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Organic Amendments to Avocado Crops Induce Suppressiveness and Influence the Composition and Activity of Soil Microbial Communities

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One of the main avocado diseases in southern Spain is white root rot caused by the fungus *Rosellinia necatrix* Prill. The use of organic soil amendments to enhance the suppressiveness of natural soil is an inviting approach that has successfully controlled other soilborne pathogens. This study tested the suppressive capacity of different organic amendments against *R. necatrix* and analyzed their effects on soil microbial communities and enzymatic activities. Two-year-old avocado trees were grown in soil treated with composted organic amendments and then used for inoculation assays. All of the organic treatments reduced disease development in comparison to unamended control soil, especially yard waste (YW) and almond shells (AS). The YW had a strong effect on microbial communities in bulk soil and produced larger population levels and diversity, higher hydrolytic activity and strong changes in the bacterial community composition of bulk soil, suggesting a mechanism of general suppression. Amendment with AS induced more subtle changes in bacterial community composition and specific enzymatic activities, with the strongest effects observed in the rhizosphere. Even if the effect was not strong, the changes caused by AS in bulk soil microbiota were related to the direct inhibition of *R. necatrix* by this amendment, most likely being connected to specific populations able to recolonize conducive soil after pasteurization. All of the organic amendments assayed in this study were able to suppress white root rot, although their suppressiveness appears to be mediated differentially.

Soil organic matter is fundamental to the long-term sustainability of agroecosystems, and it plays a critical role in global biochemical cycles (1). In the agriculture of the past century, the use of manure and other organic waste material was progressively supplanted by synthetic agrochemicals. This change has led to a decline in soil structure and health that is often related to an increase in plant diseases (2). Therefore, the use of organic amendments has reemerged as an environmentally benign alternative to improve soil quality. This practice has been related to increases in crop yield and plant health and the enhancement of natural suppressiveness of soil against several phytopathogens (3). However, the type and nature of the amendment and the rate of application must be carefully selected for each specific pathosystem. Thus, several authors have reported on the possible negative effects of organic amendments in certain conditions, such as phytotoxicity and increased disease incidence (4–7).

The use of organic amendments or mulches in avocado crops (*Persea americana* Mill.) has produced beneficial effects such as increased root growth and health, reduced plant stress in adverse climatic conditions, and increased avocado yield (8–10). Several organic amendments have shown an obvious suppressive effect against the oomycete *Phytophthora cinnamomi*, the pathogen that causes the widespread *Phytophthora* root rot of avocado (11, 12). However, there is a lack of information about the potential positive or adverse effects of organic amendments on white root rot in avocado caused by *Rosellinia necatrix*. This fungus mostly affects avocado crops in Mediterranean countries, where the presence of the pathogen in the soil, together with the favorable environmental conditions, has turned this disease into one of the main limiting factors for avocado production (13).

The quantity and quality of organic matter input affect both the physicochemical properties of the soil and biotic factors related to soil microbiota such as microbial biomass and diversity, community structure, and soil activities (14–20). The suppressive effects of organic amendments and compost appear related to their influence on soil microbiota because soil pasteurization usually leads to the loss of suppressiveness (21, 22). However, the specific nature of disease suppression is unknown in most cases, and the particular mechanisms involved have not yet been identified (23). To determine which amendments have potential suppressive capability, it is important to identify the microbial populations and associated processes that could account for disease suppression (24).

Several attempts have been made to identify the key factors involved in soil suppressiveness and to find predictive parameters

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TABLE 1 Main characteristics of the treatments used in the plant assays

Treatment	Code	Composition and composting procedure	Composting time	
			Assay 1	Assay 2
Unamended control	UC	No organic amendment was added		
Almond shells	AS	Commercial almond shells derived from almond industry were piled and traditionally composted; the compost pile was only watered with rainwater	5 yr	1 yr
Pruning waste	PW	Avocado wood derived from pruning waste was finely chopped and composted; the compost pile was watered and turned for aeration every month	5 mo	5 mo
Yard waste	YW	Yard and garden wastes (mostly grass) collected from gardens of the area and composted; the compost pile was watered and turned for aeration every month	5 mo	5 mo

for the suppressive potential of soil organic amendments (25–27). Many authors have correlated the suppression of certain plant diseases with organic parameters related to physicochemical properties (28, 29), microbial biomass (30, 31), microbial diversity (23, 32), microbial community composition (33, 34), microbial overall activity (30, 35), metabolic and enzymatic profiles (34, 36), and particular enzymatic activities (37, 38). Even if certain of these parameters are better predictors than others, none is universally related to disease suppression, and their correlation with suppressiveness is largely dependent on the pathosystem, the type of amendment used, and the environmental conditions (26, 27). In this sense, each type of organic amendment must still be empirically tested for different pathogens, crops, and environments (39).

The availability of suitable methodologies for obtaining detailed information about soil community composition and functioning in each of the suppressive models is a key to unraveling the variety of subjacent mechanisms of disease suppression (40). Limitations and biases of culture-based methods for studying soil microorganisms have been overcome by molecular methods that can analyze a large portion of the nonculturable microorganisms in soil (41). Denaturing gradient gel electrophoresis (DGGE) is a well-established fingerprint method in microbial ecology (42), and it has been successfully used in comparative analyses to study the influence of a wide range of parameters and conditions on the microbial community composition of soil and the rhizosphere (14, 43–47). However, microbial activities and functional diversity might be as important as phylogenetic traits when studying soil microbial communities (48, 49). Organic amendments are known to affect soil functional diversity (18), and the analysis of changes in metabolic and enzymatic abilities can potentially discriminate between suppressive and conductive soils (34, 36).

The objective of the present study was to assess the effects of three different vegetal organic amendments on the development of avocado white root rot caused by *R. necatrix* and to monitor their influence on plant growth under controlled conditions. Microbial communities in the soil and rhizosphere of amended plants were characterized and compared to those of control plants using a polyphasic approach. This analysis includes both culture-based and culture-independent methods for assessing chemical and biological parameters that are potentially involved in the effects of organic amendments on the development of avocado white root rot.

MATERIALS AND METHODS

Greenhouse inoculation assay. An experimental microplot platform was designed and constructed for the plant assays to mimic semifield conditions. The greenhouse was built as an open structure with double roofing to allow air passage for improved ventilation, and the microplots (35 liter plant pots) were earthed up in a white gravel bank to reduce oscillation of the soil temperature (see Fig. S1 in the supplemental material). Two independent 1-year-long experiments were conducted and are named in the present study as assay 1 and assay 2. Two-year-old commercial avocado seedling plants (cv. Topa-Topa) were transplanted to 35 liter pots filled with a blend (1:1) of disinfested natural soil and peat. All plants, except for those in the control treatment, had the top layer of soil mulched with 19 liters of one of the experimental organic amendments (or disinfected soil for the control) as described in Fig. S1B in the supplemental material. Seventeen avocado plants were used for each of the four treatments assayed in these studies as listed in Table 1. After application, the organic amendments matured for six additional months in the greenhouse as the plants grew, and then the soil was inoculated using wheat grains colonized by *R. necatrix* as described by Sztejnberg and Madar (50). Four holes per pot were made on the soil surface using a punch, and 16 g of wheat colonized with *R. necatrix* strain CH53 was distributed in the holes before filling with the surrounding soil.

Eleven plants for each treatment were inoculated with the pathogen, and the remaining six noninoculated plants were used as a control. Plant disease was monitored for 6 months after inoculation. At this point, soil and rhizosphere samples were taken from the control plants of each of the assayed treatments to study the effect of the organic amendments on the microbial community. No samples were taken from the inoculated plants because most of them were dead several weeks before the end of the assay.

Plant growth. Plant growth was monitored in both the inoculated and noninoculated plants. The circumference of the trunk was measured at a height of 15 cm above the ground and used to calculate the trunk cross-sectional area. The lateral branches were removed to maintain apical dominance and the lengths of the main and lateral stems were measured and summed for calculation of the total growth of lateral branches. Variation in plant size after 9 months of the experiment was used to calculate three plant growth parameters: increase in plant height (%), increase in trunk cross-sectional area (%), and total growth of lateral branches (cm).

