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Changes in rhizosphere bacterial populations brought about by management aid in the management of the pathogen that causes citrus greening disease.

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Research Article

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Abstract

The worldwide citrus sector is severely impacted by citrus greening, also known as Huanglongbing or HLB, which is mostly brought on by the bacterial disease *Candidatus Liberibacter asiaticus* (CLas). By lowering pathogen titre and improving root and soil health, microbial community changes brought on by agricultural management are thought to contribute to HLB resistance. The amount of HLB management is questionable, however, since we don't fully understand how management approaches affect the soil microbiome. Here, we looked at how agricultural management techniques affected the rhizosphere-associated bacterial communities, which in turn reduced CLas titer. Samples of the rhizosphere and roots were taken from two locations in Florida where various management techniques (such as the application of compost, metallized reflecting mulch ground coverings, and microbial inoculations) are now being used to avoid HLB. Amplicon sequencing was used to evaluate management-induced alterations in the rhizosphere bacterial population. The titer of the pathogen CLas in roots was measured using qPCR assays. We also assessed the characteristics of the soil and the activity of microbial enzymes involved in the cycling of soil nutrients. According to our findings, certain management techniques cause changes in the rhizosphere bacterial communities' community structure, which have an adverse effect on the HLB pathogen. Management techniques decreased CLas titer and enhanced soil quality. Furthermore, we discovered that Actinobacteria were often concentrated in the effective treatment areas, indicating that Actinobacteria species may serve as markers for the soil's ability to reduce HLB.

KEYWORDS

soil health, rhizosphere microbial populations, ground cover, compost, and citrus greening

INTRODUCTION

The Asian citrus psyllid (ACP, *Diaphorina citri* Kuwayama) spreads the phloem-limited, picky α -proteobacteria *Candidatus Liberibacter asiaticus* (CLas), which causes citrus greening disease (also known as Huanglongbing, HLB). One of the most dangerous and ancient citrus diseases in the world is HLB (N. Wang & Trivedi, 2009). In many citrus-producing nations worldwide, including those in Africa, Asia, Oceania, and the Americas (South, North, and Central Americas, as well as the Caribbean), HLB is a serious issue (Zhang et al., 2021). In addition to endangering citrus output in California, Texas, Australia, and the Mediterranean area, it has already completely destroyed the Florida citrus sector (McCollum & Baldwin, 2017). Uneven yellow leaf mottling, corky veins, branch dieback, tiny, deformed fruit that stays green, fruit loss due to early dropping, and eventually tree mortality are some of the unique signs that infected trees exhibit (Bě, 2006; N. Wang & Trivedi, 2009). As of right now, the industry lacks both a treatment and a

financially feasible plan for dealing with ill trees. In order to lower the density of ACP, disease management now relies on the effective application and sufficient dispersion of insecticides. Even sophisticated pesticide management systems, however, are unable to stop the development of HLB, according to recent research (Rehberg et al., 2021). Thus, the future of the citrus business worldwide is uncertain, particularly for Florida's citrus sector, which runs the danger of becoming irrelevant and economically significant if crop yields continue to decline and trees continue to die. Although there is no known treatment for HLB, the significance of root health for HLB management has come to light. Before the above-ground symptoms ever manifest, the illness may cause up to 30%-50% of root density to be lost (Graham et al., 2013; Johnson et al., 2014). According to Castellano-Hinojosa et al. (2021), Johnson & Graham (2015), Strauss & Albrecht (2018), and Stokes et al. (2020), these findings established the groundwork for short-term treatments that specifically lower the pathogen's levels in infected citrus trees and reverse the effects of the disease by enhancing root and soil health. In order to manage a few plant diseases, agronomic management approaches affect soil health, modify the soil microbiome, and trigger natural biocontrol processes that result in the creation of disease-suppressive soils (Carrión et al., 2019; Ou et al., 2019; Trivedi et al., 2021). One of the few logistically and financially feasible ways to manage some phytopathogens that are otherwise challenging to control, like *Ca. Liberibacter* spp., may be to harness the advantageous potential of native microbiota (Blaustein et al., 2018; Munir et al., 2022; Trivedi et al., 2016; Zhang et al., 2021). Nevertheless, there is little actual data to back up this theory. The degree of HLB control is questionable since different management techniques have different effects on the soil microbiome. Here, we looked at how management strategies affected the titer (population density) of the HLB pathogen by altering the bacterial communities in the rhizosphere. Our main theory is that specific management techniques will change the physiochemical and biological characteristics of the soil, resulting in the accumulation of strong populations of advantageous microorganisms. These microorganisms' actions will lower the pathogen titer, making plants less vulnerable to HLB infection. We gathered samples from two Florida locations where various management techniques (such as ground coverings, compost application, and microbial inoculations) are now being used to suppress HLB in order to test our hypothesis. Using high throughput sequencing, we evaluated the composition of the bacterial community and estimated soil characteristics and microbial functional groups that carry out crucial soil functions in agroecosystems (Trivedi et al., 2017). Certain qPCR assays were used to track the HLB pathogen's titers (Trivedi et al., 2012). To assess how various management strategies affected microbial coexistence patterns, we used network analysis. Next, we identified microbial groups that have a strong correlation with a lower CLas titer using machine learning techniques (Trivedi et al., 2017).

