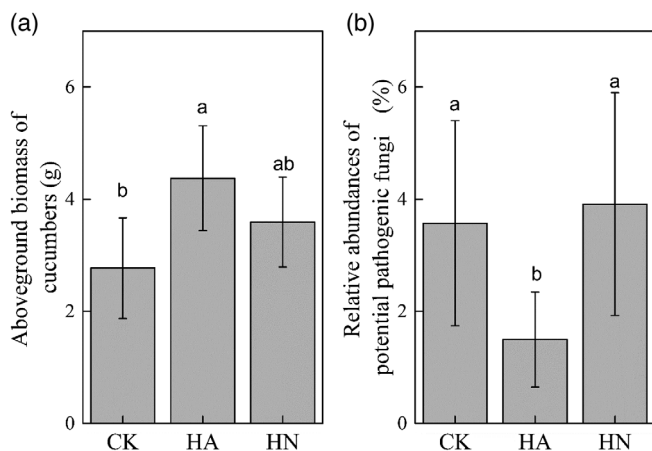

FIGURE 1 Aboveground biomass of cucumbers (g) and relative abundance of potentially pathogenic fungi (%) under different treatment. Different letters in a same plot indicate significant differences between the different treatments according to the Duncan test ($p < 0.05$, $n = 8$). CK, control; HA, humic acid from weathered coal; HN, sodium humate from peat

3.3 | Microbial community compositions

For bacterial responses, 16S amplicon sequencing yielded 47,442 high-quality sequences for each sample (on average). The relative abundances of *Bacteroidetes* (17.6%) and *Gemmatimonadetes* (5.2%) in the rhizosphere soil were significantly ($p < 0.05$) more abundant in the HA treatment than in the CK and HN treatments, while *Acidobacteria* (12.0%) and *Firmicutes* (4.7%) phyla were significantly ($p < 0.05$) more abundant in the HN treatment than in the other treatments (Figure 2a). For fungal communities, the relative abundances of *Zygomycota* (4.8%) in the rhizosphere soil of cucumbers were significantly ($p < 0.05$) more abundant and *Chytridiomycota* (1.6%) phyla were significantly ($p < 0.05$) less abundant in the HN soil than in the CK soil and the HA soil (Figure 2b).

At the OTU level, for the rhizosphere bacteria, 64 OTU with relative abundance >0.1% were identified as significantly ($p < 0.05$) enriched in the CK treatment (Figure 3). Meanwhile, 33 OTU with relative abundance >0.1% were identified as significantly ($p < 0.05$) enriched in the HA treatment. Furthermore, 88 OTU with relative abundance >0.1% were identified as significantly enriched in the HN treatment (Figure 3 and Table S5).

For the rhizosphere fungi, 5 OTU with relative abundance >0.1% were identified as significantly ($p < 0.05$) enriched in the CK treatment (Figure 3). These OTU were affiliated with the *Ascomycota* and *Basidiomycota* phyla. In addition, 6 OTU and 36 OTU with relative abundance >0.1% were identified as significantly ($p < 0.05$) enriched in the HA and HN treatments, respectively. The enriched OTU in the HA treatment were affiliated with the Ascomycota, Basidiomycota, and Chytridiomycota phyla. The enriched OTU in the HN treatment were mainly affiliated with the Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota phyla (Figure 3 and Table S5).