Disease assessment. Disease progression was measured by evaluating the aerial symptoms of white root rot in symptomatic plants using a symptom scale modified from Ruano-Rosa et al. (51): 0, healthy plant; 1, plant with first symptoms of wilt; 2, overall wilted plant; 3, wilted plant with first symptoms of leaf desiccation; and 4, completely dried plant (dead plant) (see Fig. S2 in the supplemental material). The disease index (DI) in each treatment was calculated according to the method described by Cazorla et al. (52). The experiment was considered finished 165 days postinoculation in both of the assays. For statistical comparison of the treatments, the area under the disease progress curve (AUDPC) was calculated for each plant (51, 52).

Chemical analysis of the soil. Three bulk soil samples from each treatment were collected at the end of assay 2, air dried, milled, and sent to an external laboratory (CEBAS-CSIC, Murcia, Spain) for chemical analyses. The parameters shown in Table S1 in the supplemental material were used for principal component analysis (PCA).

Soil and rhizosphere sampling. Fifteen-centimeter-deep soil core samples were obtained using a 4-cm-diameter core sampler. Three plants per treatment (named A, B, and C) were randomly selected, three equidistant points around each plant (named R1, R2, and R3) were sampled, and each sample was independently processed and analyzed.

The soil samples were placed in cold storage and transported to the laboratory. Moist field soil was passed through a 2-mm-pore-size sieve, and roots were separated from the bulk soil. The fine avocado roots contained in the sample together with the surrounding adhering soil were defined in the present study as the rhizosphere samples, which included rhizosphere, rhizoplane, and endorhizosphere habitats. The sieved soil that was carefully cleared from the roots was considered the bulk soil sample. Fresh soil and rhizosphere samples were used for culturable microbial population analysis and for community-level Biolog and APIZYM assays (bioMérieux SA, Lyon, France). DNA extraction from the rhizosphere samples was also performed immediately after sample collection, and three subsamples of the bulk soil were stored at −80°C for subsequent DNA extraction.

Microbial isolation and plate counts. For the microbial analysis, the three replicate samples from the same pot were pooled to provide a single composite sample from each plant, and three composite samples per treatment (pots A, B, and C) were analyzed. For the bulk soil analysis, subsamples of 10 g of the bulk soil were suspended in 90 ml of saline solution (0.85% NaCl) with 5 g of sterile gravel (2 to 4 mm in diameter) and mixed at 250 rpm for 30 min on an orbital shaker, which was followed by 20 min of decantation. For the rhizosphere analysis, one gram of the fine roots was homogenized for 2 min in a Stomacher bag with 10 ml of saline solution. In both cases, 10-fold serial dilutions of the supernatant were plated on different selective media.

Selective media were used for the specific isolation of fast-growing heterotrophic bacteria, pseudomonads, sporulating bacteria, actinomycetes, and fungi as described by Larkin and Honeycutt (53). To isolate sporulating bacteria, soil suspensions were pretreated at 80°C for 10 min before plating. Plates were incubated at 23°C for 48 h for the enumeration of fast-growing heterotrophic bacteria, pseudomonads, and sporulating bacteria and for 10 days for actinomycetes and fungi. The average values from triplicate analyses were expressed as CFU per gram of dry soil (oven-dried soil at 105°C for 24 h) or per gram of fresh root.

Sample preparation for the Biolog and APIZYM assays. Soil and rhizosphere suspensions were prepared for the inoculation of community-level physiological profiling and hydrolytic activity assays. The soil suspensions were prepared as follows: 3 g of bulk soil was suspended in 30 ml of saline solution (0.85% NaCl) with 2 g of gravel and horizontally mixed at 250 rpm for 30 min on an orbital shaker. For the rhizosphere suspensions, 2-g portions of fine roots were homogenized for 2 min in a Stomacher bag with 20 ml of saline solution. Every suspension was centrifuged at 50 × *g* for 5 min, and then the supernatant was transferred to a sterile 50-ml tube and centrifuged again at 130 × *g* for 5 min. This low-speed centrifugation has been described as the most efficient method for decreasing the optical density (OD) of a soil suspension by settling the largest soil particles with minimum effect on cell density (54). These suspensions were used for the inoculation of both the Biolog and APIZYM assays.

Community-level physiological profiling assay. The ability of soil microbial communities to use 31 useful carbon sources was assessed using Biolog EcoPlates (Biolog, Inc., Hayward, CA) developed for soil community analysis. Three microtiter plates per treatment and one for each replicate sample (pots A, B, and C) were filled with 150 μl of soil or rhizospheric suspension per well, followed by incubation at 23°C. The utilization rate of the carbon sources was monitored by measuring OD at 590 nm. Color development was analyzed two or three times a day using a microplate reader until the curve of the average well color development (AWCD) reached the saturation point (8 days). The incubation period in which the increase in the AWCD for each plate became maximal was determined and used for the selection of the closest common reading time point for all of the treatments. The OD values were used to compare the physiological profiles, and raw OD data were corrected by subtracting the control OD value (no carbon source provided). Negative values were considered "zero" in subsequent data analyses of net OD. To reduce biases from variations in inoculum density or differences in AWCD, the data were normalized by dividing the net OD of each well by the AWCD (55). A PCA was performed on the normalized data. Substrate richness (S), substrate evenness (E), and metabolic diversity by Shannon index (H) were calculated based on net OD data as described by Zak et al. (56).

Hydrolytic activity assays. Nineteen enzymatic activities were analyzed by using the semiquantitative APIZYM system. APIZYM strips were inoculated with 90 μl of soil or rhizosphere suspension prepared as described below and incubated for 48 h at 23°C. A value ranging from 0 to 5 was assigned according to the colorimetric standard table provided by the manufacturer that relates color intensity with the quantity of hydrolyzed substrate. Three samples per treatment (A, B, and C) and two assay replicates were used to calculate average values.

DNA extraction. DNA was extracted from nine bulk soil samples per treatment (pots A, B, and C and soil core replicates R1, R2, and R3) and nine equivalent rhizosphere samples per treatment. DNA extraction from all soil and rhizosphere samples (0.25 g of soil and 0.3 g of fine roots) was performed using a Power Soil DNA kit (MO-BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's protocol.

PCR amplification of 16S rRNA gene fragments. All DNA samples from soil and rhizosphere were analyzed separately by PCR-DGGE. PCR amplification of the variable region of the bacterial 16S rRNA gene was performed with the universal bacterial primers 341F-GC and 907R described by Muyzer et al. (57, 58). The amplifications were carried out in 50-μl reaction mixtures that consisted of 1 μl of DNA template (ca. 5 ng), 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM concentrations of each deoxynucleoside triphosphate, 1 μM concentrations of primers (each), 2.5 U of *Taq* DNA polymerase (all components were from Invitrogen, Carlsbad, CA), and 5% (vol/vol) dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO). Touchdown PCR was carried out as described by Muyzer et al. (57, 58).

DGGE profiling. DGGE analyses were conducted using a D-Code Universal detection system (Bio-Rad Laboratories, Inc., Hercules, CA). Equal amounts of PCR product were loaded into the wells of a 6% polyacrylamide gel (acrylamide–*bis*-acrylamide [37.5:1]) containing a gradient of 30 to 60% denaturants (a 100% denaturant concentration was defined as 7 M urea and 40% [vol/vol] deionized formamide). Electrophoresis was performed in 1× Tris-acetate-EDTA buffer at 60°C with a constant voltage of 75 V for 14 h. The PCR products amplified from nine replicates per treatment (pots A, B, and C and soil core replicates R1, R2, and R3) were loaded in the same gel. The lanes on the outsides of the gels were loaded with an unrelated DGGE marker to assist in the normalization and comparison among gels. However, to eliminate potential "gel effects," one replicate from each sample (pots A, B, and C) of the different treatments was loaded in the same gel for a direct comparison among treatments. Gels were stained with ethidium bromide (0.5 μg/ml), destained in distilled water and photographed under UV illumination using a Gel Doc XR+ imaging system (Bio-Rad Laboratories, Inc.).