1 | MATERIAL AND METHODS

1.1 | Study sites

In two separate research locations, we looked at how management techniques affected the reduction of CLas titer.

1.1.1 | Site A

This is located in Venus, Florida, near Amelia Farms. Microbial application and metallized reflecting mulch layer (mulch treatment) are examples of management treatments. For comparison, a bare area without any mulch or microbial treatment was used (referred to as uncovered based on the farm manager's opinion). For every therapy, there were three separate blocks. Four trees were chosen at random from each block. Before planting, a six-foot-wide reflective plastic film called Shine N' Ripe XL, made by Imaflex, was placed on top of the soil and secured in place by furrowed dirt on both sides. The commercial microbial treatment Pathway's "PowerBlend™" was administered to the grove's microbial treatment blocks via irrigation (roots and soil) and aerial spraying (foliage) at a rate of 16 fluid ounces per acre once a month starting in March 2015. At the time of sampling, the trees were five years old.

1.1.2 | Site B

This location is at Vero Beach, Florida, at the Florida Research Center for Agricultural Sustainability. Mulch cover and compost made from urban plant debris were two management strategies. For comparison, bare land without any mulch or compost treatment was used (uncovered). A block of trees that were two rows wide made up the treatments. Four trees in each block were used to gather samples. Two months following planting, the compost treatment was applied at a rate of 12 tonnes per acre using shredded yard-trimming waste. When the trees were about a year old, the second application of compost was made. The mulch treatment was similar to Site A.

1.2 | Sample collection and DNA extraction

Both experimental locations used the same sampling procedure. The samples were taken around 30 cm from each tree's trunk in each of the four ordinate directions. Fine roots (approximately 1 mm in diameter) from a depth of 5 to 15 cm were gathered after the top 5 cm of soil was removed. Using a shovel, the roots were extracted from the ground, and any dirt that wasn't firmly affixed to the roots was then gently brushed away. The Colorado State University laboratory received samples that were transported on ice. Samples were received within a day and processed right away. Each tree's roots were combined from the four areas around it. Using brush pencils, the residual dirt that stuck to the roots was softly brushed in order to swiftly and carefully gather the rhizosphere soil. On ice, the soil collecting process was carried out. This soil, known as the rhizosphere section, was utilised to clarify how management techniques affected the structure, functions, and characteristics of the microbial community. The CLas titer was measured using DNA that was isolated from the remaining root samples. Following the manufacturer's recommendations, we extracted DNA from rhizosphere and root samples using the Qiagen PowerSoil® DNA isolation kit and the DNeasy Plant Pro kit, respectively. Qubit was used to quantify the extracted DNA, which was then kept at -80° C until needed.

1.3 | Amplicon sequencing and data processing

Illumina MiSeq 2 × 300 bp paired-end sequencing was used at the Colorado State University Next Generation Sequencing facility (Fort Collins, USA) to sequence amplicons targeting the V4 region of the bacterial 16S rRNA gene using primer-pair 515F-806R (Caporaso et al., 2012) (Supporting Information: Section S1). The supplemental material (Supporting material: Section S2) contains comprehensive details on the procedure used to handle sequencing data. In short, after connecting the paired-end reads using FLASH (Magoč & Salzberg, 2011) and many rounds of quality filtering, operational taxonomic units (OTUs) were constructed at 97% sequence similarity using UPARSE (Edgar, 2013). As advised by Edgar et al. (2011), singletons and chimeric sequences were eliminated. The SILVA database (v119; Quast et al., 2012) for bacteria and the naïve Bayesian classifier (Q. Wang et al., 2007) with a minimum bootstrap support of 60% were used to assign taxonomies to OTUs in mothur. OTUs that represented sequences originated from mitochondria, chloroplasts, and other plants were eliminated. For Sites A and B, samples were rarefied to 7700 and 11,800 OTUs, respectively, which is the minimal number of sequences for a single sample collected across sites.