Through a random forest classification approach, we explored the major factors that caused the difference in the aboveground biomass of cucumbers among the CK, HA, and HN treatments. For the random
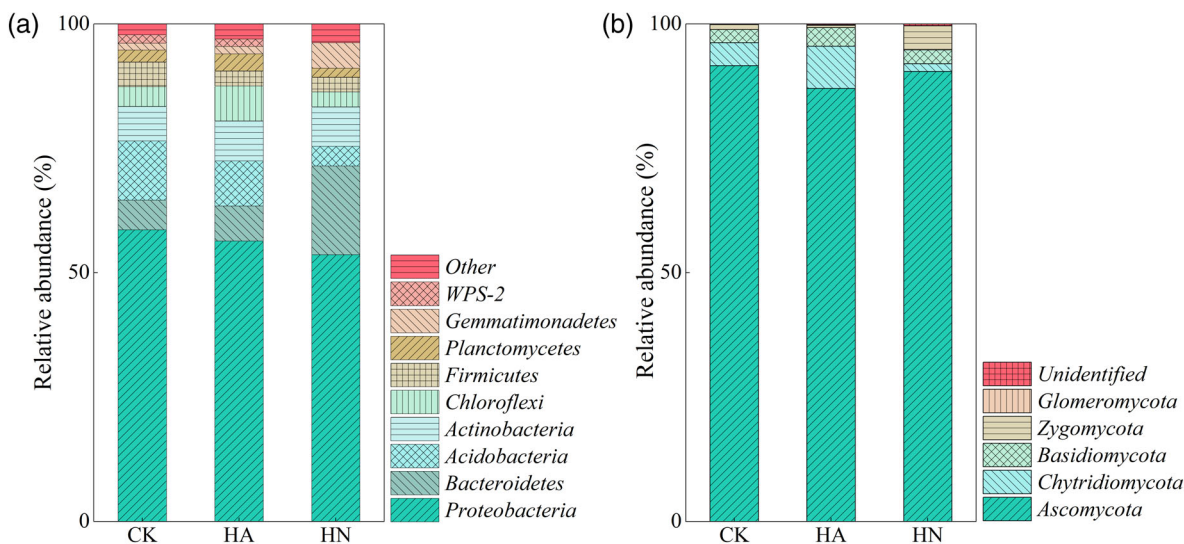

FIGURE 2 The relative abundance of various phyla of bacterial and fungal communities in the rhizosphere soil of cucumbers. CK, control; HA, humic acid from weathered coal; HN, sodium humate from peat [Colour figure can be viewed at wileyonlinelibrary.com]
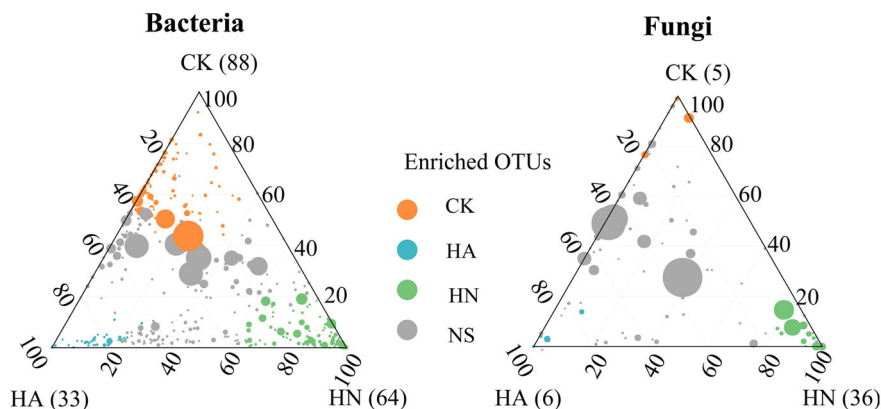
FIGURE 3 Each circle represents one OTU. The size of each circle represents its relative abundance. The position of each circle is determined by the contribution of the indicated compartments to the total relative abundance. Orange circles mark OTU significantly enriched in the CK treatment; blue circles mark OTU significantly enriched in the HA treatment; green circles mark OTU significantly enriched in the HN treatment; gray circles (NS) mark OTU did not significantly enriched in any of the three treatments; CK, control; HA, humic acid from weathered coal; HN, sodium humate from peat [Colour figure can be viewed at wileyonlinelibrary.com]

forest classifier (R package RandomForest, ntree = 500), the relative abundance of potential phytopathogenic fungi and the fungal community structure were recognized as playing the most important role in the determination of the aboveground biomass of cucumbers.

The aboveground biomass of cucumbers was significantly ($p < 0.05$) and negatively correlated with the relative abundances of the potential pathogenic fungi (Figure 4). We further explored the major OTU (the OTU annotated as pathogens that were excluded) that caused the difference in the relative abundance of potential phytopathogenic fungi among the three treatments. For the random forest classifier (R package RandomForest, ntree = 500), 28 OTU with relative abundance > 0.1% were recognized as playing the most important role in the determination of the relative abundance of potential phytopathogenic fungi. The CK-enriched OTU were affiliated with the *Pseudozasmidium*, *Aggregatilinea*, *Povalibacter*, *Acidibacter_Unidentified*, and *Longilinea* genera. The HA-enriched OTU were affiliated with the *Davidiella*, *Penicillium*, *Zopfiella*, *Chaetomium*, *Spizellomyces*, and *Nocardioides* genera. The HN-enriched OTU were affiliated with the *Tistlia*, *Nakamurella*, *Tautonia*, *Chujaibacter*, *Gaiella*, *Roseisolibacter*, *Gp14_Unidentified*, *Gp6_Unidentified*, and *Micropepsis* genera.