Analysis of DGGE profiles. Gel images were normalized and analyzed with InfoQuest FP 5.10 software (Bio-Rad Laboratories, Inc.). The Pearson correlation coefficient for each pair of lanes within a gel was calculated as a measure of similarity between the community fingerprints and used to perform cluster analysis by the unweighted pair group method with arithmetic mean (UPGMA). Cophenetic correlation coefficients were calculated to assess the robustness of the assigned clusters. The number and relative intensity of the DGGE bands in each fingerprint were determined using InfoQuest FP software. The number of bands was used as an estimate of the apparent bacterial richness (*S*). Genetic diversity, measured by the Shannon index (*H*), was calculated as $H = -\Sigma \mathrm{pi} \ln(\mathrm{pi})$, where pi is the intensity of each DGGE band divided by the total area of the fingerprint, and the evenness (*E*) was calculated as $E = H/\log(S)$.

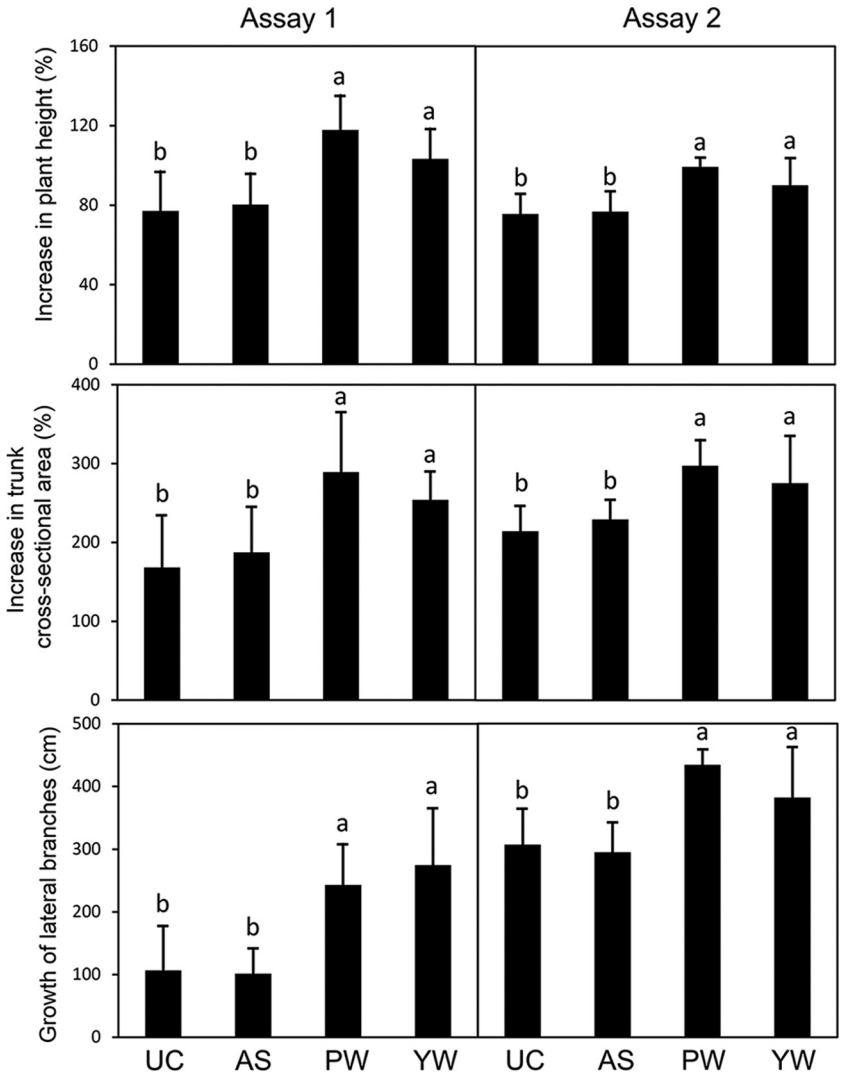

FIG 1 Effect of the organic amendments on avocado plant growth during 9 months of assay. Comparison between amended and unamended control treatment in plant height, trunk cross-sectional area, and growth of lateral branches of noninoculated plants in the two greenhouse experiments. Different letters mean significant differences between treatments (ANOVA, $P < 0.05$). UC, unamended control; AS, almond shells; PW, pruning waste; YW, yard waste.

DGGE band excision, cloning, and sequence analysis. DGGE dominant and/or differentiating bands were excised with a scalpel from the DGGE gels and transferred to 1.5-ml sterile tubes containing 20 μl of sterile MilliQ water, cut into pieces to facilitate DNA elution, and incubated overnight at 4°C. Two microliters of the resulting suspension was used in a PCR to reamplify the excised 16S rRNA gene fragment using the same primers and PCR conditions described for the soil DNA samples. The resulting PCR products were analyzed on a DGGE gel, together with the original community DNA sample, to check their electrophoretic mobility. Excised bands displaying the same melting behavior as the original bands in the community profiles were used as the templates for further PCR amplification with the primers 341F/907R (without a GC clamp). The PCR fragments were ligated into a pGEM-T Easy vector (Promega, Madison, WI) and transformed into competent cells of *Escherichia coli* DH5-α as recommended by the manufacturers. Positive clones were verified by colony PCR and rechecked for comigration with the original band in a new DGGE gel. Selected clones were sent to Macrogen, Inc. (Amsterdam, Netherlands) for sequencing with T7 and SP6 primers. Contigs were assembled with the forward and reverse sequences using the Contig Express software (Vector NTI Advance 10; Invitrogen). The presence of chimeric sequences was detected with DECIPHER's Find Chimeras web tool (59). The resulting sequences were verified for similarity with previously published bacterial 16S rRNA gene sequences in the GenBank database using the BLAST web tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Microcosm assay. Direct inhibition of *R. necatrix* was tested in a microcosm system to check the role of soil microorganisms in the suppressiveness of composted almond shell (AS) amended soil. This microcosm assay was carried out using unamended control (UC) soil, AS-amended soil and several modified versions of these soils. Pasteurized soil (UCp and ASp) was prepared using moist heat treatment at 100°C for 15 min to reduce microbial biomass (60). Four different complemented soils were also prepared to partially recover the microbial community, mixing pasteurized and fresh soil in a 9:1 (wt/wt) proportion (always nine parts of pasteurized soil per one part of fresh soil): UCp+UC, UCp+AS, ASp+UC, and ASp+AS.

Microcosm assays were conducted using a diffusion chamber experimental design adapted from Epstein, 2013 (61). A fungal disk (0.6-cm in

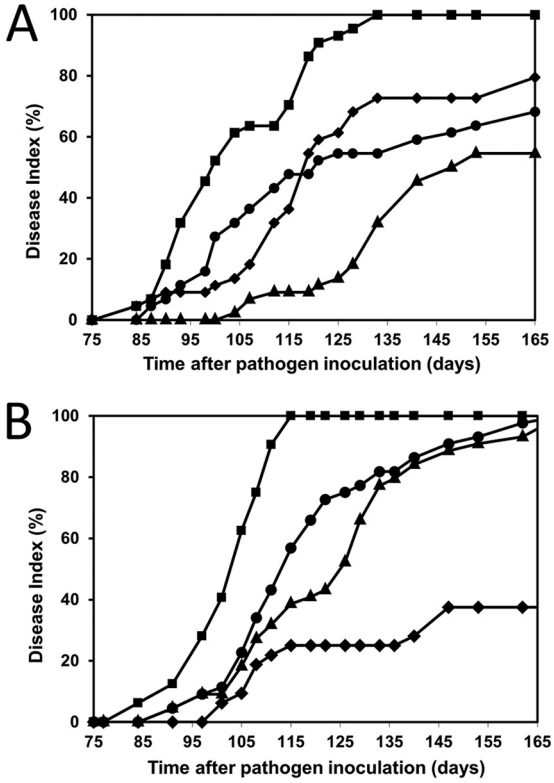

FIG 2 Effect of the organic amendments on avocado white root rot. Time course of the disease index, calculated by evaluation of the aerial symptoms of white root rot in the inoculation assays. (A) Assay 1; (B) assay 2. Symbols: ■, unamended control; ▲, almond shells; ●, pruning waste; ◆, yard waste.