1.4 | Quantification of CLas

Using an ABI Prism 7500 Sequence detection system (Applied Biosystems), quantitative real-time PCR (qPCR) experiments in 96-well plates were used to assess the CLas titer. The β -operon region of CLas was targeted using the primer/probe set CQULA04F-CQULAP10P-CQULA04R, and qPCR reactions were carried out using the QuantiTect probe qPCR kit (Qiagen Inc.) in accordance with the parameters outlined in Trivedi et al. (2012; Supporting Information: Materials S2). To translate individual Ct values into pathogen tite as genome equivalents gram⁻¹ of samples, target copy numbers for each reaction were determined from the standard curve (Supporting Information: Materials S3).

1.5 | Soil properties and enzymatic activities

Using a Delta pH meter (Mettler-Toledo Instruments Co.), the field-moist soil-to-water ratio of 2.5 was used to measure the pH of the soil. After soil samples were first dried at 105° C and then ignited at 360° C in a muffle furnace, organic matter (OM) was quantified (Nelson & Sommers, 1996). OM-loss-on-ignition (%) is the percentage of weight lost during the ignition process. A 1:5 soil:extract ratio was used to calorimetrically analyze nitrate from 0.5 M K₂SO₄ soil extracts (Sims et al., 1995). 4-methylumbelliferyl (MUB) and 7-Amino-4-methylcoumarin (MUC; only for LAP) substrates were used to measure soil enzymatic activities, including β -Glucosidase, β -D-celluliosidase, β -Xylosidase, α -Glucosidase, N-acetyl- β -Glucosaminidase, phosphatase, and Leucine-amino-peptidase (LAP) (Bell et al., 2013; details in Supporting Information: Section S4).

1.6 | Bacterial coexistence patterns

Using the SparCC-based method Fastspar, co-existing associations of bacterial OTUs were predicted by network analysis (Friedman & Alm, 2012; Watts et al., 2018). OTUs with less than three occurrences or fewer than five total reads were eliminated from the computation in order to reduce the misleading correlations from uncommon taxa. Only strong ($r > 0.6$) and significant ($p < 0.01$) correlations between OTUs were kept in the network after 100 bootstraps to reduce the false discovery rate. Microbial OTUs are represented by nodes, while correlations between OTUs are represented by edges, the width of which is proportionate to the strength of the link. Gephi was used to characterize the network's modularity (clusters of closely related OTUs), and nodes were colored according to their modules.

1.7 | Statistical analysis

We examined the microbial community structure using the R package mctoolsr (Leff, 2019). Bray-Curtis distances were computed and then ordained in multidimensional scaling utilizing a canonical analysis of principal coordinates (CAPs) study limited to management treatments in order to investigate beta-diversity. To identify significant beta-diversity changes associated with management interventions, permutational multivariate analysis of variance (PERMANOVA) models were created. General linear models were used to assess differences in soil characteristics and CLas titer, and Tukey HSD tests were used to ascertain the impact of management strategies on various variables. R (www.r-project.org) was used for the statistical analyzes, with a significance threshold of $\alpha < 0.01$. The direction and intensity of the correlations (positive or negative) between soil and microbial factors and CLas titer were described using Spearman correlation analysis. The hmisc package in R was used to perform Spearman correlations (Harrell & Harrell, 2015). We first normalized (log-transformed when necessary) and standardized each of our seven ecosystem functions (activity of soil enzymes) using the method outlined in L. Wang et al. (2019) in order to obtain a quantitative index of microbial functioning and activity (i.e., the capacity of the soil microbes to maintain multiple processes simultaneously) for each sample. A soil functioning index was then calculated by averaging the standardized ecological functions (L. Wang et al., 2019). The ability of various communities to sustain multiple functions at once is measured by this function averaging index, which is commonly used in the literature on soil functioning (Delgado-Baquerizo et al., 2016; Trivedi et al., 2017; L. Wang et al., 2019). We identified keystone microorganisms important in lowering pathogen titer using random forest (RF) analysis based on data from the management experiment. Using an ensemble of decision trees based on bootstrapped samples from a data set, RF is a potent machine learning approach that provides excellent prediction accuracy (Breiman, 2001). The RF and rfPermute tools in R were used to conduct RF analysis with 999 permutations (Archer, 2019). The importance and varImpPlot functions were used to determine the most significant predictors. The randomForestExplainer package in R was used to assess the predictors' relevance based on increases in node purity and mean square error values (Paluszynska & Biecek, 2017). Keystone taxa were predicted by the relevant variables ($p < 0.01$). The two locations underwent separate analyzes.

