Soil Sterilization Eliminates Beneficial Microbes That Provide Natural Pest Suppression Ecosystem Services Against *Radopholus similis* and *Fusarium Oxysporum* V5w2 in the Endosphere and Rhizosphere of Tissue Culture Banana Plants

Dennis M. W. Ochieno *†

Laboratory of Entomology, Wageningen University & Research, Wageningen, Netherlands

Endosphere and rhizosphere microbes offer plant growth promotion and pest suppression ecosystem services in banana-based agroecosystems. Interest has been growing towards the use of such beneficial microbes in protecting vulnerable tissue culture banana plants against pathogens such as *Radopholus similis* and *Fusarium oxysporum*. A screenhouse experiment with potted tissue culture banana plants was conducted using sterile and non-sterile soil to investigate the effect of soil biota on *R. similis* and *F. oxysporum* strain V5w2. Plants grown in non-sterile soil had lower damage and *R. similis* density in roots and rhizosphere, while most plant growth-related parameters including root freshweight, shoot freshweight, total freshweight, plant height, and leaf size were larger compared to those from sterile soil. Shoot dryweight and Mg content were higher in plants from sterile soil, while their leaves developed discolored margins. *R. similis*-inoculated plants in sterile soil were smaller, had more dead roots, higher nematode density, and produced fewer and smaller leaves, than those from non-sterile soil. For all plant growth-related parameters, nematode density and root damage, no differences were recorded between controls and *F. oxysporum* V5w2-inoculated plants; and no differences between those inoculated with *R. similis* only and the ones co-inoculated with the nematode and *F. oxysporum* V5w2. Banana roots inoculated with *F. oxysporum* V5w2 were lighter in color than those without the fungus. Independent or combined inoculation of banana plants with *F. oxysporum* V5w2 and *R. similis* resulted in lower optical density of root extracts. *In vitro* assays indicated the presence of *Fusarium* spp. and other root endophytic microbes that interacted antagonistically with the inoculated strain of *F. oxysporum* V5w2. It is concluded that, soil sterilization eliminates beneficial microbes that provide natural pest suppression ecosystem services against *R. similis* and *F. oxysporum* in the endosphere and rhizosphere of tissue culture banana plants. I recommend the integration of microbiome conservation into tissue culture technology through the proposed "Tissue Culture Microbiome Conservation Technology."

Keywords: biological control, endophyte, *Fusarium oxysporum* V5w2, *Radopholus similis*, rhizosphere, tissue culture

INTRODUCTION

Banana and plantains are important food crops among many communities in the developing countries around the world (Scott, 2021). The annual global production of banana and plantains has been estimated at about 145 million metric tons (Ploetz and Evans, 2015; Rocha et al., 2021), of which around 30% comes from Africa (Kikulwe and Asindu, 2020). Disease complexes involving plant-parasitic nematodes and Fusarium of Banana Complex (FOBC) remain a major threat to banana production and hence food security in Africa and many other regions around the world (Maryani et al., 2019; Rocha et al., 2020; Bedoya et al., 2021). In Eastern Africa, the production of highland cooking banana "*Matooke*" (*Musa* spp. genomic group AAA-EA), which is a very important staple food crop, is threatened by disease interactions between various species of plant parasitic nematodes especially *Radopholus similis* and the devastating Fusarium of Banana Complex (FOBC) (Kangire et al., 2000; Kangire and Rutherford, 2001; Talwana et al., 2016; Coyne et al., 2018; Van der Veken et al., 2021). *R. similis* among other nematode species have the potential to inflict as high as 50% yield loss (Speijer and Kajumba, 2000; Ssango et al., 2004), while the highly virulent stains of *Fusarium* species could just wipe out susceptible banana cultivars (Dita et al., 2018; Maymon et al., 2020). *R. similis* burrows into the root cortex of banana plants causing necrotic lesions and death of roots (Seenivasan, 2017). The consequences of root damage include impaired nutrient uptake and toppling of the banana trees. Besides this, wounds inflicted by the plant parasitic nematodes allow entry of pathogenic microbes especially the *Fusarium* species associated with root-rot and wilt diseases (Mai and Abawi, 1987; Speijer and Sikora, 1993; Back et al., 2002). Depending on prevailing conditions such as the availability of nutrients and agro-climatic factors (Gaidashova et al., 2009; Olivares et al., 2021), *Fusarium*-nematode disease interactions in susceptible plant cultivars are mostly synergistic characterized by increased nematode penetration and multiplication (Back et al., 2002; Rocha et al., 2020), enhanced root tissue and xylem colonization by the fungus (Dita et al., 2018; Pegg et al., 2019), resulting in stunted growth coupled with intensified rotting of roots, rhizomes and pseudostems and the ultimate wilting and death of banana plants (Kangire et al., 2000; Kangire and Rutherford, 2001; Jamil et al., 2019; Liu et al., 2020; Ochieno, 2020).