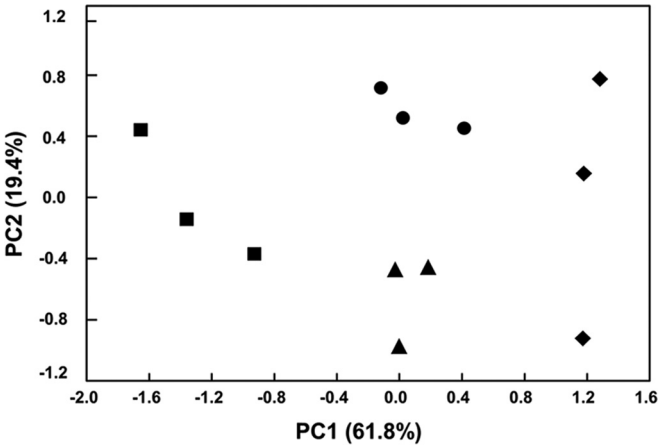

FIG 3 Effect of organic amendments on the chemical properties of the soil. Scatter plot based on PCA of the soil chemical properties of the assay 2. The symbols refer different treatments: ■, unamended control; ▲, almond shells; ●, pruning waste; ◆, yard waste. The data for the chemical composition of the amended soils and the correlation of chemical parameters to ordination axes derived from PCA analysis are available in Tables S1 and S2 in the supplemental material.

diameter) from a 1-week-old culture of *R. necatrix* on potato dextrose agar (PDA) was transferred to a 5-cm-diameter disk of water-agar medium (1%) and placed on top of a nitrocellulose filter (0.45-μm pore size). These multilayer systems were placed on containers on top of the different soils and covered to reduce aerial contamination. A picture of this device is shown in Fig. S3 in supplemental material. Twelve replicate chambers per soil type were incubated for 5 days at 25°C. At the end of the assay, the initial disk area and total growth area of *R. necatrix* were measured using Quantity One 1-D analysis software (Bio-Rad Laboratories, Inc.), and the variation of the area (Δarea) was calculated.

Data analyses. Data distributions were tested for normality using the Shapiro test for normality ($P = 0.05$). For data following a normal distribution, i.e., microbial counts, plant growth parameters, and fungal growth inhibition (microcosms), the differences between treatments were tested using analysis of variance (ANOVA) followed by a Fisher least-significant-difference test ($P = 0.05$). Population density values were $\log_{10}$ transformed before analysis. Disease progression data and diversity indices, which did not show a normal distribution, were compared by nonparametric Mann-Whitney U test ($P = 0.05$). All of these analyses were performed using SPSS software version 15.0 (SPSS, Inc., Chicago, IL). PCA was performed on soil chemical data and Biolog physiological profiles using the demo version of the Multivariate Statistical Package (MVSP, v3.12e; Kovach Computing Service, Anglesey, United Kingdom).

Nucleotide sequence accession numbers. Nucleotide sequence accession numbers were as follows: KF733465 to KF733499 and KF733500 to KF733507 (see Table 4).

RESULTS

Plant growth. In the present study, plants amended with pruning waste (PW) and yard waste (YW) showed a significant increase in growth in comparison to control plants (ANOVA, $P < 0.05$). The effects of YW were more evident in assay 1, where such increase was significant in all of the growth parameters, whereas in assay 2, only trunk cross-sectional area was significantly higher than control (Fig. 1). The treatment with almond shells (AS) showed no significant differences in plant growth from the unamended control (UC).

White root rot progression. The first root rot aerial symptoms appeared ~84 days after inoculation with *R. necatrix* in both of the independent microplot assays. The evolution of the disease index for each treatment with time is shown in Fig. 2. In both of the assays, the unamended control treatment was the first to show aerial symptoms and reach 100% disease index (all of the inoculated plants were dead) at 115 to 135 days postinoculation (assays 1 and 2, respectively). In contrast, the three organic amendments assayed in the present study induced a delay and/or a decrease of white root rot symptoms. The most evident suppressive effect was produced by the AS treatment in the first assay (Fig. 2A) and by the YW treatment in the second assay (Fig. 2B). Statistical comparisons of AUDPC data showed that all of the assayed organic amendments produced a significant reduction (Mann-Whitney U, $P < 0.05$) in white root rot progression compared to the unamended plants.

Chemical soil properties. The chemical characteristics of the amended and unamended soils at the end of the assay (12 months after plant transplantation) are shown in Table S1 in supplemental material. PCA clustered together the three replicate samples from each treatment and separated the different treatments (Fig. 3). This differentiation by treatment indicates both a clear effect of the organic matter on the soil chemical composition and a differential effect depending on the nature of the amendment.

The first principal component (PC1), which explained more than half (61.8%) of the total variance, allowed differentiation between amended and unamended soils and among YW and the other organic treatments. Amended soils, especially the YW treatment, were generally associated with higher levels of total N, total

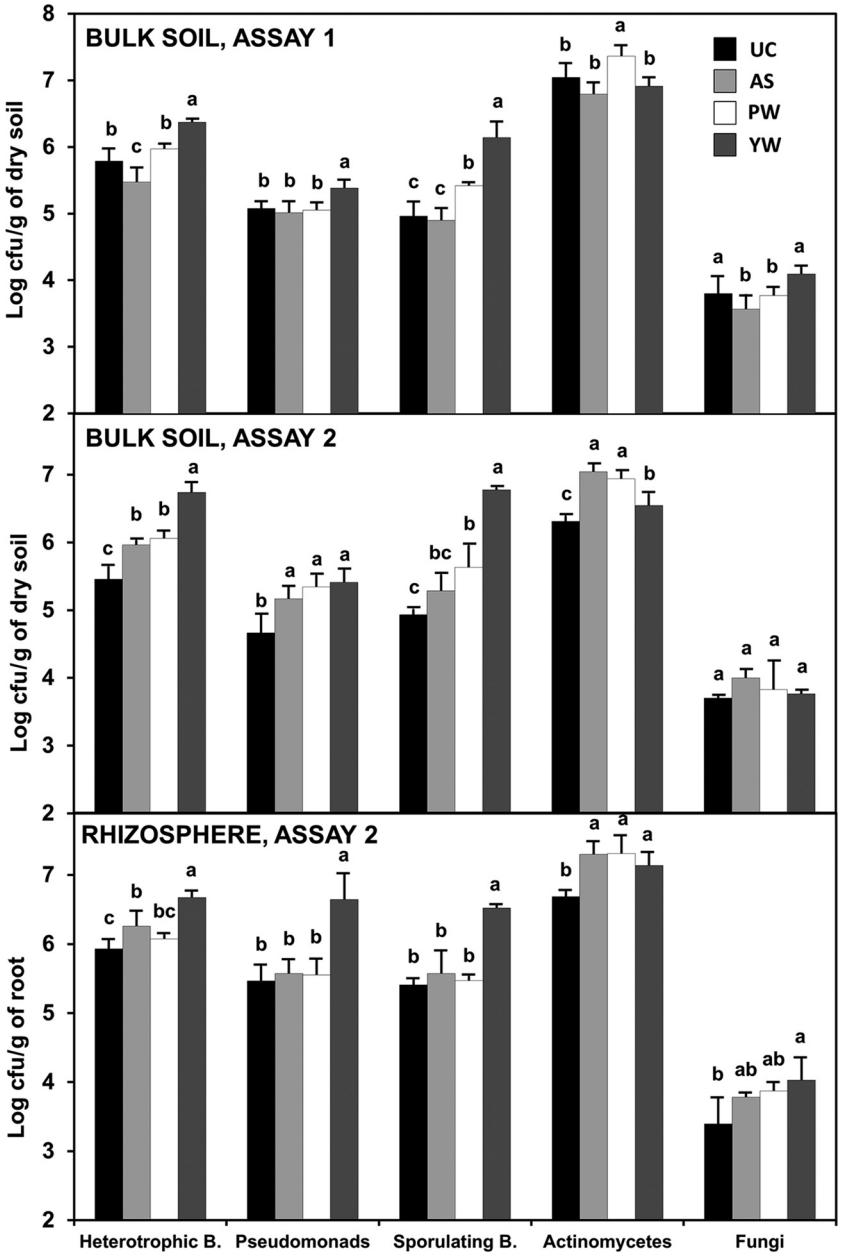

FIG 4 Effect of organic amendments on culturable microorganisms. The population densities of fast-growing heterotrophic bacteria, pseudomonads, sporulating bacteria, actinomycetes, and fungi were assessed by plate counts. Different lowercase letters indicate significant differences (ANOVA, $P < 0.05$). UC, unamended control; AS, almond shells; PW, pruning waste; YW, yard waste.