2 | RESULTS AND DISCUSSION

2.1 | Management practices impact pathogen suppression

Citrus roots' CLas titer, which is expressed as genome equivalents of CLas per g⁻¹ of root samples, was affected by management approaches at both locations (Figure 1A,B). The greatest CLas titer is found in uncovered plots at both sites ($\log_{10} 8.56 \pm 0.53$ and $\log_{10} 9.37 \pm 0.12$ for Sites A and B, respectively). CLas tier was substantially lower in mulch-covered treatments ($\log_{10} 6.11 \pm 0.47$ and $\log_{10} 7.52 \pm 0.12$ for Sites A and B, respectively) than in uncovered samples at both sites ($p < 0.001$). The most successful management techniques in Sites A and B were microbial ($\log_{10} 5.42 \pm 0.36$) and compost ($\log_{10} 6.27 \pm 0.20$) treatments, which each reduced CLas titer by 35% in comparison to conventional, uncovered management techniques. Reports from other pathosystems have demonstrated that environmental factors, such as management practices, climate, and soil characteristics like nutrient content, can impact interactions between plants, their associated microbiome, and pathogens (Campos et al., 2016; Carrión et al., 2019; Hannula et al., 2019; Ou et al., 2019; Trivedi et al., 2017; Wei et al., 2019). The idea that soil agroecosystems can be altered to improve disease suppression by boosting the antibiosis capabilities of plant-associated microbial communities is supported by management techniques like reducing or eliminating tillage and crop rotations, which can lead to the development of disease-suppressive soils (Campos et al., 2016; Peralta et al., 2018).

2.2 | Management practices impact microbial community structure and composition

In agricultural settings, management choices may have a significant role in community changes in the soil or plant microbiome that result in the accumulation of soils that inhibit disease. We used CAPs and PERMANOVA on Bray-Curtis dissimilarities to visualize and quantify the differences between microbial communities (β -diversity) in order to investigate if rhizosphere bacterial community structure and composition connect with management techniques (Figure 1C,D). The unique microbial community composition under various management approaches at both locations was shown by the distinct separation pattern. PERMANOVA further validated the changes in bacterial communities, demonstrating a considerable variation in the makeup of microbial communities in various managements within both sites ($p < 0.001$). The structure and functions of the rhizosphere microbial population are affected by the application of compost, according to a few studies. Improvements in plant performance and a reduction in the severity of soil-borne illnesses were strongly associated with changes in microbial communities brought about by compost (Antoniou et al., 2017; Tsolakidou et al., 2019; Zhao et al., 2018). According to Antoniou et al. (2017), Tsolakidou et al. (2019), and Zhao et al. (2018), compost amendment boosts microbial taxon and functional diversity, microbial activity, and the abundance of numerous groups strongly associated with biocontrol and plant growth-promoting chemicals. Mulching has been shown to change the makeup of the soil microbiome (Dong et al., 2017; Huang et al., 2019; Luo et al., 2019). Microbial treatments (8.90 ± 2.81) in Site A had significantly higher ($p < 0.01$) relative abundances of bacteria belonging to Firmicutes than uncovered (2.38 ± 1.22) and mulch (5.89 ± 2.61) treatments because the microbial inoculants applied in Site A comprised a bacterial consortium made up of several strains belonging to Firmicutes. Nevertheless, consistent with previous research (Deng et al., 2019; Guo et al., 2019; Schmidt et al., 2014), our findings show that a microbial amendment therapy with nonnative