3.4 | Diversities of the microbial communities

The bacteria alpha diversity indices, involving the chao1 index and the acquired OTU, were significantly ($p < 0.05$) greater in the HA and HN assays in contrast to the CK assay, whereas no significant ($p < 0.05$) diversity was observed between the HA and HN assays (Figure 5a,b). Similar to the bacteria alpha diversities, fungus alpha diversities were significantly greater in the HA and HN treatments than the CK treatment (Figure 5d,e). However, compared with the HA treatment, the HN treatment significantly increased the soil fungi observed_OTU by 20%.

PCoA on the foundation of the Bray-Curtis distances suggested that the bacterial community compositions were separated by

treatments. The CK, HA, and HN assays displayed diverse microbiota structures (Figure 5c). The fungal community structures in the CK assay were significantly ($p < 0.05$) diverse from those n the HN assay (Figure 5f, Table S4). No significant diversity was observed in the fungal community structures between the CK assay and HA assay.

3.5 | Microbial co-occurrence networks

Co-occurrence networks were established to determine the interrelationships between the micro-organisms (cucumber rhizosphere bacteria and fungus) from the HA, HN, and CK assays (Figure 6). For a more meaningful contrast of the net performances from every treatment, 24 sub-networks were produced from the microbiota.

The topology attributes of the sub-networks and the ANOVA outcomes were presented by Table. Specifically, HA significantly ($p < 0.05$) increased the degree (by 243.5%), density (by 84.9%), clustering coefficient (by 19.8%), average degree of potentially pathogenic fungi (by 38.4%), positive correlations of the potentially pathogenic fungi with the other microbes (by 82.1%), and ratios of the P/N of the potentially pathogenic fungi to the other microbes (by 151%) of the networks in contrast to those of the CK treatment. Meanwhile, HA significantly ($p < 0.05$) reduced the modularity (by 58.6%) and the average path length (9.8%) of the networks. HN significantly ($p < 0.05$) elevated the degree (by 58.8%), modularity (by 47.5%), average degree of potentially pathogenic fungi (by 12.1%), and positive correlations of the potentially pathogenic fungi with the other microbes (by 17.4%) but significantly ($p < 0.05$) decreased the diameter (by 18.8%), density (by 15.6%), clustering coefficient (by 6.3%), average path length (by 0.8%), and ratios of the P/N of the potentially pathogenic fungi to other microbes (by 39.9%) of the networks in contrast to those of the CK treatment. For the Random Forest classifier (R package RandomForest, ntree = 500), the network degree and the average degree of potential pathogenic fungi were recognized as the

most vital factors for the determination of the aboveground biomass of cucumbers.

4 | DISCUSSION

For the purpose of reducing the effects of agriculture on the environment, it is pressing to develop eco-friendly measures to improve soil resistance or resilience to diseases. Here, novel organic amendments,
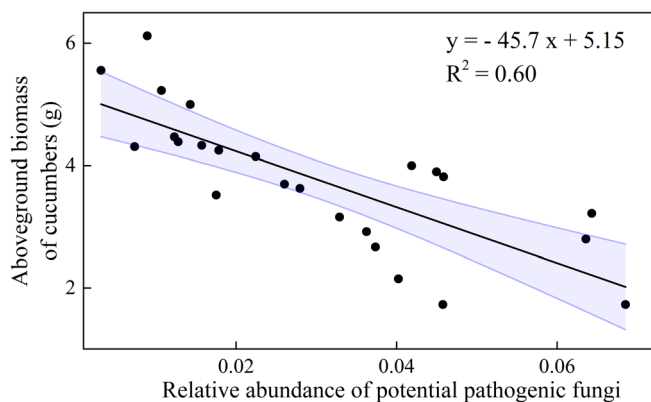

FIGURE 4 Correlations between the aboveground biomass and the relative abundance of potential pathogenic fungus ($n = 24$) [Colour figure can be viewed at wileyonlinelibrary.com]

humic substances, which can potentially result in crop growth promotion and soil suppression against multipathogens are described.

