Plant Soil (2019) 438:421–433
https://doi.org/10.1007/s11104-019-04037-6

REGULAR ARTICLE

Characterizing differences in microbial community composition and function between *Fusarium* wilt diseased and healthy soils under watermelon cultivation

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Received: 12 September 2018 / Accepted: 13 March 2019 / Published online: 23 March 2019

Abstract
Aims Continuous cropping of watermelon is known to result in the disruption of the rhizospheric bacteria and fungi that contribute to the occurrence of Fusarium wilt disease. However, the underlying changes in microbial composition and function as a response to monocropping are less studied.
Methods In this study, differences in composition and potential function of the microbiome between healthy and diseased soils were investigated using MiSeq targeted sequencing and the functional GeoChip array, respectively.
Results Twenty years of continuous watermelon monoculture was found to significantly alter the soil microbial communities by increasing bacterial diversity but decreasing fungal diversity. Compare to bacterial network, fungal co-occurrence networks were less robust and less connected in the monoculture diseased soil. Identified keystone species, belonging to the Proteobacteria, Bacteroidetesand Acidobacteria, were present in both the diseased and healthy soils. Key fungal species from the healthy soil belonged solely within the Ascomycete, while in the diseased soil Basidiomycota were dominant. As such, overall variations in the composition of the soil microbiome are accompanied by changes in the identities of the keystone species when comparing healthy versus diseased soils, further suggesting that soil function may also be altered. Relative abundances of genes associated with the degradation of hemicelluloses and chitin, the Calvin circle, ammonification, stress responses, iron uptake, and nitrogen fixation were significantly higher under long-term monoculture. Particularly, Fusarium spp. relative abundance was positively correlated with the relative abundances of genes involved in adherence, cellular metabolism, and immune evasion which may facilitate pathogen infection of plant roots.
Conclusions In conclusion, these results highlight the significant compositional and functional differences in microbial communities between Fusarium wilt diseased soils and healthy soils under watermelon cultivation. This provides insight into the complex array of microorganisms in soils that suffer from Fusarium disease and illustrates potential directions towards the manipulation of the soil microbiome for suppression of this disease.

Keywords Fusarium wilt of watermelon · Microbial composition · Microbial function · Continuous monocropping · GeoChip

Responsible Editor: Birgit Mitter.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-019-04037-6) contains supplementary material, which is available to authorized users.

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Introduction

Watermelon is a globally cultivated cash crop though continuous mono-cropping can lead to severe infections by *Fusarium* wilt caused by the fungal pathogen *F. oxysporum* f. sp. *niveum*. This pathogen has resulted in the disruption of watermelon production in many areas of the world (An et al. 2011). In general, continuous mono-cropping can lead to alterations in soil properties. Horst and Härdter (1994) found that nutrient accumulation was much lower in mono-cropping system. Clermont-Dauphin et al. (2010) found that tillage decreased soil organic matter as well as microbial respiration in banana mono-cropping. The variation in soil properties can induce changes in microbial composition and declines in the abundances of soil microbes (Utkhede 2006). *Fusarium* wilt disease incidence rises when this disruption influence the rhizosphere environment. As such, understanding the response dynamics of the soil microbial community to continuous watermelon cropping is paramount in the control of this disease.

Recently, studies have hypothesized that continuous cropping resulted in changes to the soil microbial community composition (Li et al. 2014; Lu et al. 2013a). For example, the bacterial and fungal community structures altered significantly after banana long-term monoculture (Shen et al. 2018). The assessment of the status of soil microbial community was critical to the identification of the impact of management practices on microbial populations (Mazzola 2004). Therefore, there is a continuing interest in assessing the composition and function of the fungi and bacteria associated with healthy and diseased soils in order to parse the characteristics of the microbiome that may be manipulated for disease suppression (Agnelli et al. 2004; Salles et al. 2004).

Soil microbes are of a fundamental significance in nutrient cycling and energy flow (Kennedy and Smith 1995; Yao et al. 2000). Changes in microbial community composition are usually accompanied by alterations in microbial functional properties, such as biogeochemical cycling, such as C, N and S cycling, phosphorus utilization (Yao et al. 2006; He et al. 2007). Key soil ecological functions such as organic matter decomposition, nutrient mineralization, virulence, stress resistance, and heavy metal resistance are mediated by the soil microbiome and are of prime interest in the assessment of impacts due to long-term continuous mono-cropping. Previous studies have demonstrated that continuous cropping can influence specific soil functions, however, changes in the functional diversity of the soil microbial community due to mono-cropping remains unclear. For example, different watermelon cropping systems has been shown to lead to significant differences in the production of carboxylic acids, amino acids, and polymers as well as changes in amine utilization efficiencies (Zhang et al. 2015). However, detailed effects of continuous cropping on the soil microbiome composition and linkages between composition and variations in soil functions remain unclear.

In this study, the soil microbial community composition and function of a diseased soil after long-term continuous watermelon monoculture was compared to that of a healthy soil using targeted Miseq sequencing and functional Geochip microarrays. The overall aim was to unravel how the soil microbial community composition and function responds to long-term continuous monoculture in order to provide insight on potential bioremediation for the suppression of *Fusarium* wilt disease.

Materials and methods

Site description, management and sampling

Soil samples were collected from watermelon *Fusarium* wilt resistant cultivar breeding experiment fields (800 m^2) in Huaian, Jiangsu, China (N33°11′, E119°21′) in May 2014 (Fig. 1). Diseased soil were collected from a field that was continuously planted with watermelon for more than 20 years. This resulted in a *Fusarium*-infested soil that prevented survival of all watermelon varieties. Soil from the adjacent field (1.5 m far away from diseased soil), which has been randomly cultivated with *Oryza sativa*, pepper, soybean and tomato, free from *Fusarium* wilt disease, served as the control (healthy soil). Both soils which belonged to Inceptisols exhibited similar edaphic properties and fertilization regimes. The pH, organic carbon, and total N of the diseased soil were 6.64, 16.99 g kg^{-1} and 1.94 g kg^{-1}, respectively. While the pH, organic carbon, and total N of the healthy soil were 6.27, 13.43 g kg^{-1} and 1.23 g kg^{-1}, respectively.