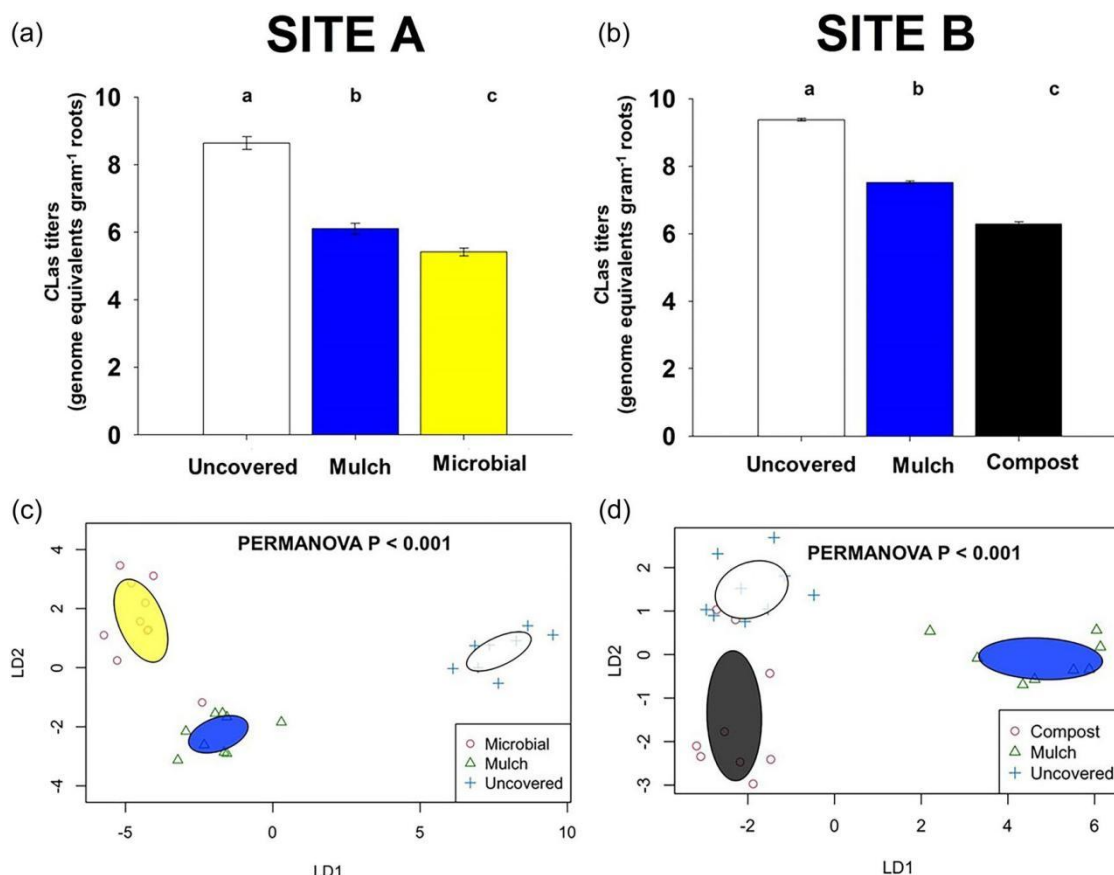


FIGURE 1 Bacterial β -diversity (c, d) and pathogen titer (a, b) in rhizosphere samples from various management techniques at Amelia farms (Site A) and Vero Beach (Site B). The CLas titer is shown as genome equivalents of CLas g⁻¹ root samples in panels (a) and (b). Statistically significant differences at $p < 0.001$ are shown by different letters. The ordination biplots for canonical analysis of principal coordinates (CAP) in panels (c) and (d) indicate how management approaches affect bacterial populations in the rhizosphere. The Bray-Curtis dissimilarity serves as the foundation for CAP analysis. Uncovered, covered, microbial, and compost treatments are represented by white, blue, yellow, and black colored bars (a and b) and symbols (c and d). Increases in the quantity of the microbes present in the exogenously applied microbial therapy itself are not the main cause of the substantial changes in microbial community composition brought about by bacteria. Overall, we observed distinct alterations in the bacterial population in the rhizosphere in response to various management techniques that were linked to a lower CLas titer. Overall, our findings point to the possibility of managing HLB by manipulating the microbiome via selected management techniques.

| Changes in management resulted in soil properties and microbiome assemblies that suppressed CLas titre

Management techniques had a substantial influence on soil parameters, according to analysis of the collected soil samples ($p < 0.001$; Figure 2). Compared to unprotected soils, the soils under mulch (both sites), compost (Site B), and microbial treatments (Site A) had greater OM and nitrate contents. In contrast to other management practices (6.69 ± 0.11 and 6.61 ± 0.14 for mulch and microbial treatments, respectively in Site A and 6.8 ± 0.11 and 6.7 ± 0.17 for mulch and compost treatments, respectively in Site B), the pH of the soil from the uncovered was significantly higher (7.02 ± 0.13 and 7.32 ± 0.13 for Sites A and B, respectively).

In comparison to uncovered treatments, soil microbial functioning and activity (used here as a proxy of soil health) were three times greater in mulch and microbial treatments in Site A and twice higher in mulch and compost treatments in Site B. Our findings demonstrated a strong

correlation ($p < 0.001$) between CLas titer and changes in microbial community composition and functions brought about by management (Figure 3). The suppression of bacterial and fungal pathogens is associated with the soil activities performed by soil microbial communities (Jaiswal et al., 2017; Trivedi et al., 2017). Microbiota-mediated disease resistance may result from changes in the makeup and activity of the soil microbiome in response to management techniques. Changes in the abundance of some taxa that have many characteristics linked to plant defense, such as direct pathogen antagonism or the stimulation of plant defensive responses, are the cause of this. With CLas titer, we found strong relationships between management-induced changes in soil pH and soil nutrients (Figure