In this study, it was shown that different types of humic substances promoted the cucumber growth to different degrees (Figure 1). The most commonly proposed explanations can be related to soil fertility and microbial communities with the application of humic substances (Afifi et al., 2017; Olk et al., 2018). In our study, however, HA and HN application failed to significantly change soil fertility (Table S1). According to Li, Fang, et al. (2019) and Li, Liu, et al. (2019) and Magdoff and Weil (2004), the improvement in soil fertility dramatically depends on the quantity and duration of the humic substances that have been applied. For example, 1000 kg ha^{-1} humic acid application did not significantly affect soil fertility in the first application year. Similarly, 4.4 g kg^{-1} HA and HN application also did not significantly affect soil fertility in our 1-year study. In addition, the soil fertility failed to show a significant correlation with pathogen abundance and the aboveground biomass of cucumber. According to the results obtained by us, it was also shown by many reports that the soil microbiome contributed to pathogen suppression on the basis of humic application. Thus, it is speculated that the soil microbiome under the influence of humic substances contributes to the suppression of potential pathogenic fungi in the rhizosphere soil of cucumbers.

Humic substances, falling within the scope of organic amendments, have the capability of inhibiting a variety of pathogenic fungi in vitro (Wei et al., 2018). In comparison with CK, the abundance of
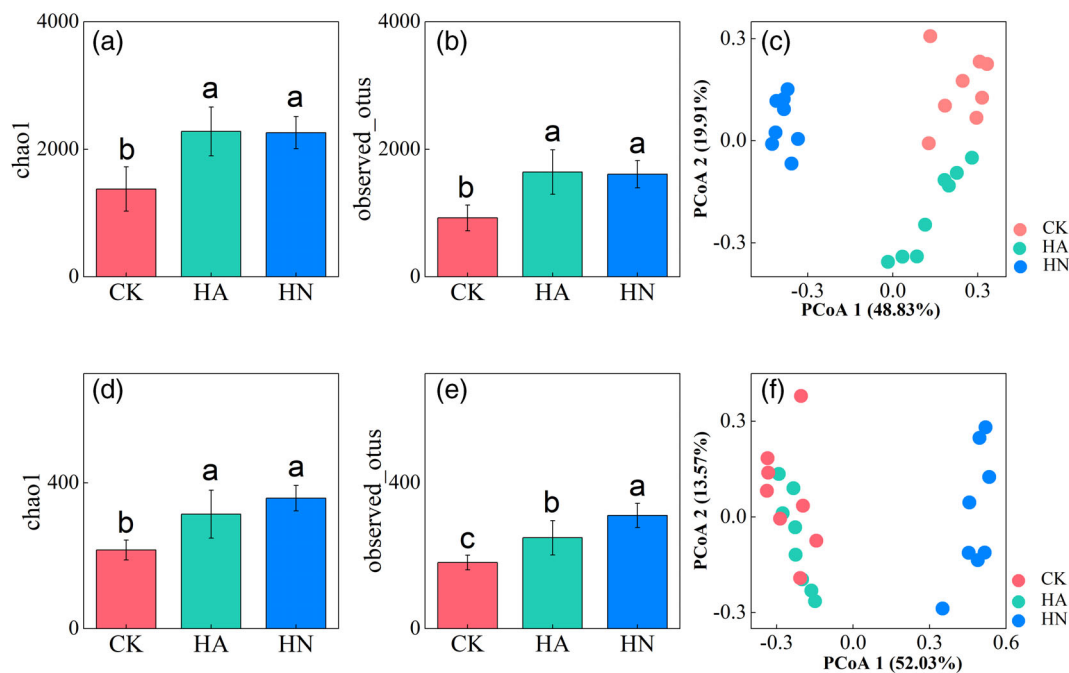