In the face of the global rise in food demand, ecosystem services, and technologies that limit the damage caused by plant-parasitic nematodes and fungal pathogens in banana and other food crops become of increasing importance. Plant-parasitic nematodes including *R. similis* and microbial pathogens such as the Fusarium of Banana Complex (FOBC) are readily transmitted through infected planting material (Dita et al., 2018; Jacobsen et al., 2019; Kumar et al., 2021). The highly aseptic tissue culture technology is efficient for mass production of certified clean banana planting material (Kumar et al., 2021). However, stringency in aseptic production of tissue culture banana plants not only eliminates harmful organisms, but also beneficial endophytic microbes that provide various ecosystem services including pest suppression (Dubois et al.,

2006). Besides elimination of beneficial endophytic microbes, tissue culture banana plants are propagated in soil sterilized by various methods including steam-sterilization (Ochieno, 2010). This again eliminates beneficial rhizosphere biota associated with various plant growth promoting ecosystem services (Yu et al., 2019). Deficiency in pest-protective endosphere and rhizosphere microbes renders tissue culture banana plants susceptible to re-infestation by plant-parasitic nematodes and microbial pathogens in the field (Jacobsen et al., 2019).

To address this draw-back, the innovative concept of re-introducing beneficial pest-protective endophytic microbes such as *Fusarium oxysporum* V5w2 into tissue culture banana plants was developed (Dubois et al., 2004; Paparu et al., 2006a,b; Sikora and Pocasangre, 2006; Sikora et al., 2008), while this endophyte-enhanced banana tissue culture technology had already been availed to farmers in Kenya and Uganda from the year 2006 (Dubois et al., 2006, 2011; zum Felde, 2011; Waweru et al., 2014). Induced systemic resistance is the main mechanism through which *F. oxysporum* V5w2 among other strains suppress various pests and diseases of banana (Vu et al., 2006; Paparu et al., 2007, 2009a,b, 2010, 2013). However, future research is still necessary to elucidate the mechanisms underpinning nematode suppression and increased banana yield observed after treatment with *F. oxysporum* V5w2 (Kisaakye et al., 2022). Besides this, *F. oxysporum* V5w2 has been reported to be a non-beneficial endophyte that interacts with *R. similis* in a wilt disease complex of banana (Ochieno, 2020, 2021). Therefore, more information has still been pending in the registration of *F. oxysporum* V5w2 as a biopesticide through the Kenya Standing Technical Committee on Imports and Exports of live organisms (KSTCIE) (Hoeschle-Zeledon et al., 2013; Avedi et al., 2014; KEPHIS, 2014; Ochieno, 2020). This is partly because in the year 2008, the concept of using various strains of *F. oxysporum* as endophytic beneficial microbes in banana and other crops was considered not well-understood (Sikora et al., 2008).