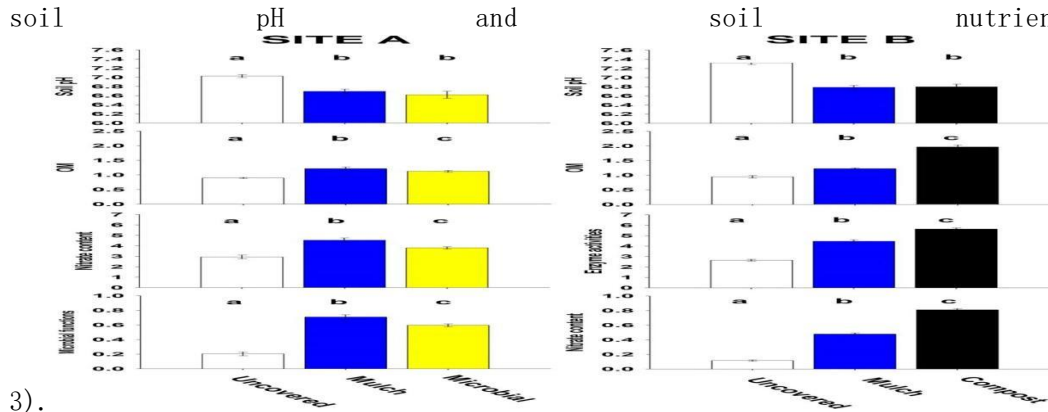
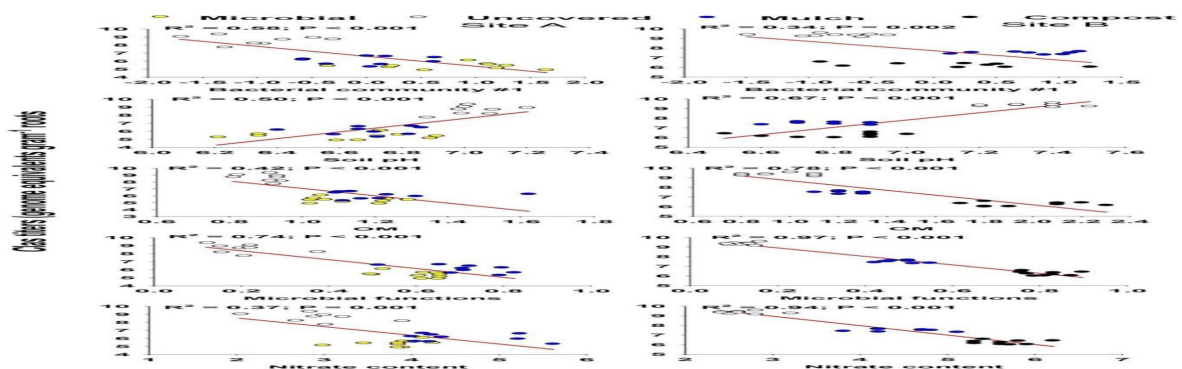


FIGURE 2 Differences in the physicochemical soil properties and microbial functions between different management practices at Amelia farms (Site A) and Vero Beach (Site B).

OM-loss-on-ignition (%) is used to represent organic matter (OM). Nitrate-N ppm N is the unit of measurement for nitrate content. To determine microbial functions, the activity of seven soil enzymes was standardized, and the results were averaged to create a soil functioning index (L. Wang et al., 2019). Figure 3 shows the correlation between physicochemical soil parameters and microbiological functions with CLas titer under various management approaches in two field locations. Different letters indicate statistically significant differences at $p < 0.001$. OM-loss-on-ignition (%) is used to represent organic matter (OM). Nitrate-N ppm N is the unit of measurement for nitrate content. To determine microbial functions, the activity of seven soil enzymes was standardized, and the results were averaged to create a soil functioning index (L. Wang et al., 2019). It's interesting to note that in Florida's central and south-central citrus producing areas, reducing pH by acidifying irrigation water has been shown to lessen the decrease in root density linked to HLB (Morgan & Graham, 2019). According to Xu et al. (2013), management approaches have an impact on alterations in rhizosphere bacterial coexistence networks. Similarly, improvements in soil OM and N content are reported to boost growth by reducing the CLas titer in both young and elderly citrus trees. To determine how management techniques affected the coexistence patterns of rhizosphere bacterial populations, we built bacterial networks (Figure