Both the healthy and diseased soils were collected from 9 replicated plots (80 m^2 for each). For each sample, 10 random soil cores (0–20 cm in depth) from each plot were collected and mixed to form one composite sample. The 18 soil samples were placed into separate sterile plastic bags and transported to the laboratory on ice. Each soil sample was sieved through a 2-mm sieve, thoroughly homogenized, and stored at −80 °C for subsequent DNA extraction.

Fig. 1 Photos showing the experiment field for the diseased and healthy soils

DNA extraction and sequencing

Total soil DNA was extracted from 0.25 g of freeze-dried soil using the PowerLyzer™ PowerSoil® DNA Isolation Kit (MOBIO Laboratories, Inc. Carlsbad, CA), according to the manufacturer's directions. The extracted DNA was quantified using a Nanodrop ND-2000 spectrophotometer (Nanodrop Technologies, Wilmington DE).

The barcoded primers 341F/805R (5′-CCTACGGGNGGCWGCA-3′/5′-GACTACHVGGGTATCTAATCC-3′) (Klindworth et al. 2013) and ITS3/ITS4R (5'-GCATCGATGAAGAACGCAGC-3′/5′-TCCTCCGCTTATTGATATGC-3′) (Mello et al. 2011) were used to amplify the V3-V4 region of the 16S rRNA gene and the fungal ITS2 region, respectively. A 20 μl mixture was prepared for each reaction and included 1x reaction buffer (TAKARA), 2 mM Mg2+, 0.2 mM dNTP, 0.1 μM of each primer, 1 U HotStarTaq polymerase (takara) and 2 μl template DNA. The cycling program was 95 °C for 2 min; 35 cycles of 94 °C for 20 s, 55 °C for 40 s, 72 °C for 1mins; 72 °C for 2 min. Amplicon libraries were pooled and sequenced using the 2 × 250 base paired Illumina MiSeq platform.

Geochip

GeoChip 5.0 was employed to profile potential functions of the soil microbiome. GeoChip 5.0 contains 167,044 distinct probes, covering 395,894 coding sequences (CDS) from ~1500 functional gene families involved in microbial carbon (degradation, fixation, methane), nitrogen, sulfur, and phosphorus cycling, energy metabolism, metal homeostasis, organic remediation, "Other" (phylogenetic genes and CRISPR system), secondary metabolism (e.g. antibiotic metabolism, pigments), stress responses, viruses (both bacteriophages and eukaryotic viruses), and virulence (Zhou et al. 2008). DNA extracts were sent to the Institute for Environmental Genomics at the University of Oklahoma for GeoChip analysis.

Sequence analysis

Raw reads were processed using the open source bioinformatic software package UPARSE following the MiSeq SOP protocol (Schloss et al. 2009). Briefly, paired-end raw sequences were assembled and short (<300 bp) and low quality (mismatched with two bases and the total base error rate > 1) reads were removed. Chimera reads were removed using Uchime (Edgar et al. 2011). Primers were removed before clustering. Both bacterial and fungal sequences were clustered at 97% similarity. Singletons, one sequence present in only one sample were removed and all samples were rarefied to the same number of reads as the sample with the lowest reads (31,000 16S rRNA and 37,000 ITS reads

per sample). Each representative sequence was assigned with a RDP classifier for taxonomical identification with a threshold of 0.8 for bacteria and 0.7 for fungi. All sequences were deposited in the NCBI Sequence Read Archive (SRA) database (Accession number: SRP151181 for bacteria and SRP151184 for fungi).

Data analysis and statistics

Shannon diversity, Chao1 richness and rarefaction curves were calculated and ordinations of community patterns of both bacteria and fungi illustrated using PCA (Principal Component Analysis). Co-occurrence networks were constructed in order to explicate interactions within the microbiome by calculating all possible Pearson rank correlations between bacterial or fungal genera using the script from Mothur v.1.30.2. Correlation data was filtered with a cut-off at an absolute r value of 0.6–0.93 (Steinhauser et al. 2007). After applying Benjamini-Hochberg's (Benjamini and Hochberg 1995) false discovery rate correction, edges with merged *P*-values below 0.05 were retained in order to improve network precision. Network OTUs were grouped at the genus level and only those genera with more than five sequences were considered in the following analyses. To describe the topology of the resulting networks, a set of parameters (average clustering coefficient, average path length, and modularity) were calculated (Newman 2006). The network structure was explored and visualized with the interactive platform gephi (Bastian et al. 2009) using the Fruchterman–Reingold layout.

To compare the differences in the potential function between healthy and diseased soils, we conducted an analysis in which we assessed separately the read counts based on functional gene-category, sub-category 1 and sub-category 2 levels. Volcano plots were utilized to graphically represent the results of the moderated t-tests using the R package ggplot2 (v.2.0.0). To graphically represent the results obtained at gene-category and sub-category level, a script was utilized (Bulgarelli et al. 2015), in which the relative abundances of read counts per million was used, as well as box plot representations using the R package ggplot2. Taxa above 5‰ relative abundance were plotted for sub-category 1 level analyses. Treemap (v.3.7.3) was used to visualize the significantly abundant functions, the adjusted *P* value, and per mil relative abundance in bubble graphs, in which the size of the bubbles indicates the relative abundance per mil of the raw read counts. Correlation between key species and function were calculated using mothur (version 1.29.2), and the visualization of these correlations was implemented by gephi. Key genera were selected by the relative abundance at or above 0.005 and the functions were aggregated at the sub-category 1 level. This workflow was implemented for both the bacterial and fungal data.