In the year 2010, induced systemic resistance was found to be unlikely mechanism of *F. oxysporum* V5w2, while its pathogenicity remained unresolved despite the fungus not being genetically very closely related to the three pathogenic strains of *F. oxysporum* f. sp. *cubense* (Foc) tropical race 4 that included Fo001 (VCG 0120), JC1 (VCG 01210), and II5 (VCG 01213) (Kurtz, 2010). Furthermore, *F. oxysporum* V5w2 was among 16 strains originating from Uganda, Kenya, Costa Rica, Cuba and Indonesia that were found not to be compatible with any known vegetative compatibility group (VCG) of *F. oxysporum* f. sp. *cubense* or with any non-pathogenic isolate (Niere, 2001; Kurtz, 2010). Also, it was concluded that *F. oxysporum* V5w2 was a plant growth suppressing microbe (Ochieno, 2010) (**Figures 1A,B**), which has the potential of invading banana xylem through the rhizosphere just like the known pathogenic strains (Paparu et al., 2009a; Warman and Aitken, 2018; Ochieno, 2020). *F. oxysporum* V5w2 was therefore considered not to be a beneficial endophyte that offers biological control of banana pests through induced systemic resistance mechanisms (Ochieno, 2020). However, between the year 2010 and 2014, there were more evidences supporting the retention of *F. oxysporum* V5w2 as an endophytic biological control agent of banana pests (Paparu et al., 2010,
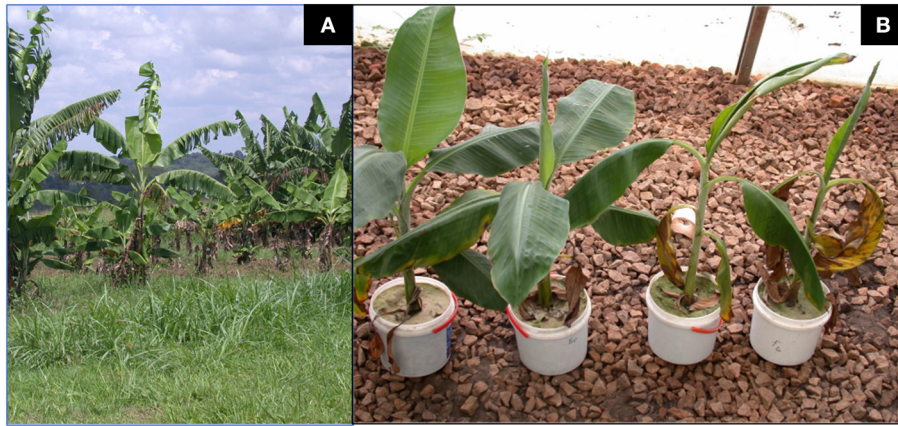
FIGURE 1 | Tissue culture banana plants inoculated with strains of *Fusarium oxysporum*. **(A)** An experimental plot of East African Highland Cooking Banana plants (genomic group *Musa* AAA-EA, cv. *Kisansa*) inoculated with wild-type *F. oxysporum* V5w2 expressing stuntedness and yellowing of leaves flanked by two plots with relatively healthier plants of the same cultivar. The plants had been transferred to the field on 18th January 2007 and the photograph taken 10 months later on 19th November 2007 (Source: Own work). **(B)** Potted East African Highland Cooking Banana plants (genomic group *Musa* AAA-EA, cv. *Kibuzi*) co-inoculated inoculated with chlorate-resistant *F. oxysporum* V5w2 and *Radopholus similis* expressing wilt-like symptoms *(2 right)* and those inoculated with *F. oxysporum* V5w2 only *(2 left)*. All plants were grown in sterile beach sand collected from Lake Victoria and supplied with phosphorus-deficient nutrient solution (Source: Own work).

2013; Dubois et al., 2011; zum Felde, 2011; Waweru et al., 2013, 2014). Issues concerning the application of *F. oxysporum* V5w2 as an endophytic biological control agent have since remained in an open debate requiring further evidences toward consensus (Ochieno, 2020).

The objective of the present study was to determine the effect of soil sterilization, and hence elimination of soil biota, on the suppression of *R. similis* and *F. oxysporum* V5w2 in tissue culture banana plants grown under adequate supply of nutrients. It is hypothesized that under proper nourishment, rhizosphere biota positively interact with banana roots to suppress *R. similis* and *F. oxysporum* V5w2 thereby promoting plant growth.

MATERIALS AND METHODS

Experimental Design

The treatments, viz. *R. similis* nematode vs. no nematode, *F. oxysporum* vs. no fungus, sterile vs. non-sterile soil were studied in a full factorial experiment ($2 \times 2 \times 2$). This resulted in 8 treatment combinations that comprised the untreated controls, *R. similis*, *F. oxysporum*, and *R. similis* + *F. oxysporum*, each having been conducted in sterile and non-sterile soil. The eight (8) treatment combinations, each having n=15 plants, were in three (3) replicates and repeated twice (2) over time to result in nine (9) sets of plants per treatment combination. The plants were completely randomized in the screenhouse. One hundred days after the start of the experiment, data were collected on parameters that included (i) biomass (root, shoot dry weight, total biomass), (ii) percentage dead roots, (iii) percentage root necrosis, (iv) *R. similis* densities in roots and soil, as well as the presence of other nematode species (vi) number of standing leaves, (vii) leaf length and width, (viii) percentage root piece colonization by *F. oxysporum*, (ix) identities of root-invading

microbes and their interactions with *F. oxysporum*, (x) root color, (xi) optical density of root extracts, and (xii) leaf nutrient content.