4). We observed significant changes in



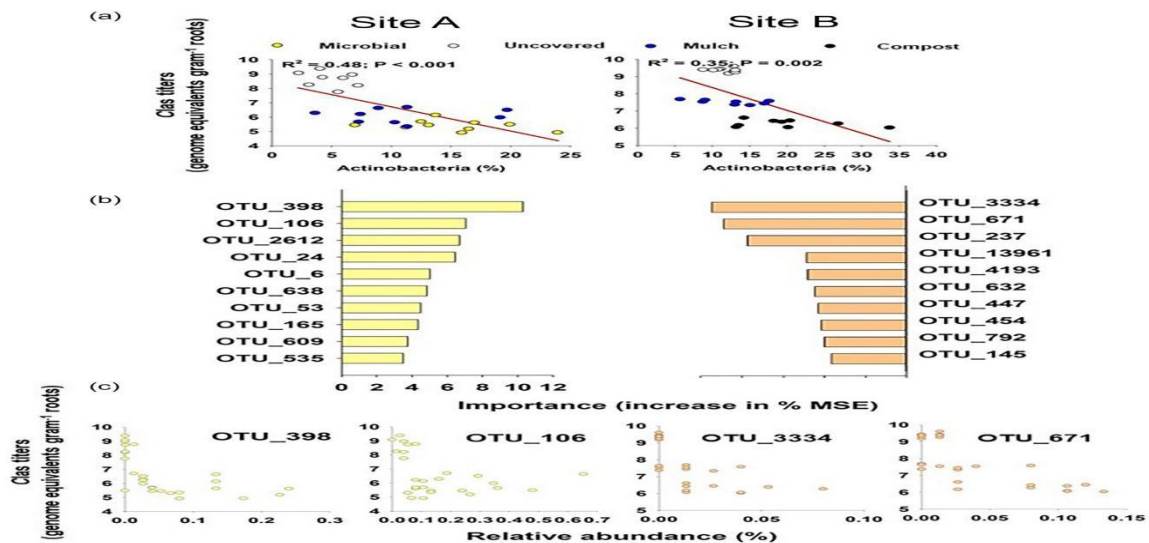
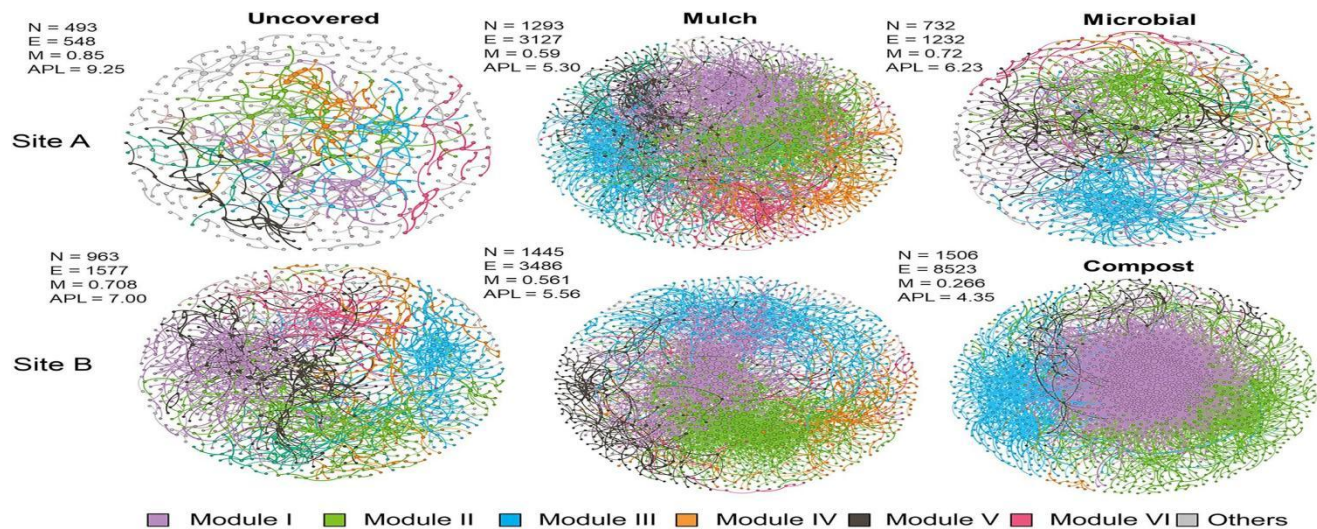


FIGURE 4 bacterial correlation networks with many nodes (taxa) and various management techniques. Different modules are represented by different colors. Each network's top displays its topological characteristics. APL, average path length; E, edge; M, modularity; N, node. modular and various topological features of the bacterial coexistence patterns in response to management strategies for both locations. Increased network complexity is indicated by higher node and edge counts, lower modularity, and longer average paths. Reports on how the pathogen affects the stability of microbial coexistence networks are few. Our findings show that the least complicated of the treatments for both locations were the bacterial coexistence networks of the uncovered treatment with the greatest CLas titer. Furthermore, as compared to mulch, microbial, or compost treatments, the uncovered treatments at both sites had the fewest nodes and edges. Other research (Gao et al., 2021; Wei et al., 2019) have shown reduced network complexity with higher pathogen load, which is consistent with our findings. Increased availability of resources (such as water, soil carbon, and nutrients) and improved soil functionality that favorably affects microbial diversity and network complexity may lead to increased network complexity in the mulch, microbial, and compost treatments (Banerjee et al., 2016, 2019; de Vries et al., 2018; Qi et al., 2019). According to our findings, bacteria under microbial management, mulch, or compost have strong interactions that may improve community stability during pathogen infection in comparison to control (Wei et al., 2015, 2019). Researchers can forecast and develop new theories about microbial interactions using microbial coexistence networks. Our findings imply that microbial interactions are crucial for lowering CLas titer in citrus roots, even if these theories need experimental confirmation.