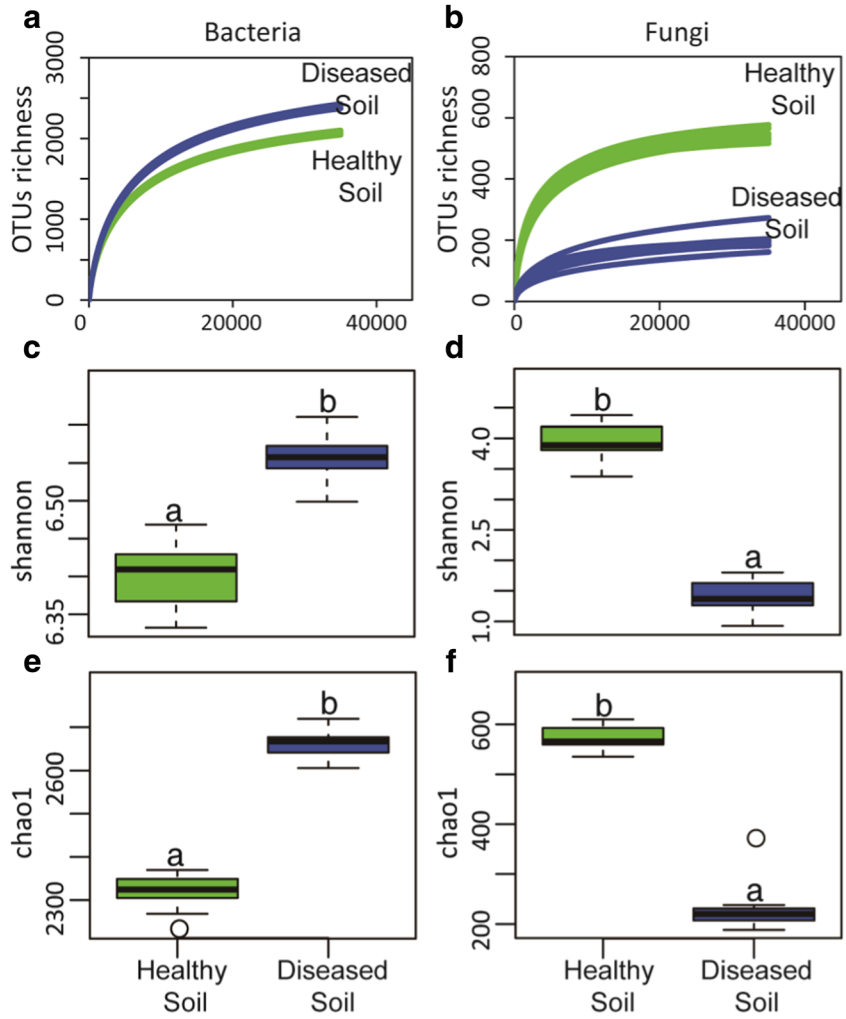
Results

Diversity of bacterial and fungal communities in healthy and diseased soil

The bacterial α-diversity (Shannon index, Chao 1 index) of the healthy soil was significantly lower than that of the diseased soil (Fig. 2C, E) while the α-diversity of fungal community exhibited an opposite relationship (Fig. 2D, F). Principal component analysis showed a clear separation between healthy soil and diseased soil bacterial and fungal communities (ANOSIM for bacteria, $P = 0.001$; for fungi, $P = 0.001$) (Fig. 3A, B). The principal component axes explained a total of 89% and 95% of the variation in the bacterial and fungal communities, respectively. The relative abundance of bacterial phylum showed that Bacteroidetes and Actinobacteria were much higher in diseased soil (Fig. S1A). And the relative abundance of Ascomycota increased obviously in diseased soil (Fig. S1B).

Differential co-occurrence networks of bacteria and fungi between healthy and diseased soil

Biodiversity encompasses not just the presence of species but also interactions among species. The healthy and diseased soils exhibited markedly different bacterial and fungal co-occurrence patterns. The bacterial networks in the healthy and diseased soil had a similar number of nodes meanwhile the number of links between nodes in the diseased soil was close to the number of links in the healthy soil. The topology of healthy bacterial networks, for instance, clustering coefficient and average path length were very similar to the topology of diseased bacterial networks. For fungi, the number of nodes and links were lower in the diseased than the healthy soil (Fig. 4, Table S1). We defined the nodes in the top ten degree (the number of links belonging to specific OTU) as keystone species potentially active in the mediation of microbial community interactions.