FIGURE 5 Comparison of alpha diversities (a, b, d, and f) and beta diversities (c and f) of the rhizosphere microbial communities between different treatments. (a,b) alpha diversity indices of bacterial communities across different treatments; (d,e) Alpha diversity indices of fungal communities across different treatments; (c,f) principal coordinate analysis based on the Bray-Curtis distances showing the effects of the HA and the HN on bacterial (c) and fungal (f) communities. Chao1 and observed_otu were calculated from the rarefied bacterial and fungal OTU table. Different letters in a same plot indicate significant differences between the different treatments according to the Duncan test ($p < 0.05$, $n = 3$). CK, control; HA, humic acid from weathered coal; HN, sodium humate from peat [Colour figure can be viewed at wileyonlinelibrary.com]
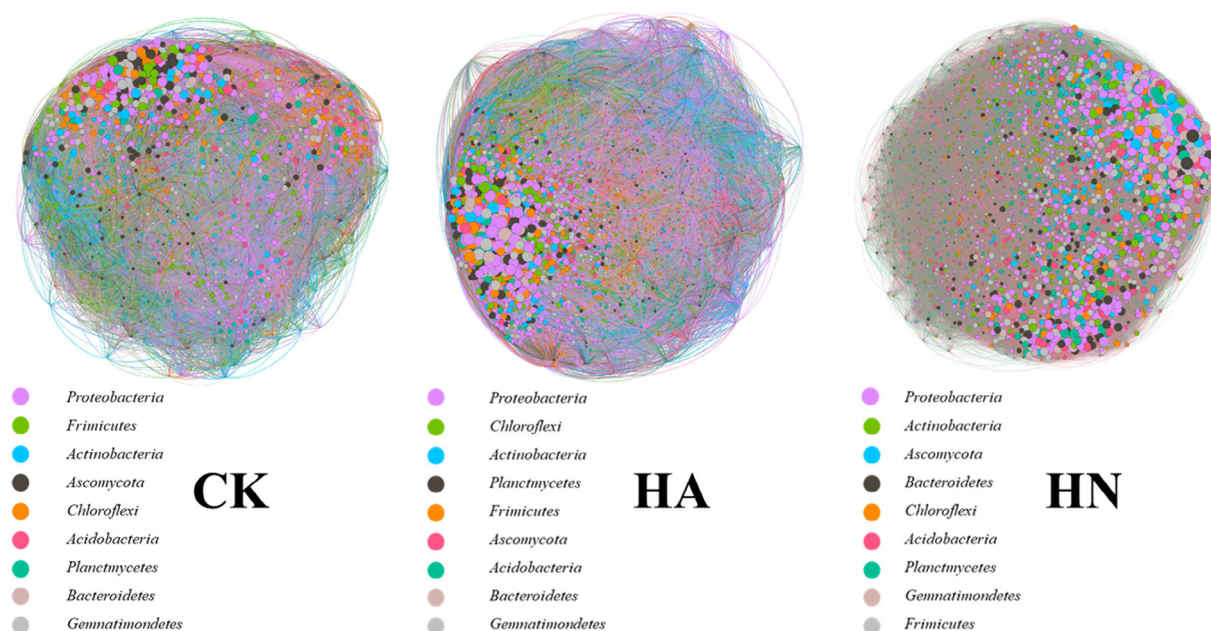
FIGURE 6   Co-occurrence networks of microbial (bacterial and fungal) communities in samples. A connection stands for a significant ($p < 0.01$) correlation between two OTU. The size of each node represents the number of connections (i.e., degree), and the thickness of each connection between two nodes (i.e., edge) represents the value of the spearman correlation coefficients. A blue edge indicates a positive relationship, while a red edge indicates a negative relationship [Colour figure can be viewed at wileyonlinelibrary.com]

potential phytopathogenic fungi categorized by FUNGuild in cucumber rhizosphere soils decreased with the application of HA (Figure 1). Similar results were also found in the diseased soil of cucumber and banana in response to the application of humic substances (Afifi et al., 2017; Shen et al., 2014). However, although both HA and HN could result in soil suppressiveness, variations in the relative abundance of potentially phytopathogenic fungi in cucumber rhizosphere soil between the two treatments were still observed. Compared with the CK, the relative abundance of potentially phytopathogenic fungi categorized by FUNGuild significantly ($p < 0.05$) decreased with the HA application rather than the HN application (Figure 1). Similar results could also be found in previous studies in which different humic substances extracted from different materials imposed different effects on the suppression of fungal pathogens (Wei et al., 2018; Wu et al., 2016). Wei et al. (2018) attributed this to the higher sulphur (S) and active composition (carbonyl C, for example) contents in HA compared to HN.

Moreover, it is often expected that soil microbial communities are of great significance to the health of soil and plants (Dungan et al., 2003; Reeve et al., 2010). In our study, it was shown that humic substances caused high turnover in both bacterial and fungal compositions, signifying that humic acid (from weathered coal) and sodium humate (from peat) could regulate the variation in microbial composition (Figures 2 and 5; Table S4). Previous studies have also well documented that the relative abundances of *Bacteroidetes* and *Firmicutes* in soil are greatly influenced by organic amendments (Li et al., 2020; Li, Fang, et al., 2019; Li, Liu, et al., 2019; Ren et al., 2018). We also obtained similar results on bacterial compositions under the influence of HA and HN (Figure 2a). In addition,

studies have also suggested that organic amendments can enhance the abundances of fungi that involved in soil carbon cycling and sequestration, *Zygomycota* and *Chytridiomycota* for instance (Fan & Wu, 2021; Zhao et al., 2018). HN had a higher C content, and the relative abundances of *Zygomycota* and *Chytridiomycota* were therefore significantly increased with HN application (Figure 2b). In addition to the above two phyla, there was no significant differences in the relative abundance of the other fungal phyla between the three treatments (Figure 2b).