Banana Plants

Tissue culture banana plants (genomic group AAA-EA, cv. *Kibuzi*) (Onguso et al., 2004; Akankwasa et al., 2021), were obtained from the Banana Tissue Culture Laboratory at Sendusu Field Station in IITA-Uganda. The plants had been micro-propagated by the shoot-tip culture technique in which plants are produced by multiplication of the meristematic tissue of banana corms (Vuylsteke, 1998). The banana plants had been maintained for 6 weeks in nutrient solution (1 g/L, Polyfeed™, Haifa Chemicals, Israel), after which they were graded into four sizes and distributed to eight groups of 15 plants. Four groups were marked for inoculation with *F. oxysporum* and the other four were unmarked.

Pre-planting initial plant growth parameters were recorded through a slight modification of the method described by Akello et al. (2007) and Paparu et al. (2009b). Initial plant height (the distance from the base of the plant to the youngest leaf axil), initial number of open leaves, and initial width (widest part of the lamina) and initial length (the distance from the leaf apex to the leaf stalk) of the largest open leaf were recorded using a measuring tape. Initial fresh weight of each plant was measured using a toploading laboratory balance (Mettler Toledo™, Columbus, Ohio, United States), and the number of healthy-looking functional roots recorded.

Fusarium oxysporum and *Radopholus similis* Inoculums

The fungus used was chlorate-resistant *Fusarium oxysporum* strain V5w2 that had originally been meant to be an endophytic biological control agent (Niere et al., 1999; Paparu et al., 2009b; Dubois et al., 2011; zum Felde, 2011; Waweru et al., 2013,

2014), but currently considered to be a non-beneficial plant growth suppressing microbe (Ochieno, 2010, 2020). Inoculum was prepared by sprinkling the soil culture onto 90 mm diameter glass Petri dishes containing half-strength PDA (19 g/L, Sigma-Aldrich, Germany). Ten days later, the fungal spores (conidia) and mycelia were scraped from the media into a 500 mL beaker containing sterile water. The spore mixture was sieved (1 mm aperture) and homogenized, and then adjusted to 1.5×10^6 spores/mL.

Radopholus similis were obtained from cultures aseptically maintained on carrot discs at the Nematology Laboratory in Sendusu Field Station of IITA-Uganda (Speijer and De Waele, 1997; Ochieno, 2010). The nematodes were supplied in sterile water suspension containing mixed stages of 250 *R. similis* /mL.

Soil Substrate

Loamy top soil (0–10 cm) was collected from a bare 5-year-old banana field at the Sendusu Field Station of IITA-Uganda. The soil at this site has been classified as isohyperthermic Rhodic Kandiudalf (USDA taxonomy) (McIntyre et al., 2000). The soil was sieved (5 mm aperture) and thoroughly mixed. Soil samples were analyzed for chemical properties at the National Agricultural Research Laboratories at Kawanda-Uganda. Available phosphorus (P) and exchangeable potassium (K) were extracted using the Mehlich-3 method (Mehlich, 1984). Phosphorus in the extract was determined using the molybdenum blue colorimetric method and potassium using a flame photometer (Okalebo et al., 2002). Total nitrogen (N) was analyzed by Kjeldahl oxidation and semi-micro Kjeldahl distillation (Bremner, 1960). Organic matter (OM) was determined using the Walkley-Black method (Walkley, 1947). Soil pH was analyzed using de-ionized water with a soil to water ratio of 1:2.5. The soil contained N (0.15%), P (3.6 ppm), K (0.38 $cmol_c$/kg), OM (2.4%) and a pH of 5.1. Part of the loamy soil was steam-sterilized at $100°C$ for 1 h using an electrode steam conditioner (Model ESC40, Marshall-Fowler, South Africa).

Fungal Inoculation, Planting, and Nematode Infestation

Plants that were marked for *F. oxysporum* V5w2 treatment were inoculated by the root-dipping technique (Paparu et al., 2006a; Ochieno, 2010), except that in the present study the roots were left undamaged. The roots were immersed for 4 h into a 1.5 L spore suspension contained in tubs (30 × 25 × 15 cm, length × width × height). Control plants were immersed in distilled water.