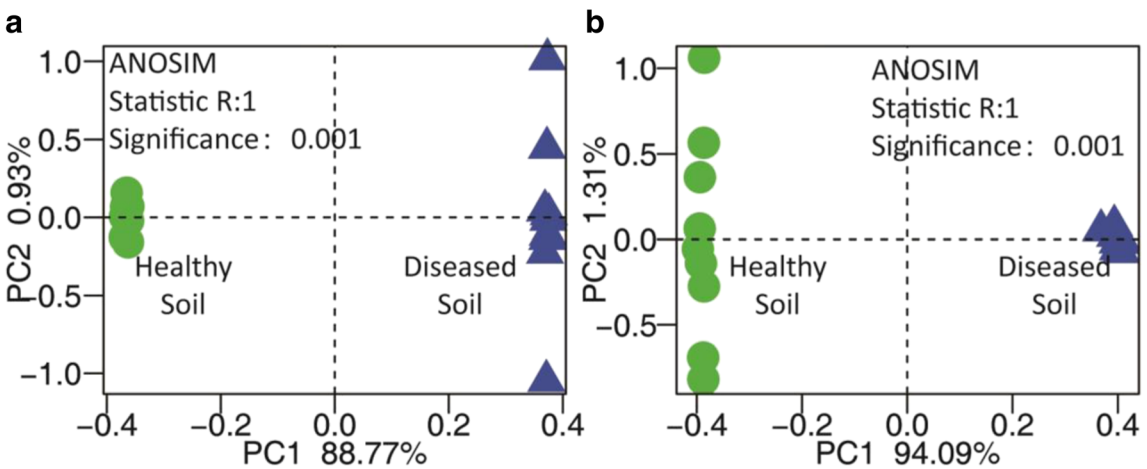


Fig. 2 The rarefaction curves (**a** bacteria, **b** fungi) and Shannon index (**c** bacteria, **d** fungi) and Chao1 (E bacteria, F fungi) alpha-diversity of the healthy and diseased soils. For each panel, the results of a Tukey HSD post hoc comparison for the overall treatment effect is also presented. Treatments with different letters indicate significant differences at $P < 0.05$

Fig. 3 Principal component analysis (PCA) of bacterial (**a**) and fungal (**b**) community beta-diversity based on Bray-Curtis dissimilarity between all samples of the healthy and diseased soils

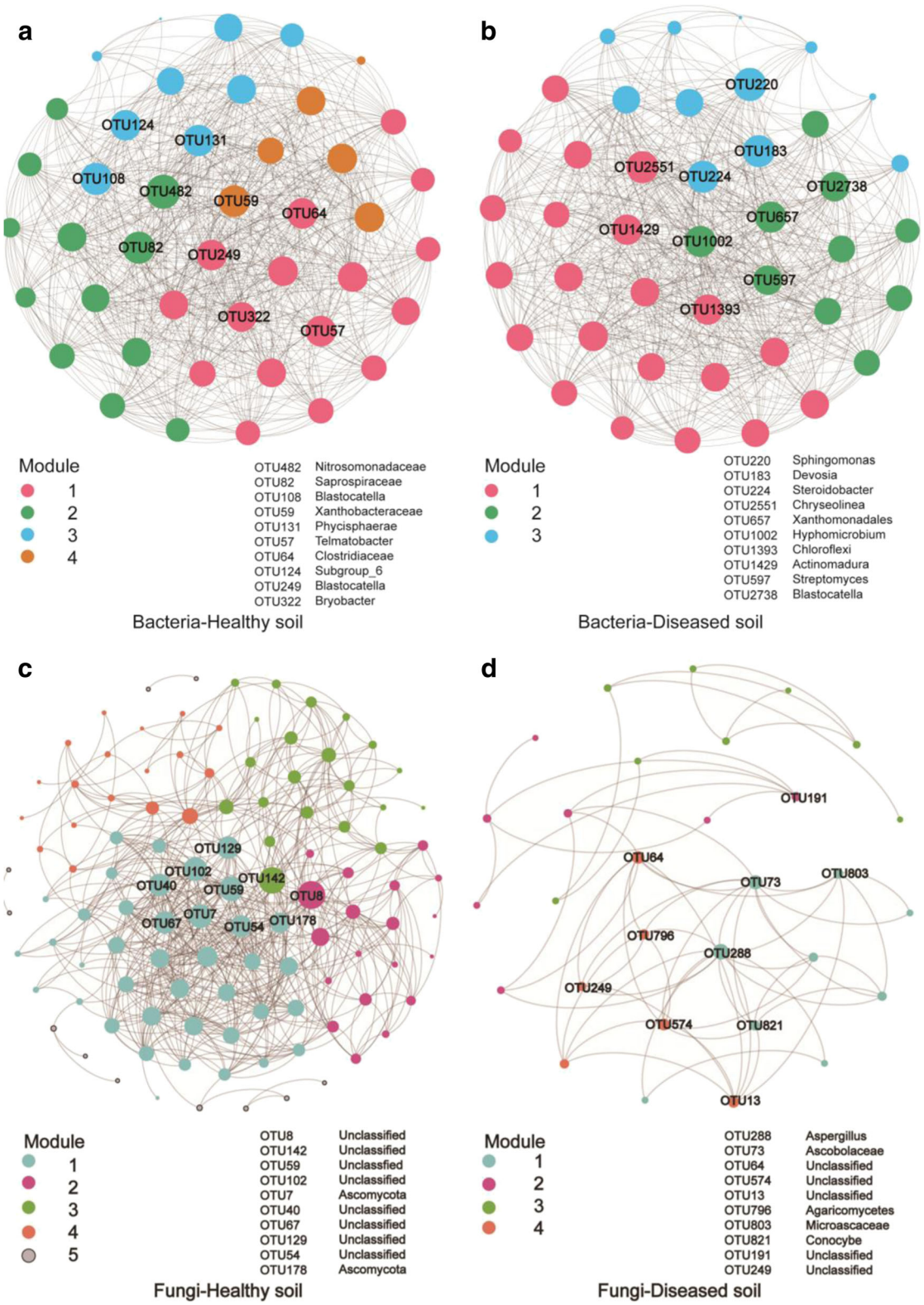


Fig. 4 Co-occurrence patterns of the bacterial and fungal community in the healthy and diseased soils. The top ten abundant key species (OTUs) in each network were labeled in black

Keystone species belonging to Proteobacteria, Bacteroidetes and Acidobacteria were commonly present in both the diseased and healthy soils although there were subtle differences in the order or family levels. For fungi, the healthy soil community was dominated by key species belonging to Ascomycete, while those of the diseased soil were affiliated with Basidiomycota, such as OTU796 (Basidiomycota|Agaricomycetes) and OTU821 (Basidiomycota|Agaricomycetes|*Conocybe*).