Third, at the genus level, it was observed that higher abundances of both *Penicillium* and *Nocardioides* were significantly more enriched in the HA treatment (Figure 3). It has been proposed that *Penicillium* and *Nocardioides* could directly or produce potentially resistant substances (rifamycin for instance) to suppress pathogenic fungi (El-Refai et al., 2011; Hemtasin et al., 2016). Therefore, we speculate that the increase in the abundance of these species displays an inhibiting effect following the application of HA.

Fourth, humic substances can facilitate the improvement of microbial activity and influence the diversity and structure of microbial communities (Laura et al., 2014; Luo et al., 2019). Usually, there is a positive correlation between soil microbial diversity and pathogen suppression (Jaiswal et al., 2017). In our study, a significant ($p < 0.05$) and negative correlation was observed between bacterial alpha diversity indexes (chao1 and observed_OTU included) and the suppression of potentially pathogenic fungi (Figure S2). Similar findings can also be found in suppressive soil systems induced by bioorganic fertilizer or organic amendments identified previously (van Elsasa et al., 2012). Thus, it was deduced that humic substances suppressed fungal pathogens in promoting soil bacterial diversity.

TABLE 1 Topological features of microbial (including bacteria and fungi) subnetworks

Treatment	Degree	Diameter	Density	Modularity	Clustering coefficient	Average path length	Average degree of potentially pathogenic fungus	Positive correlations	P/N
CK	76 c	4.0 a	0.1 b	1.2 b	0.5 b	2.3 a	133.5 c	633 b	2.1 b
HA	261 a	4.0 a	0.2 a	0.5 c	0.6 a	2.1 b	184.7 a	1152 a	5.2 a
HN	121 b	3.3 b	0.1 c	1.8 a	0.4 c	2.3 a	149.7 b	742 b	1.2 c

Note: different letters in a same column indicate significant differences at the 0.05 probability level according to the Duncan test ($n = 8$).
Abbreviations: CK, control; HA, humic acid from weathered coal; HN, sodium humate from peat.

Furthermore, it is also expected that the complexity of soil microbial networks is of great significance to the health of soil and plants (Wei et al., 2015). In our study, it was shown that humic substances (HA and HN included) induced higher complexity (with significantly ($P < 0.05$) higher degrees of microbial co-occurrence networks) in microbial networks (Table 1; Xu et al., 2018). The network degrees were negatively related to the relative abundance of potential phytopathogenic fungi, indicating that humic substances could induce higher microbial complexity to potentially suppress pathogenic fungi (Figure S2).

In summary, the results obtained from this study confirm that the application of humic substances represents an effective strategy to control multiple phytopathogenic fungi in the rhizosphere soil of cucumber. Humic substances suppressed fungal pathogens through changes in bacterial and fungal communities as well as the topological characteristics of co-occurrence networks. Specifically, the beneficial microbiome is induced by humic substances, which stimulates suppression in an indirect manner. It is rather evident that the mechanism of multipathogen suppression remains a subject for study to further elucidate its role in disease outcome and allow for the design of better agricultural management, and it can also be used in sustainable strategies for plant health.

5 | CONCLUSIONS

HA and HN were capable of increasing the soil bacterial and fungal alpha-diversities and degrees of co-occurrence networks in comparison with the unamended control. By affecting the relative abundance of potential pathogenic fungi, the fungal community structures, and the network degree and the average degree of potential pathogenic fungi, humic substances significantly increased the aboveground biomass of cucumbers. In addition, there was a negative correlation between aboveground biomass and the relative abundance of the potential pathogenic fungi. HA increased the relative abundance of *Penicillium* and *Nocardioides* genera and the microbial network complexity of the rhizosphere soil and subsequently decreased the relative abundance of phytopathogenic fungi, which consequently caused an increase in the aboveground biomass of cucumbers. Overall, this study highlights the significance of humic substances from weathered coal and peat in regulating microbial community compositions and network topological patterns in the suppression of phytopathogenic fungi and promotion of cucumber growth.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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