C, organic C, S, Ca, B, and Mn (significant positive correlations with PC1) and lower levels of Cr, Al, Fe, K, Cd, and Ni (significant negative correlation with PC1) compared to the control soil (see Table S2 in supplemental material). PC2 was closely associated with Zn and Cu values, which explained the differences between the AS and PW treatments and within the YW treatment.

Culturable microbial populations. Microbial counts in the bulk soil and rhizosphere samples showed low levels of fungi (nearly 10^3 to 10^4 CFU per g of dry soil) and higher counts of the different bacterial groups, which has been widely described for soil microbial communities. The actinomycetes generally showed the largest populations (nearly 10^7 CFU per g of dry soil), whereas fast-growing heterotrophic bacterial populations numbered ~10^6 CFU per g of dry soil, indicating a difference greater than one order of magnitude depending on the treatment. The counts of pseudomonads and sporulating bacteria were approximately 10^5 to 10^6 CFU per g of dry soil, also showing significant differences depending on the amendment used.

The YW amendment showed the largest effect, inducing a significant increase (ANOVA, $P < 0.05$) in all of the analyzed bacterial groups in both bulk soil and rhizosphere except for actinomycetes in the soil of assay 1 (Fig. 4). The amendment with PW also increased bacterial numbers, although this effect was lower and mainly restricted to culturable actinomycetes and sporulating bacteria. The AS

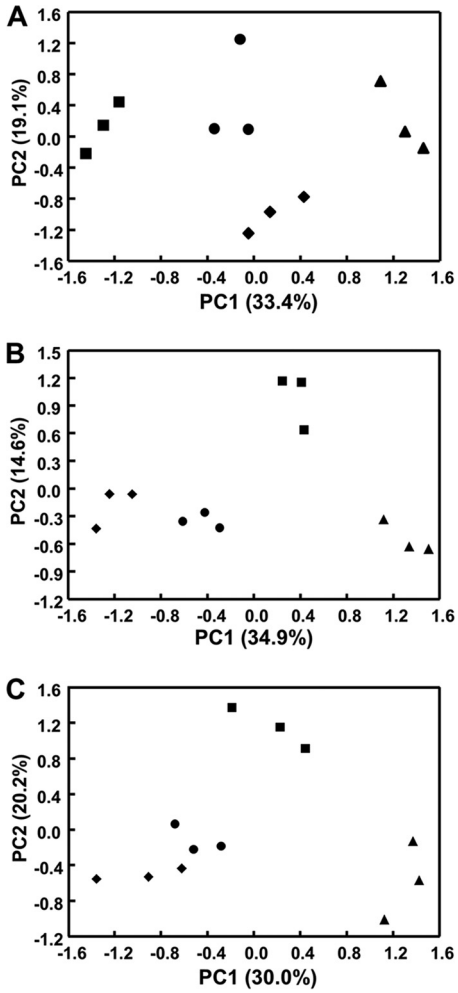

FIG 5 Effect of organic amendments on the metabolic profiles of the microbial community. Scatter plots were prepared based on PCA of normalized OD data of Biolog Ecoplates. (A) Bulk soil of the assay 1; (B) bulk soil of the assay 2; (C) rhizosphere of the assay 2. Symbols: ■, unamended control (UC); ▲, almond shells (AS); ●, pruning waste (PW); ◆, yard waste (YW).

treatment showed the lowest effect on culturable populations and did not affect bacterial populations in assay 1 except for fast-growing heterotrophic bacteria levels, which were even lower than those found in the unamended soil. In assay 2, however, this treatment yielded a slight increase in heterotrophic bacteria and actinomycetes, both in bulk soil and in the rhizosphere (Fig. 4).

Physiological profiles of soil and rhizosphere microbial communities. The initial comparison between Biolog EcoPlate profiles from the soil and rhizosphere samples by PCA clearly differentiated the rhizosphere samples from the bulk soil, but the effect of the treatments was not clear (data not shown). Individual analyses were performed for the bulk soil and rhizosphere samples to detect the effect of the amendments on catabolic profiles. The three replicate samples from the unamended control clustered separately from the amended treatments in both the soil and rhizosphere biplots (Fig. 5). This spatial distribution revealed a clear effect of each different amendment on the metabolic profiles of soil and rhizosphere microbial communities (Fig. 5).

The diversity indices based on Biolog profiles showed slight but statistically significant differences (Mann-Whitney U test, $P < 0.05$) between amended and unamended plants (see Table S3 in supplemental material). In assay 1, the addition of organic amendments increased soil metabolic diversity as determined by Shannon index (H) and richness ($P < 0.05$) compared to the UC, especially for *H*, which was increased by every organic amendment. However, in assay 2, the organic treatments did not show such a clear effect on the diversity indices. The amendment with AS was the only treatment that showed an overall enhancement of metabolic diversity, increasing *H* in both assays in the soil and in the rhizosphere (Mann-Whitney U test, $P < 0.05$) (see Table S3 in the supplemental material).

Extracellular enzyme profiles. The analysis of 19 extracellular hydrolytic enzymes using the APIZYM system revealed a clear influence of the organic amendments on the enzymatic activity profiles of the soil and the rhizosphere. The highest overall activity corresponded with the YW treatment in the soil and rhizosphere, whereas the lowest overall activity always corresponded with the UC (Table 2). Each of the organic treatments induced an increase in the activity of 6 enzymes in bulk soil: acid phosphatase, naphthol-AS-BI-phosphohydrolase, leucine arylamidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, and β-glucosidase. In contrast, the only common effect of the addition of organic matter to the enzymatic activities in the rhizosphere was a decrease in alka-

TABLE 2 Enzymatic profiles of soil and rhizosphere of assay 2 based on the hydrolytic activities assessed by the API ZYM system[a]

	Bulk soil				Rhizosphere			
	UC	AS	PW	YW	UC	AS	PW	YW
Phosphatases								
Alkaline phosphatase	2.3	4.7	3.0	5.0	4.3	3.5	3.5	3.5
Acid phosphatase	2.0	4.7	4.0	4.3	4.0	4.5	5.0	4.0
Phosphohydrolase	1.3	3.3	2.0	2.0	4.0	4.0	3.5	3.5
Esterases								
Lipase	0.0	0.3	0.0	1.3	1.7	1.0	3.0	1.0
Esterase Lipase	1.7	2.3	1.3	1.7	2.3	2.5	3.0	2.5
Esterase	1.3	2.3	1.0	1.7	1.3	1.5	2.0	2.0
Amino-peptidases								
Leucine arylamidase	1.0	2.0	2.7	4.3	3.0	2.0	1.5	1.0
Valine arylamidase	1.0	1.0	1.0	1.0	1.3	1.5	0.5	1.5
Cystine arylamidase	0.0	0.0	0.0	0.0	0.0	1.0	0.5	1.0
Proteases								
Trypsin	0.0	0.0	0.0	0.0	0.7	1.0	0.5	3.0
Chymotrypsin	0.0	0.0	0.0	0.0	0.3	1.0	0.0	1.5
Glycosyl-hydrolases								
α-galactosidase	0.0	0.0	0.3	1.0	2.0	2.0	2.0	3.0
β-galactosidase	1.0	2.3	2.3	2.7	2.3	3.0	3.5	3.0
N-acetyl-β-glucosaminidase	1.7	3.3	2.3	2.7	3.0	2.5	3.5	3.0
α-glucosidase	1.3	1.0	3.0	4.0	1.7	1.0	1.5	3.0
β-glucosidase	1.0	3.3	2.3	4.3	2.3	2.5	3.5	3.0
β-glucuronidase	0.0	0.0	0.0	0.0	1.3	2.5	1.0	2.5
α-mannosidase	0.3	0.7	0.0	0.0	1.0	1.0	1.0	2.5
α-fucosidase	0.0	0.7	1.0	1.7	0.3	0.5	1.0	2.0
Total activity	16.0	32.0	26.3	37.7	37.0	38.5	40.0	46.5

[a] Different shadings indicate different intensities of the enzymatic reactions: white, low intensity (0.0 to 1.9); light gray, moderate intensity (2.0 to 3.9); and dark gray, high intensity (4.0 to 5.0). UC, unamended control; AS, almond shells; PW, pruning waste; YW, yard waste.