Variation of functions between healthy and diseased soils

In addition to the alterations in microbiome composition, significant variations were detected with respect to potential microbial functions. Functional composition was distinct between the healthy and diseased soils (Fig. 5A, B). The relative abundances of carbon cycling genes involved in hemicellulose and chitin degradation and the Calvin cycle in the diseased soil were significantly higher than those of the healthy soil. In contrast, the relative abundances of starch degradation and glyoxylate cycle genes were more abundant at the healthy soil, with the exception of pathways for cellulose degradation, which did not differ between soils. Fewer genes associated with organic remediation were detected in the healthy soil and abundances of genes involved in the degradation of aromatics and herbicide-related compounds were much lower in the healthy soil as well.

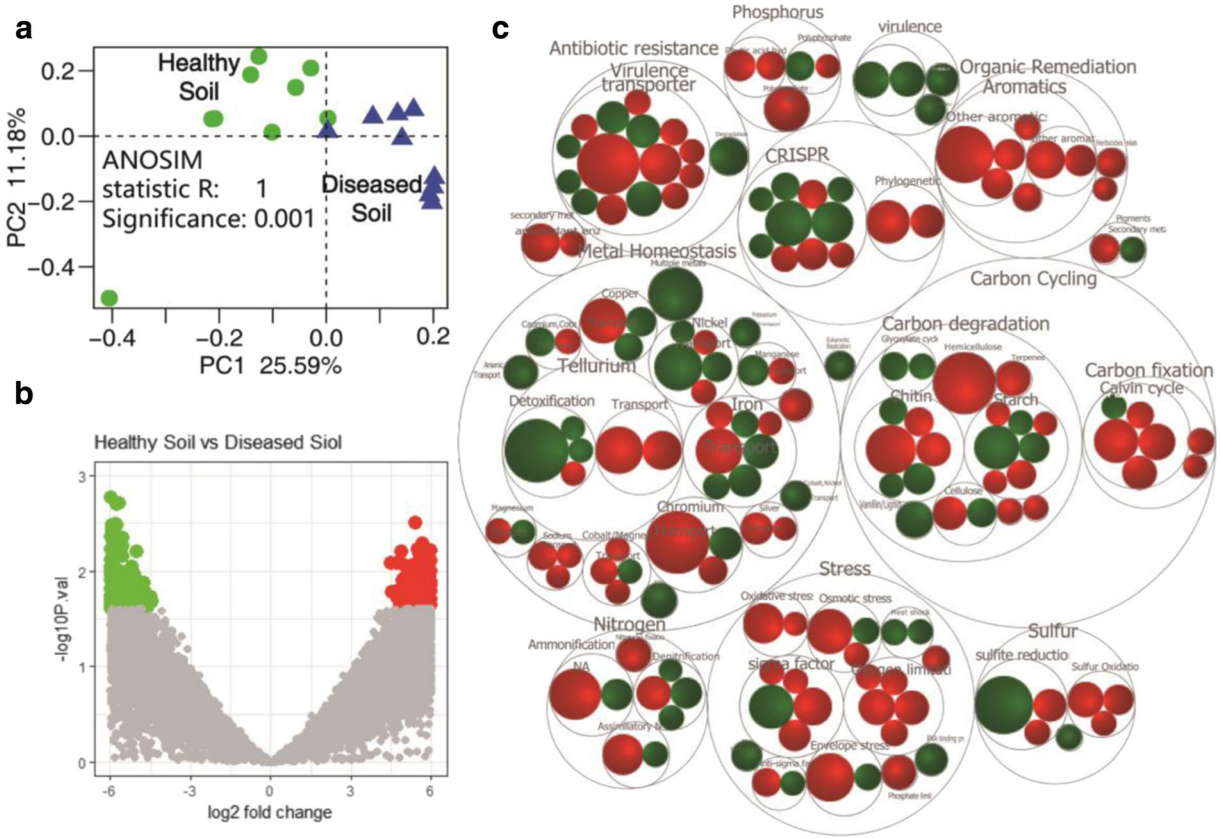


Fig. 5 Differential abundance of functions between the healthy and diseased soils. Principal component analysis of function beta-diversity based on Bray-Curtis dissimilarity between all samples of the healthy and diseased soils (**a**). Differences in the abundance of taxa between treatments were considered significant when adjusted *P*-values were lower than 0.05 at gene level. Volcano plots were built to graphically represent the results of the moderated t-tests (**b**). Differential abundance of functions was made using a zero-inflated Gaussian distribution mixture model followed by moderated t-test and a Bayesian approach (**c**). Only functions significantly enriched in one of the two soils are shown (FDR < 0.05). The largest circles represent gene category level. The inner circles represent sub-category1 and sub-category2 level. The color of the circles represents the functions enriched in the healthy soil (green) or diseased soil (red). The size of the circle is the mean relative abundance of the differentially abundant function

With respect to nitrogen cycling, the relative abundances of ammonification and nitrogen fixation genes were significantly higher in the diseased soil while the relative abundances of denitrification genes were lower in the diseased soil though not significant. For the stress related genes, relative abundance of the oxidative stress, osmotic stress, oxygen limitation, and envelope stress were significantly higher in diseased soil while genes associated with heat shock and nitrogen limitation were more abundant in the healthy soil. For metal homeostasis, ion (cadmium, zinc, copper) transporters were more prevalent in diseased soil while nickel and tellurium detoxication were more abundant in the healthy soil.

Linking microbial community composition with functions

We determined if the composition of the bacterial or fungal community was correlated with the GeoChip-derived potential function of the community. When links between community and function were taken into consideration, OTUs with relative abundances greater than 0.001 and sub-category1 functions were subjected to Pearson correlations and subsequently visualized as networks (Fig. 6A, B).