2.3 | Microbial modulators of CLas suppression

In comparison to the uncovered treatment, the relative abundance (%) of the phylum Actinobacteria was considerably greater ($p < 0.01$) in the mulch (both Sites A and B), microbial (Site A), and compost (Site B) treatments. The relative abundance in the uncovered, mulch, and microbial treatments at Site A was 4.86 ± 1.76 , 10.91 ± 3.34 , and 15.05 ± 3.60 , respectively. At Site B, the relative abundance of Actinobacteria in the uncovered, mulch, and compost treatments was 11.60 ± 1.54 , 14.36 ± 2.24 , and 19.37 ± 2.70 , respectively. The relative abundance of Actinobacteria and CLas titer were shown to be significantly correlated negatively ($p < 0.001$ and 0.002 for Sites A and B, respectively).



ctively; Figure 5A). Both

FIGURE 5 Actinobacteria as the HLB suppression modulator. (a) The relationship between Actinobacteria relative abundance and CLas titer under various management strategies in Sites A and B. (b) The key Actinobacteria groups associated with the decline in CLas titer at both locations. Supporting Information contains OTU taxonomic information. Table 1. (c) The relationship between the relative abundance of the most productive bacterial groups found by RF analysis and the CLas titer. The bacterial OTUs from Sites A and B are shown in yellow and light brown, respectively; MSE stands for mean square error. In a variety of soil types, the relative abundance of Actinobacteria is increased by compost additions (Antoniou et al., 2017; Zhao et al., 2018) and plastic film mulching (Dong et al., 2017). Actinobacteria are vital to the metabolic processes of soil (Bhatti et al., 2017). greater soil functions relating to plant health and disease control may result from this group's greater relative abundance. In soils that inhibit illness, actinobacteria are acknowledged as keystone species (Cha et al., 2016; Mendes et al., 2011). Soil suppressiveness against *Fusarium oxysporum* may be predicted by the presence of Actinobacteria on a continental scale (Trivedi et al., 2017). It has been shown that management-induced increases in Actinobacteria's relative abundance lower pathogen pressure and manage soil-borne plant disease (Cha et al., 2016; Cordovez et al., 2015; Lee et al., 2021). It's interesting to note that a number of Actinobacterial isolates linked to citrus are hostile to CLas and other citrus pathogens (Riera et al., 2017; Trivedi et al., 2011). We hypothesize that by lowering pathogen titer, gradual increases in Actinobacteria member abundance by artificial inoculation or modifications to management techniques may be able to control HLB. Actinobacteria phylotypes that are strongly correlated with the prevalence of HLB infections were identified using RF analysis. Several groups were shown to be substantially connected with a decrease in CLas titer using RF analysis (Figure 5B,C, Supporting Information: Table 1). According to reports, soils resistant to soil-borne fungal diseases, such as fusarium-induced wilt (Peng et al., 1999) and Panama disease (Xue et al., 2015) of bananas, are dominated by members of the microbial groups identified in our study, such as those belonging to Gaiellaceae, Streptomycetaceae, and Mycobacteriaceae. Several Actinobacteria groups, including Gaiellaceae, have been linked to healthy plants, according to Poudel et al. (2016). A mutually exclusive link between *Liberibacter* spp. and members of the Micromonosporaceae and Streptomycetaceae was found by network analysis of microbial community time series in citrus leaf and root samples (Blaustein et al., 2017). Isolating and screening beneficial bacteria that are hostile to the pathogen and creating microbial consortiums to suppress the pathogen are two options for disease prevention (Berendsen et al., 2018; Vannier et al., 2019). Our findings has the potential to start systematic initiatives that screen beneficial

microorganisms and create microbial consortiums for HLB control by using knowledge of how management techniques affect the native soil microbiome.

CONCLUSIONS

Changes in the makeup of the bacterial population, microbial activities, and soil physiological characteristics brought about by management combine to form the suppressive potential of soils, which lowers the CLas titer. It may be feasible to create a generic strategy for creating disease-suppressive soil microbiomes for HLB management, according to the comparable findings reported for two separate field locations. Additionally, the relative abundance of soil Actinobacteria might be a useful biological indicator for creating prediction models to pinpoint and describe management strategies that exacerbate HLB suppression. These results make it possible to lower HLB occurrences both directly via the use of microbial amendments and indirectly through management strategies. The widespread use of sustainable management techniques that use the microbiome to slow the evolution of HLB and lessen plant damage will be guided by future research that offers a mechanistic understanding of the interactions between antagonistic microorganisms and CLas.

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