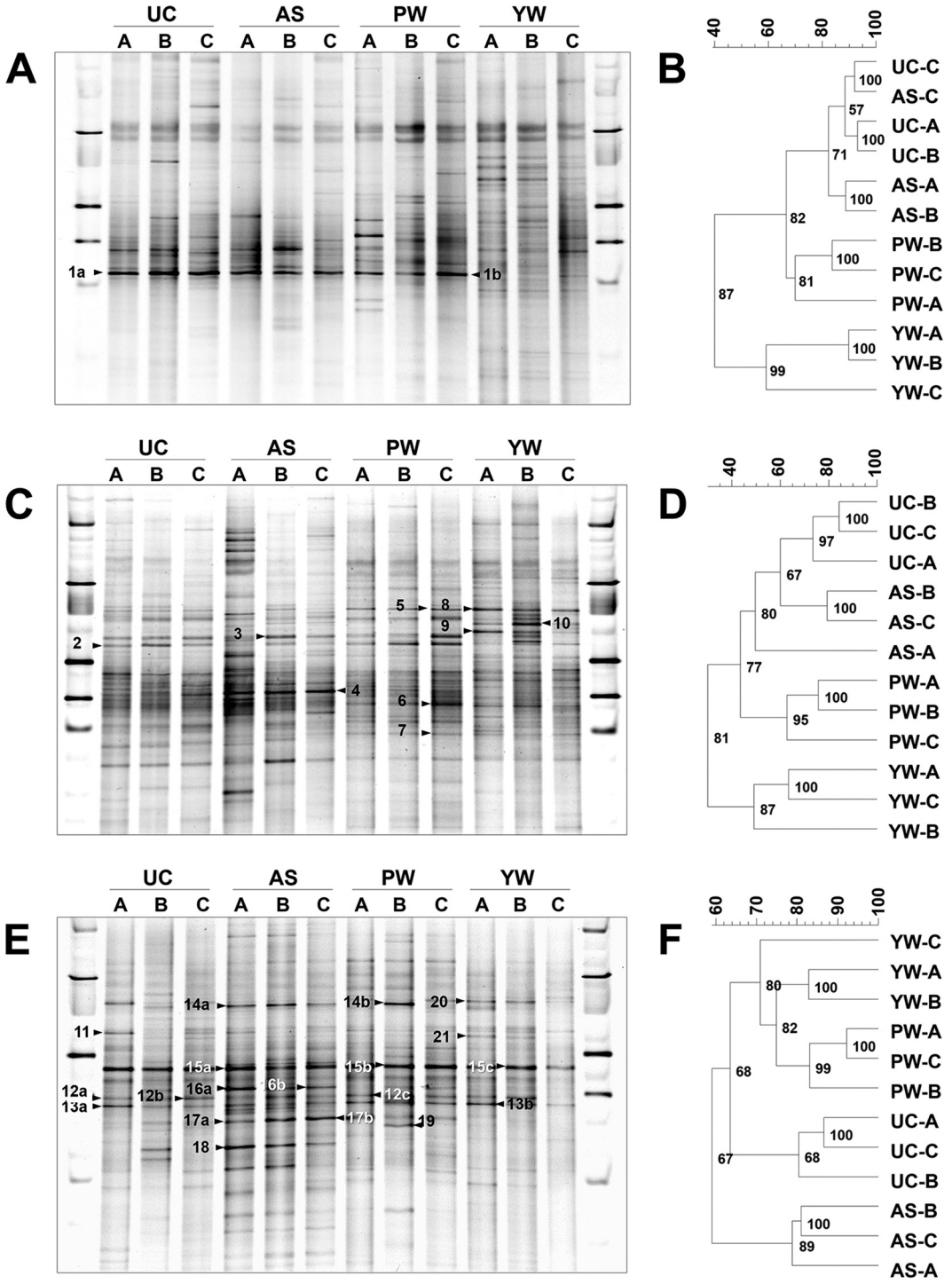

FIG 6 Effect of organic amendments on soil bacterial communities. (A, C, and E) PCR-DGGE fingerprints of bacterial 16S rRNA gene fragments. One replicate from each pot (named A, B, and C) of the different treatments were loaded in the same gel. (B, D, and F) Cluster dendrograms based on Pearson correlation coefficient and UPGMA algorithm showing similarity between 16S DGGE profiles. Numbers at the nodes represent cophenetic correlation values in percent. (A and B) Bulk soil of the assay 1; (C and D) bulk soil of the assay 2; (E and F) rhizosphere of the assay 2. UC, unamended control; AS, almond shells; PW, pruning waste; YW, yard waste. The codes of numbers and letters marked on the DGGE bands from panels A, C, and E correspond to the band codes of 16S rRNA gene sequences shown in Table 4.

line phosphatase activity. Moreover, certain enzymatic activities were specifically enhanced in the soil by the different amendments, whereas their responses to the same amendment in the rhizosphere were more variable (Table 2).

Soil bacterial community analysis by DGGE. In an initial approach that included all of the replicates and used one or two treatments per gel, cluster analysis showed a general treatment-based clustering. Nevertheless, the clustering between treatments

TABLE 3 Genetic diversity indices based on DGGE profiles

DGGE profile	Mean (SD)[a]			
	UC	AS	PW	YW
Soil assay 1				
Shannon	2.53 (0.08)	2.63 (0.20)	2.94 (0.36)	2.83** (0.15)
Richness	17.00 (2.00)	20.00 (3.61)	26.33 (7.02)	22.67** (2.52)
Evenness	0.89 (0.01)	0.88 (0.01)	0.90 (0.04)	0.91 (0.02)
Soil assay 2				
Shannon	2.90 (0.08)	2.95 (0.25)	2.99 (0.08)	3.21** (0.08)
Richness	20.33 (1.15)	24.67 (5.51)	24.00 (2.65)	30.00** (2.65)
Evenness	0.96 (0.01)	0.92* (0.02)	0.94 (0.03)	0.94 (0.01)
Rhizosphere				
Shannon	2.49 (0.12)	2.89** (0.13)	2.46 (0.16)	2.35 (0.10)
Richness	19.00 (1.00)	28.00** (1.00)	18.33 (2.08)	15.33* (1.15)
Evenness	0.85 (0.03)	0.87 (0.03)	0.85 (0.02)	0.86 (0.03)

[a] UC, unamended control; AS, almond shells; PW, pruning waste; YW, yard waste. *, significantly lower than UC ($P < 0.05$); **, significantly higher than UC ($P < 0.05$).

suggested the possibility of a "gel effect" that is commonly related to slight differences in gel quality or running conditions (62, 63). To eliminate this effect, the comparison among treatments was subsequently performed using common gels that contained only one replicate from each sample (pots A, B, and C) of the four assayed treatments. The gels and resulting cluster analyses are shown in Fig. 6. In general, the fingerprints were clustered according to the treatments. The soil samples showed a similar clustering in both of the independent greenhouse experiments. The most similar profiles were displayed by the AS and UC treatments, whereas the YW treatment showed the most dissimilar fingerprints in both of the assays (Fig. 6B and D). In the rhizosphere analysis, the treatment with AS showed the greatest effect on the bacterial community, which was observed both by visual and clustering differences (Fig. 6E and F).

The bacterial genetic diversity based on the number of DGGE bands and their relative intensity was overall higher in the soil of assay 2 than in assay 1. Despite this difference, the treatment YW showed a higher diversity than the other treatments in both assays as revealed by the Shannon and evenness parameters (Mann-Whitney U test, $P = 0.05$), but it did not affect diversity in the rhizosphere (Table 3). However, the treatment with AS increased the bacterial diversity of the rhizosphere but did not affect any of the diversity indices in the soil (Table 3).