The banana plants were grown in 2.5 L buckets containing either sterile or non-sterile soil under screenhouse conditions (25 ± 3°C, 70–75% RH, 12L: 12D photoperiod). Post-planting initial height, plus length and width of the youngest open leaf were recorded. The plants were supplied with rain water (100 mL) daily and a complete nutrient solution (CNS, 100 mL) weekly. One liter of CNS contained 1,650 mg NH_4NO_3, 1900 mg KNO_3, 440 mg $CaCl_2.2H_2O$, 370 mg $MgSO_4.7H_2O$, 170 mg KH_2PO_4, 37.3 mg $Na_2EDTA.2H_2O$, 27.8 mg $FeSO_4.7H_2O$, 6.2 mg H_3BO_3, 22.3 mg $MnSO_4.4H_2O$, 8.6 mg $ZnSO_4.7H_2O$, 0.83 mg KI, 0.25 mg $Na_2MoO_4.2H_2O$, 0.025 mg $CuSO_4.5H_2O$, and 0.025 mg $CoCl_2.6H_2O$ (Murashige and Skoog, 1962). Twenty days after

planting, three holes (5 cm deep) were made into the soil around the base of each plant, by using a disinfected stick. Each plant was inoculated with 500 *R. similis* in 2 mL of the nematode suspension distributed across the three holes before covering them with soil.

Data Collection

At 100 days after nematode inoculation, final plant growth parameters that included plant height (the distance from the base of the plant to the youngest leaf axil), number of fully developed leaves, and width (widest part of the lamina) and length (the distance from the leaf apex to the leaf stalk) of the youngest leaf were recorded using a measuring tape based on Akello et al. (2007) and Paparu et al. (2009b). Plants were harvested and the number of dead and healthy roots were recorded and used to calculate the percentage dead roots. The shoots and roots were detached and their fresh weights recorded using a toploading laboratory balance (Mettler Toledo™, Columbus, Ohio, United States). Root and shoot biomasses were combined to obtain total fresh weight. The roots were preserved at 4°C within 3 days. The shoots were oven-dried at 70°C for 14 days to determine dry weight. From these drying banana shoots, two youngest open leaves were pre-selected from seven randomly chosen banana plants for the determination of nutrient contents. Samples for each banana plant were mixed and analyzed separately for nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and Zinc (Zn) in the Soil and Plant Analytical Laboratories at Kawanda Agricultural Research Institute, Kampala, Uganda.

From the preserved banana roots, 5 roots per plant were randomly selected for assessment of necrosis caused by nematodes, and for estimation of nematode densities. Three roots were randomly selected per plant to estimate the level of colonization by *F. oxysporum* V5w2 and identify other endophytic microbes.

Assessment of root necrotic lesions caused by *R. similis* and quantification of nematode densities were undertaken based on the methods of Speijer and De Waele (1997) and Brooks (2004). Each of the five roots selected per plant for necrosis assessment was cut into 10 cm length. The pieces were then split longitudinally and one half selected from each of them. The cumulative length of necrotic lesions of cortical tissue on each 10 cm side of the stele was recorded as a fraction of the total 20 cm length per root. The sum length of the necrotic tissue for five roots per plant was then expressed as percentage necrosis. These roots were chopped into small pieces and 5 g sub-samples were mixed with 50 mL of water, and macerated at medium speed for 20 s using a Waring® laboratory blender. Nematodes were extracted from the suspension using a Baermann tray for 24 h. The extracts were transferred into 100 mL glass bottles and stored at 4°C for 24 h to allow the nematodes to sediment. The water volume was standardized to 25 mL by gentle siphoning from the surface. The suspension was homogenized by stirring, and nematodes quantified on a counting dish at ×10 magnifications from triplicate 2.5 mL pipette sub-samples. The nematode densities were expressed as *R. similis* counts per 100 g of root material. To investigate whether

densities of *R. similis* varied in the rhizosphere between sterile and non-sterile soil, eight 100 mL soil samples were selected from each soil treatment. This was done for plants that were treated with *R. similis* only. *R. similis* and other nematode species in the soil samples were extracted using a Baermann tray over a period of 48 h. The extract was concentrated to 25 mL followed by concurrent morphological identification nematode species and

absolute counts of *R. similis* in the sample based on nematode identification keys (Fortuner, 1988; Luc et al., 2005).