For bacteria, OTU5 (Acidobacteria|Acidobacteriaceae), OTU38 (Acidobacteria|RB41), OTU218 (Acidobacteria|*Blastocatella*), and OTU357 (Acidobacteria|RB41) were significantly positive correlated with nitrogen and sulfur cycling such as nitrification, sulfide oxidation, sulfite reduction, and DMSP degradation (Table S2). These OTUs were also significantly positive correlated with genes associated with metal homeostasis, such as potassium, and virulence, such as immune invasion, cellular survival, and adherence. OTU183 (Proteobacteria|*Devosia*), OTU224 (Proteobacteria|*Steroidobacter*), OTU351 (Proteobacteria|Xanthobacteraceae), OTU1018 (Gemmatimonadetes|Gemmatimonadaceae), and OTU2530 (Proteobacteria|Rhodospirillaceae) were significantly negative correlated with the genes associated with nitrification, sulfide oxidation, DMSP degradation, glucose limitation, immune evasion, cell adherence, and the multiple metals homeostasis. OTU183 (Proteobacteria|*Devosia*) and OTU224 (Proteobacteria|*Steroidobacter*) were significantly negative correlated with the genes involved in the Calvin cycle and denitrification.

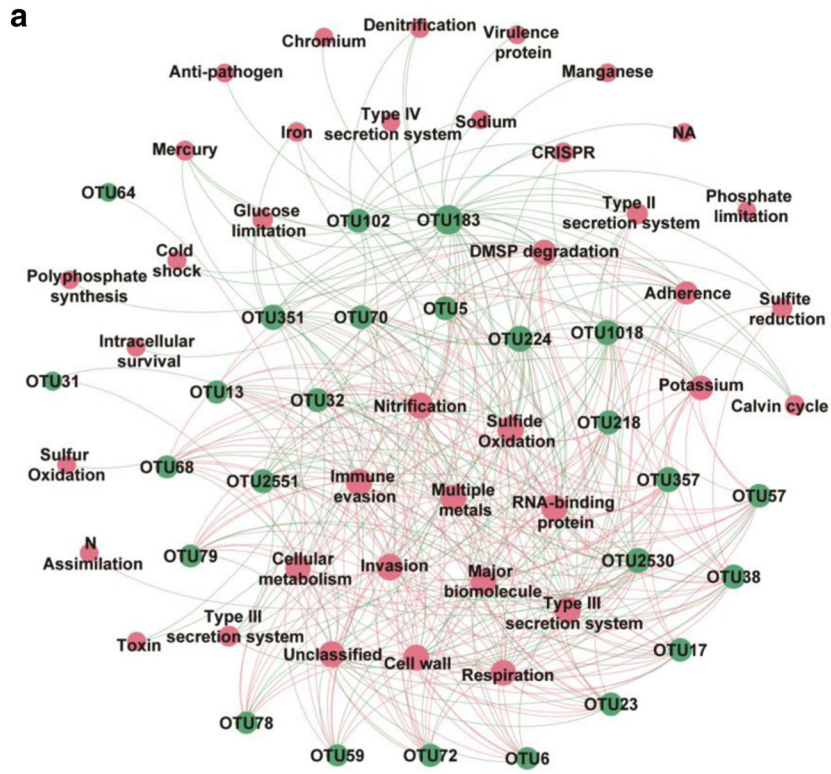
For fungi, OTU29 (Ascomycota|Chaetomiaceae), OTU358 (Ascomycota|Chaetomium), and OTU584 (Ascomycota|Sordariales) were significantly negative correlated with genes associated with multiple metals homeostasis, potassium homeostasis, nitrification, sulfide oxidation, and immune invasion (Table S3). Significant positive correlations were observed between these OTUs and carbon cycling genes, such as chitin synthesis. OTU1 (Ascomycota|*Fusarium*), OTU23 (Unclassified), OTU37 (Ascomycota|Pleosporaceae), OTU38 (Ascomycota|Chaetomiaceae), and OTU41 (Ascomycota|Nectriaceae) were significant positive correlated with genes associated with adherence (colonization), cellular metabolism, immune evasion, glucose limitation, iron homeostasis, DMSP degradation, nitrification, iron and sodium homeostasis, and sulfide oxidation and reduction. These OTUs, except for OTU1, were significantly positive correlated with genes associated with sulfite reduction. Lastly, OTU23 was also significantly positive correlated with multiple metals homeostasis, denitrification and drought tolerance.

Discussion

Soil microbial community composition change due to long-term continuous monoculture of watermelon

After long-term continuous monoculture, microbial community composition shifted as its surrounding soil environment was altered by management (Zeglin et al. 2013). Sequencing outcomes demonstrated that long-term continuous monoculture of watermelon increased bacterial diversity while decreasing fungal diversity. The results were consistent with the previous study that more diverse bacterial diversity communities were existed in potato suppressive soil (Rosenzweig et al. 2012), in keeping with that a decrease fungal diversity of silva mono-cropping (Tang et al. 2014). The increased bacterial diversity in soils disturbed by intensively continuous mono-cropping may be linked with high nutrient input caused by intensive tillage and fertilizer use that may have favored bacterial taxa.