Sequence analysis of dominant DGGE bands. In Fig. 6A, C, and E, the bands marked with numbers correspond to the dominant bands that were extracted from the DGGE gels and submitted to cloning and sequencing. Their tentative phylogenetic affiliations are shown in Table 4. The analysis of sequences obtained from the same DGGE band showed occasional heterogeneity within the clones. However, in most cases, they showed equal or very similar phylogenetic affiliations. For every single DGGE band, the number of clones with the same electrophoretic mobility as the original band that was submitted to sequencing and the number of clones displaying identical sequences are shown in Table 4. Only nonidentical sequences were submitted to GenBank under the accession numbers shown in Table 4.

Microcosm assay. The inhibition of *R. necatrix* was tested using eight types of soil to evaluate their suppressive ability and the role of soil microbial communities in disease suppression. The highest inhibition of fungal growth was displayed by the AS-amended fresh soil, with a significantly lower Δarea (ANOVA, $P < 0.05$) than the UC fresh soil and the rest of the soil types (Table 5). Pasteurized soil showed in both cases significantly lower inhibition (ANOVA, $P < 0.05$) than the corresponding fresh soil. Complementation of pasteurized soil with UC fresh soil did not have any significant effect on ASp+UC soil, but it induced a slight but significant recovery of suppressiveness in UCp+UC soil. On the other hand, soil complemented with fresh AS soil showed a clear recovery of soil suppressiveness, showing lower Δarea (ANOVA, $P < 0.05$) than pasteurized soil.

DISCUSSION

The enhancement of soil suppressiveness using organic amendments has been widely described, especially for soilborne diseases (2, 64, 65). However, this effect can be very variable depending on the pathosystem and the environmental conditions, and there are even some examples where the amendment has increased disease incidence (26, 66). Soil organic amendments have been successfully used for the control of *P. cinnamomi* in avocado crops (11, 12, 67), but they have never been tested against the white root rot caused by *R. necatrix* until now. It is therefore interesting that all of the organic amendments tested in the present study showed a suppressive effect against white root rot. The phenomenon of disease suppression has been commonly related to modifications to the soil caused by the organic amendment, including physicochemical properties, microbial populations, and associated processes (27). All of the analyses performed in the present study aimed to understand and identify factors that could account for the suppression of white root rot.

We showed that there was an effect of the organic amendments on the chemical composition of the soil. The gradient in soil nutrient content shown by PCA analysis (PC1 in Fig. 3) corresponded to the gradient of suppressiveness in the same assay (Fig. 2B), suggesting a direct relationship between the nutrient content of the soil and disease suppression. Several soil chemical parameters, which include a high content of nitrogen, carbon, and organic carbon, have been previously correlated to lower disease incidence (26). However, in some cases it is not clear whether this effect was a consequence of the influence of certain soil nutrients on soil microbiota or instead was asso-

TABLE 4 Closest phylogenetic relatives of partial 16S rRNA gene sequences derived from dominant or differentiating DGGE bands

Band code[a]	Sample origin[b]	Treatment[c]	n_{total}[d]	n[e]	NCBI accession no.	Closest phylogenetic relatives		
						Identity/strain	% identity	Accession no.
1a	Soil assay 1	UC	3	2	KF733465	Uncultured *Rhodanobacter* sp. clone AHy52	100	KC502951.1
				1	KF733466	*Rhodanobacter spathiphylli* strain B39	100	NR_042434.1
1b	Soil assay 1	PW	3	3	KF733467	Uncultured *Rhodanobacter* sp. clone AHy5	100	KC502951.1
2	Soil assay 2	UC	1	1	KF733468	*Brevundimonas lenta* strain DS-18	99	NR_044186.1
3	Soil assay 2	AS	2	2	KF733469	Uncultured *Bacteroidetes* bacterium clone BuhD-239	99	FM877553.1
4	Soil assay 2	AS	1	1	KF733470	Uncultured bacterium clone HLLCs310	99	JX100020.1
5	Soil assay 2	PW	1	1	KF733471	*Thiobacter subterraneus* strain C55	91	NR_024834.1
6	Soil assay 2	PW	2	1	KF733472	Uncultured bacterium clone 36	94	FM209350.1
				1	KF733473	Uncultured bacterium clone RamatNadiv01b09	99	JF295396.1
7	Soil assay 2	PW	2	1	KF733474	Uncultured acidobacterium clone GASP-WC2W2_D11	99	EF075273.1
				1	KF733475	"*Candidatus* Solibacter usitatus" strain Ellin6076	93	NR_074351.1
8	Soil assay2	YW	3	2	KF733476	Uncultured *Sphingobacteriales* bacterium clone AMPD3	100	AM936482.1
				1	KF733477	Uncultured betaproteobacterium clone S2-009	99	KF182945.1
9	Soil assay 2	YW	3	3	KF733478	Uncultured *Sphingobacteriales* bacterium clone AMPD3	99	AM936482.1
10	Soil assay 2	YW	2	1	KF733479	Uncultured *Sphingobacteriales* bacterium clone AMPD3	99	AM936482.1
				1	KF733480	Uncultured bacterium DGGE gel band 03_U2 clone 07	99	JX986325.1
11	Rhizos.As2	UC	2	1	KF733481	*Bacteroidetes* bacterium X3-d	99	HM212417.1
				1	KF733482	*Bacteroidetes* bacterium X3-d	99	HM212417.1
12a	Rhizos.As2	UC	3	1	KF733483	*Rubrivivax gelatinosus* strain IL-144	99	NR_074794.1
				1	KF733484	Uncultured bacterium clone sdm16	99	JQ798405.1
				1	KF733485	Uncultured *Burkholderiales* bacterium clone Plot4-E08	99	EU449563.1
12b	Rhizos.As2	UC	5	3	KF733486	Uncultured *Burkholderiales* bacterium clone Plot4-E08	100	EU449563.1
				2	KF733487	Uncultured *Burkholderiales* bacterium clone Plot4-E08	99	EU449563.1
12c	Rhizos.As2	PW	2	2	KF733488	*Rubrivivax gelatinosus* strain IL-144	98	NR_074794.1
13a	Rhizos.As2	UC	1	1	KF733489	*Albidiferax ferrireducens* strain CH1-46	99	KC855480.1
13b	Rhizos.As2	YW	2	2	KF733490	*Albidiferax ferrireducens* strain CH1-46	100	KC855480.1
14a	Rhizos.As2	AS	4	3	KF733491	*Burkholderia* sp. strain K14	100	AJ300687.1
14b	Rhizos.As2	PW	2	2	KF733492	*Burkholderia soli* strain GP25-8	99	NR_043872.1
				1	KF733503	*Cupriavidus oxalaticus* strain NBRC 13593	99	AB680453.1
15a	Rhizos.As2	AS	5	3	KF733493	*Rhizobium leguminosarum* bv. viciae strain 3841	100	NR_103919.1
				1	KF733494	*Rhizobium leguminosarum* bv. viciae strain 3841	99	NR_103919.1
				1	KF733495	*Rhizobium leguminosarum* bv. viciae strain 3841	99	NR_103919.1
15b	Rhizos.As2	PW	2	2	KF733496	*Rhizobium leguminosarum* bv. viciae strain 3841	100	NR_103919.1
15c	Rhizos.As2	YW	1	1	KF733497	*Rhizobium leguminosarum* bv. viciae strain 3841	100	NR_103919.1
16a	Rhizos.As2	AS	1	1	KF733498	Uncultured bacterium clone HLLCs310	100	JX100020.1
16b	Rhizos.As2	AS	1	1	KF733499	Uncultured bacterium clone HLLCs310	100	JX100020.1
17a	Rhizos.As2	AS	2	2	KF733500	*Frateuria aurantia* strain DSM 6220	99	NR_074107.1
17b	Rhizos.As2	AS	2	2	KF733501	*Frateuria aurantia* strain DSM 6220	99	NR_074107.1
18	Rhizos.As2	AS	2	2	KF733502	*Burkholderia tuberum* strain STM678	98	NR_027554.1
19	Rhizos.As2	PW	1	1	KF733504	*Rhodanobacter thiooxydans* strain LCS2	99	NR_041565.1
20	Rhizos.As2	YW	3	2	KF733505	Uncultured betaproteobacterium clone GASP-WC1W2_B07	99	EF074724.1
				1	KF733506	Uncultured *Sphingobacteriales* bacterium clone AMPD3	100	AM935103.1
21	Rhizos.As2	YW	2	2	KF733507	Uncultured *Bacteroidetes* bacterium clone L1-7	99	JF703503.1