Fig. 6 The pearson correlation between microbial key species and functions. The green color nodes represent the relative abundance exceed 0.1%. The red nodes are functions at the sub-catogory1 level correlated with the abundant microbes that presented as the green nodes. Green lines indicate positive correlations, and red lines indicate negative correlations


a
Denitrification
Chromium
Virulence protein
Anti-pathogen
Manganese
Type IV secretion system
Iron
Sodium
NA
Mercury
CRISPR
OTU64
Glucose limitation
OTU102
OTU183
Type II secretion system
Phosphate limitation
Cold shock
DMSP degradation
Polyphosphate synthesis
Adherence
Sulfite reduction
OTU351
OTU70
OTU5
OTU224
OTU1018
Intracellular survival
OTU31
OTU13
OTU32
Nitrification
Potassium
Calvin cycle
Sulfide Oxidation
OTU218
Sulfur Oxidation
OTU68
OTU2551
Immune evasion
Multiple metals
OTU357
OTU57
RNA-binding protein
N Assimilation
OTU79
Cellular metabolism
Invasion
Major biomolecule
OTU2530
OTU38
Type III secretion system
Toxin
Unclassified
Cell wall
Respiration
OTU17
OTU23
OTU78
OTU59
OTU72
OTU6
OTU5 Acidobacteriaceae
OTU6 Sphingomonas
OTU13 Sphingomonas
OTU17 Nitrospira
OTU23 Candidatus-Solibacter
OTU31 Incertae-Sedis
OTU32 Acidobacteriales
OTU38 RB41
OTU57 Telmatobacter
OTU59 Xanthobacteraceae
OTU64 Clostridiaceae
OTU68 SC-I-84
OTU70 Bryobacter
OTU72 Rhizobiales
OTU78 Xanthomonadales
OTU79 Candidatus_Solibacter
OTU102 Rhodospirillaceae
OTU183 Devosia
OTU218 Blastocatella
OTU224 Steroidobacter
OTU351 Xanthobacteraceae
OTU357 RB41
OTU1018 Gemmatimonadaceae
OTU2530 Lutispora
OTU2551 ABS-19
Bacteria-Function
b
Silver
Type VII secretion system
NA
Oxidative stress
Carbon degradation
Drought tolerance
Anti-sigma factor (anti-E)
N Assimilation
Nitrification
Cell wall
Manganese
Other Hydrocarbons
Sulfur Oxidation
OTU4
Potassium
Sodium
Adherence
Effector protein
Major biomolecule
Type III secretion system
Chromium
OTU3
Type III secretion system
Multiple metals
OTU1
Dissimilatory N reduction
Copper
OTU41
Adenylylsulfate reductase
OTU31
Glucose limitation
OTU29
OTU358
Respiration
OTU22
Type IV secretion system
Cadmium
OTU49
OTU23
CRISPR
Cellular metabolism
OTU37
Mercury
Invasion
OTU38
Calvin cycle
Phylogenetic
Unclassified
Immune evasion
OTU15
Toxin
Calcium
OTU584
DMSP degradation
Virulence protein
Iron
RNA-binding protein
Sulfide Oxidation
Anti-pathogen
Drug resistance
Polyphosphate synthesis
Type II secretion system
Sulfite reduction
Intracellular survival
Phosphate limitation
Zinc
Transport
OTU574
Prokaryotic
Nitrogen limitation
Aromatics
Aluminum
Cold shock
Denitrification
Effector
Others
Cobalt,Nickel
OTU1 Fusarium
OTU3 Pyronemataceae_sp
OTU4 Unknown
OTU15 Unknown
OTU22 Unknown
OTU23 Unclassified_Fungi
OTU29 Chaetomiaceae
OTU31 Chrysosporium
OTU37 Pleosporaceae
OTU38 Chaetomiaceae
OTU41 Nectriaceae
OTU358 Chaetomium
OTU584 Unclassified_Fungi
Fungi-Function

Hartman et al. (2018) found that intensive tillage soil supported higher bacterial richness and some cropping sensitive bacteria (such as Firmicutes) were responsive to tillage intensities so that they had higher abundances. Thus, selection may be towards those OTUs that are more resistant to environmental perturbation (Sessitsch et al. 2001). In contrast to bacteria, fungi are strongly reduced in abundance and richness following physical destruction of soil structure with hyphal networks requiring a longer time for re-establishment and recovery (Rousk and Bååth 2007; Sun et al. 2017). Network analysis showed that long-term continuous monoculture of watermelon resulted in extremely simplified fungal community (Fig. 3C, D). This was in line with a previous report in which the fungal network structure in a diseased soil induced by prolonged potato monoculture exhibited a poorly organized community compared to the healthy soil (Lu et al. 2013b). Indeed, variations in microbial community diversity and their interactions have implied that microbial composition and variation were significantly modified after a long-term continuous monoculture (Li et al. 2016).

Soil microbial function composition change due to long-term continuous monoculture of watermelon

Changes in the microbiome composition can influence the functional capacity of the microbial community. Recent analyses have indicated that microbial diversity positively contributes to microbial functional diversity (Balvanera et al. 2006; Cardinale et al. 2007). The variation of community composition can influence ecosystem functioning (Armitage 2017), highlighting the connection between variations in the community and functional processes. Long-term continuous cropping can induce changes in soil primary physicochemical properties thereby influencing a generalized shift in functional potential. For carbon cycling, the long-term continuous monoculture of watermelon increased the abundances of recalcitrant C degradation genes, which is supported by a previous study in which the increase of bacterial diversity had a positive effect on the rate of recalcitrant carbon decomposition (Loreau 2001). Furthermore, it has been found that an increase in the availability of specific types of dissolved organic carbon alters the activity of the microbial community in ways that increase the emergence and abundance of pathogens (Cardenas et al. 2018). These rhizosphere microbial communities subjected to pathogen proliferation shift away from utilizing labile carbon substrates towards more recalcitrant forms. This could be due to the restricted availability of readily available carbon in the soil due to the disruption of root exudation (Trivedi et al. 2012).

Anthropogenic N inputs are known to alter the interactions between plants, soil organisms, and ecosystem function (Chung et al. 2007; Eisenhauer et al. 2012). In the diseased soil, nitrogen fixation and ammonification gene abundances significantly increased while denitrification genes were relatively lower. This alteration in potential denitrification is similar to the results obtained for huanglongbing (citrus disease) diseased soils, whereas variations in abundances for the nitrogen fixation and ammonification genes were not consistent with previous results (Trivedi et al. 2012). It has been demonstrated that the *Fusarium* strain could take advantage of ammnioum and nitrate simultaneously while antagonistic fungi used these two sources of nitrogen sequentially (Celar 2003) so that *Fusarium* had the relative advantage of nutrition uptake. These differential responses are perhaps attributed to the different pathogen and plant type, which results in varying responses to the disease.