[a] Band codes refer to the DGGE bands marked in Fig. 8.
[b] Origin of the samples for sample types and assays: soil assay 1, soil assay 2, or rhizosphere assay 2 (Rhizos.As2).
[c] UC, unamended control; AS, almond shells; PW, pruning waste; YW, yard waste.
[d] n_{total}, total number of clones with the same electrophoretic mobility of the original band that had been subjected to sequencing.
[e] n, number of clones sharing identical 16S rRNA gene sequences.

ciated with an enhancement of plant growth and vigor. In fact, mulching of avocado crops in field studies increases fruit production through improving the growth and health of surface feeder roots (8, 9). These healthier roots could also potentially be more resistant to attack by soilborne pathogens such as *P. cinnamomi* or *R. necatrix*. In the present study, we did not find any evidence of this type of effect; on the contrary, we demonstrated with a microcosm assay that bulk soil amended with AS has a direct inhibitory effect on the growth of the fungus *R. necatrix*, so the plant is not necessarily involved in the mechanism of disease suppression. This experiment helped to clarify several details related to suppression mechanisms. For example, the reduction of the inhibitory effect from partial sterilization of the soil demonstrated that the suppressiveness of AS has a microbial origin. In fact, in the literature it is widely assumed that the mechanisms of disease suppression are mainly biological, whereas abiotic traits of the soil could only indirectly modulate the efficacy of suppression through their effect on the plant and/or on the pathogenic process (22, 68). Soil sup-

TABLE 5 Microcosm assay to evaluate the role of microbial community in suppressive soils[a]

Soil sample	Δarea	SD
UC	38.76[B]	1.95
UCp	46.03[A]	0.97
UCp+UC	38.81[B]	2.23
UCp+AS	34.75[C]	0.71
AS	28.75[D]	1.87
ASp	39.52[B]	1.98
ASp+UC	37.16[B]	1.86
ASp+AS	32.41[C]	0.36

[a] The average of growth area variation (Δarea) from different types of soils and their standard deviations are shown. UC, unamended control; AS, almond shells amended soil; UCp, pasteurized control soil; ASp, pasteurized amended soil. Complemented soils in a 9:1 (wt/wt) proportion were prepared by mixing 9 parts of pasteurized soil with 1 part of fresh soil in the following combinations: UCp+UC, UCp+AS, ASp+UC, and ASp+AS. Superscript capital letters indicate significant differences between treatments (ANOVA, $P = 0.05$)

pressiveness has been correlated with quantitative and qualitative changes in soil microbiota, including increases in microbial biomass (30, 31) and microbial diversity (23, 32) and changes on microbial community composition (33, 34). Many studies have focused only on phylogenetic traits, but microbial activity and functional diversity might be as important as phylogenetic traits when studying disease suppression (24, 27).

The APIZYM system has been demonstrated to be a fast but suitable method for assessing hydrolytic activities in soil. The enzymatic activity assays are not based on microbial growth, so they might reflect, at least theoretically, the *in situ* community function more closely than culture-based methods (69). In the present study, six hydrolytic enzymes were substantially enhanced in bulk soil by the addition of organic matter regardless of the nature of the amendment used. Some of these enzymes are key enzymes in primary biogeochemical cycles, frequently used as indicators of soil functioning, and their activation in soil has been widely related to the addition of vegetal composts and amendments (70–73). Especially interesting is the enhancement of *N*-acetyl-β-glucosaminidase activity, which is one of the enzymes involved in chitin degradation. Chitinolytic activity has been related to the control of several fungal diseases by single biocontrol microorganisms and to the use of compost (38, 74, 75). The sum of total hydrolytic activity in bulk soil showed that soil with higher activity was also more efficient at reducing white root rot symptoms. In the case of YW, the strong increase in soil and rhizospheric hydrolytic activity is probably related to the large increase in bacterial population levels caused by this amendment. In addition to their influence on enzymatic activities, an increase in bacterial populations (especially those of total heterotrophic bacteria and sporulating bacteria) was described by Bonanomi et al. (27) as a good predictor of the suppressive potential of an organic amendment. In this instance, an enhancement of bacterial populations might be related to the suppressive effect of the YW treatment, but it is likely not involved in the suppressive effect of AS, which barely affected microbial population size.

Previous studies have also demonstrated that organic amendments influence the composition and diversity of soil bacterial communities in avocado orchards (20). In the present study, the DGGE results provided further evidence for the ability of organic amendments to affect microbial communities, both in the soil and the rhizosphere of avocado plants. Once again YW showed the strongest effect on bulk soil, causing the most important changes in bacterial community composition and a significant increase of soil bacterial diversity. Unlike the YW treatment, the amendment with AS, which also showed high suppressive ability, scarcely affected the bacterial community composition in bulk soil as analyzed by DGGE. However, the addition of AS especially affected microbial communities in the rhizosphere, where bacterial diversity was increased and several populations were specifically enhanced. Attempts to identify these populations by sequencing of DGGE bands often leads to uncertain phylogenetic affiliations, and most soil-living bacteria have only been detected before by molecular methods and are therefore barely known. We successfully identified here two interesting bacterial populations that were enhanced in the rhizosphere of AS-amended plants. The species *Burkholderia tuberum* is part of the group of nonpathogenic *Burkholderia* species associated with plants, which include biocontrol agents and N-fixing nodulating bacteria and are considered to be potentially beneficial (76). *Frateuria aurantia* is a naturally occurring beneficial proteobacterium widely known for its ability to solubilize fixed potassium into an exchangeable form, making it assumable by plants, and commercial formulations of this bacterium are approved for use in organic agriculture (77).

As mentioned before, the microcosm assay revealed that the suppressiveness of AS has a microbial origin. Therefore, it proves that the effect of AS on soil microorganisms is responsible for turning the conducive UC soil into a suppressive soil. Thus, the AS is necessarily affecting the bulk soil microbiota, even if the PCR-DGGE method used in the present study was not able to detect large differences in bacterial community composition. The effect of AS on bulk soil was, however, detected by the analysis of soil physiological profiles and enzymatic activities. In fact, AS was the only amendment that showed a significant and consistent increase in potential metabolic diversity (based on Biolog data) both in the rhizosphere and in the bulk soil in the two plant assays. In the case of AS, the restoration of suppressiveness by complementation of sterile soil with a small proportion of fresh amended soil suggests a mechanism of specific suppression wherein some specific populations and activities should be mainly responsible for the disease control phenomenon (60). This type of mechanism agrees with the more subtle effects on soil microbiota caused by the addition of AS in the present study, where it did not show a clear enhancement of microbial populations or activities, but it induced specific structural and physiological changes.

All of the results observed here suggest that the amendments with AS and PW owe their disease suppression capacity to different mechanisms. The addition of YW increased the overall soil enzymatic activities, bacterial population levels, and diversity and caused important changes in soil bacterial community composition. These effects suggest a general increase in soil health and functioning that is presumably behind the suppressiveness of YW-amended soil through a mechanism of general suppression. This type of disease suppression is related to an overall boost of microbial communities and activities, and no specific population can be pointed to as mainly for responsible disease control (60). General disease suppression is frequently enhanced by organic matter input and has been related to increased soil fertility (2), which is in concordance with the increase in nutrient content and plant growth caused by the addition of YW. However, the suppressiveness triggered by AS seems to be related to a mechanism of specific

suppression caused by more subtle structural and physiological changes that could trigger the stimulation of specific microbial activities.

Based on the comparative analysis performed here, a single mechanism cannot be identified as the causal factor of disease suppression. In fact, the suppressive effect should be attributable not to a single mechanism but to a combination of causes, especially in the case of YW. Nevertheless, all of the organic amendments assayed in the present study were able to suppress the white root rot caused by *R. necatrix* to some extent, despite the differential nature of their suppressive effects. These amendments should be considered an effective agricultural practice for the control of white root rot in organic avocado crops.

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