Long-term monoculture increased the relative abundance of genes involved in stress responses, such as oxidative stress, osmotic stress, oxygen limitation and envelope stress, thus reflecting a critical microbial strategy of stress adaptation during long-term monoculture. This elevated potential stress response has been previously shown to also be higher in disease-associated compared to healthy soils (Zhang et al. 2017). Ion transporter acquisition genes were more prevalent in the diseased soil while genes related to ion detoxication were more abundant in the healthy soil. Some pathogens are known to produce carrier protein that sequestered ions (such as iron) from the host to support their own growth (Amin et al. 2009). In particular, the herbicide and aromatics degradation gene was more exposed to the diseased associated soil. Sorensen et al. (2002) found that *Sphingomonas* sp. could degrade phenylurea herbicides. *Sphingomonas* sp. also was a keystone species in diseased bacterial co-occurrence networks so that it contributed to the herbicide degradation. The fungi able to partly degrade herbicides cover *Aspergillus* which was a keystone species in diseased fungal co-occurrence networks (Berger 1999).

Above all, the carbon cycling of diseased soil has shifted from available carbon to recalcitrant forms so that did favor for the growth of pathogenic fungi. In addition, the nitrogen cycling of diseased soil tended to fix nitrogen and transformed to ammonium forms to

support the *Fusaium.* Continuous cropping induces alterations in nutrient acquisition that potentially promote pathogen proliferation and subsequent plant disease.

Correlations between microbial key species and functions

It is important to link microbial community composition with the microbial functional genes involved in major biogeochemical processes (Trivedi et al. 2013). In our analysis, we found that microbial richness may influence functional turnover though species identity may be as important as species richness in determining microbial ecosystem function (Covich et al. 2004). Previous studies have identified that Betaproteobacteria and Gammaproteobacteria were negatively correlated with the function of nitrification (Kimes et al. 2010). The *Devosia* and *Steroidobacter* were significantly negative correlated with the genes associated with immune invasion (Fig. 6A). Proteobacteria have been identified as the dominant bacterial genera in the rhizosphere of various plant species due, in part, to their relatively rapid growth rates (DeAngelis et al. 2009; Fierer et al. 2007). In addition, Zhao et al. (2018) found that *Pseudomonas* in Proteobacteria harbored more non-ribosomal peptides genes to supress the fusarium wilt disease. Previous study have clarified that continous tomato crroping soil treated with Jerusalem artichoke which used to reducing the occurrence of soil-borne diseases significantly increased the abundance of Proteobacteria (Shiwen et al. 2018). In turn it demonstrated that *Fusaium* wilt supressed the portion of Proteobcteria. Karpouzas et al. (2011) found that *Fusarium* strain had a negtive impact on the abundance of alpha-Proteobacteria and *Pseudomonads*. Long-term watermelon monoculture, served to suppress the abundance of some putatively beneficial microbes, such as a part of sensitive Proteobacteria. Consequently, their role within the rhizosphere as pathogen antagonists was weakened (Wei et al. 2018).

The fungi *Chaetomium* was negatively correlated with genes associated with immune invasion and it has been demonstrated that *Chaetomium* genera were associated with reduced disease and reduced *F. oxysporum* abundance (Siegel-Hertz et al. 2018). The fungi *Fusarium,* Pleosporaceae and Nectriaceae were positively correlated with adherence, cellular metabolism, immune evasion, and glucose limitation (Fig.6B). This suggests that saprotrophic ascomycete fungi are the primary degraders of plant cell wall polymers, which is supported by previous studies (Frankland 1998). Functional genes involved in adherence, invasion, and colonization have been shown to be significantly enhanced in diseased soils. These virulent genes appear necessary for pathogen infection and survival in plants (Zhang et al. 2017). The opportunistic (pathogenic fungi, for instance, *Fusarium* and Pleosporaceae) fungi express pathogenic factors allowing adherence to the host tissues or evasion to the host immune response. According to the relationship between the potential function and micro-organism, we could provide some clues to structure a beneficial microbial consortium combined with organic fertilizer so that it could reduce wilt disease (Zhao et al. 2014).

Conclusion

Long-term continuous monoculture of watermelon caused significant alterations in the soil bacterial and fungal community diversity, composition, and potential function. It did not lead to higher connected bacterial community whereas exhibiting a higher diversity, though the fungal community became less diverse with a less connected network. Genes associated with the degradation of hemicelluloses and chitin, the Calvin circle, ammonification, nitrogen fixation, iron uptake, and stress responses (including oxidative stress, oxygen limitation and envelope stress) significantly increased after long-term monoculture. This resulted in higher concentrations of dissolved organic carbon and nitrogen, which was positively correlated to pathogen proliferation. The relative abundance of *Fusarium* was positively correlated with genes associated with adherence, cellular metabolism, and immune evasion, which potentially promoted plant infection. These results provide insights into the complexity of changes in soil microbiome composition and function in soils suffering from *Fusarium* disease and provide some clues into potential methods of disease control through microbial community manipulation.

Acknowledgements This study was supported by the National Nature Science Foundation of China (31772398), Special Fund for Agro-scientific Research in the public Interest (201503110), China Science and Technology Ministry (2015CB150500), the Jiangsu Science and Technology Department (BK20160730), and the China Postdoctoral Science Foundation (2017 M621761 and 2018 T110510), the Fundamental Research Funds for the Central Universities (KYZ201720).

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