Each of the three roots selected for estimation of colonization by *F. oxysporum* V5w2 and infection by other endophytic microbes was surface-sterilized by dipping it into 96% ethanol followed by flaming. This ensured that only microbes within the tissues remained alive. Each root was then cut into six pieces. The root pieces were plated on 60 mm glass Petri dishes containing PDA enriched with $KClO_3$ for 7 days. Growth of *Fusarium* strains including V5w2 on PDA is characterized by whitish mycelia with pink pigmentation (Leslie and Summerell, 2008). The number of root pieces with growth of *Fusarium* species among six assessed pieces was recorded fungal endophyte colonization. Root piece colonization was the percentage of all root pieces yielding *Fusarium* among all assessed pieces within a treatment. Other root-invading microbes growing from the root pieces were sub-cultured and morphologically identified through the TSBF-CIAT project on Below-Ground Biodiversity (BGBD) funded by GEF-UNEP at the Soil Microbiology Laboratory, Makerere University, Uganda (Nakintu et al., 2008; Ochieno, 2010). *In vitro* growth inhibition tests between the microbes and *F. oxysporum* V5w2 were conducted. *Fusarium oxysporum* V5w2 was inoculated in the center of a glass Petri dish containing PDA using a sterile needle. The microbes were inoculated on four spots surrounding the fungal pathogen. The fungal pathogen or the test microbe was considered inhibitive if its mycelia grew into the colonies of the other marked with a boundary between them.

It was realized that roots that were stored at 4°C exhibited differences in color after 3 days. Therefore, visual and colorimetric tests on roots and their extracts was conducted to assess possible effects of the experimental treatments on root color. Roots from all plants per treatment were mixed. This was followed by random selection of up to 30 roots from every treatment; the color of the external root surface was visually recorded as either brown or light-brown by comparing with two roots that had been pre-selected as standards for the two colors. For colorimetric assessments, five roots from three plants per treatment were chopped together into a composite
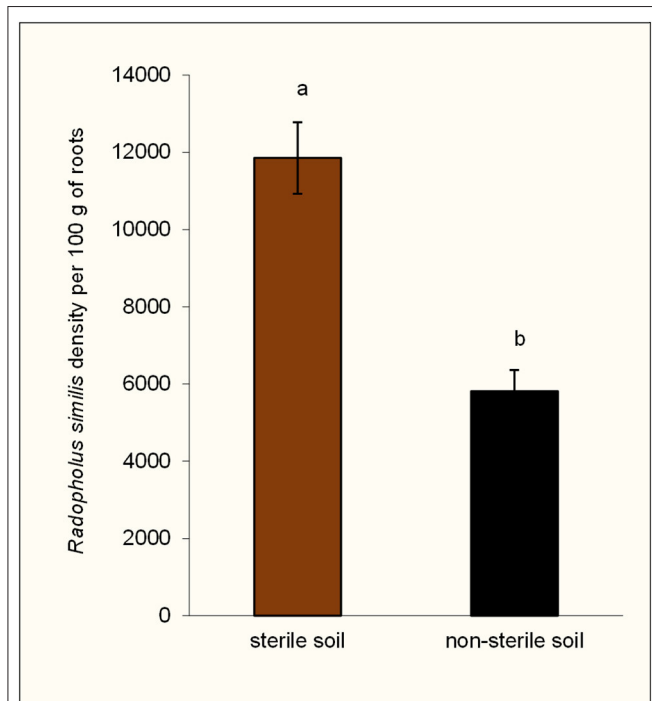
FIGURE 2 | Density of *Radopholus similis* in the rhizospheres of potted tissue culture banana plants grown in non-sterile soil and sterile soil after 100 days of inoculation with the nematode. Bars with the same letter represent values that are not statistically different (df = 1, F = 24.24, p = 0.0002).

FIGURE 3 | Banana root damage caused by *Radopholus similis*. **(A)** Healthy-looking banana roots *(left)* and those having necrotic lesions associated with *Radopholus similis* infection *(right)*. **(B)** Female *R. similis* in root cortical tissues of banana plants.

Soil Sterilization Eliminates Beneficial Microbes

TABLE 1 | Effect of soil sterility on infection symptoms of *Radopholus similis* and *Fusarium oxysporum* in tissue culture banana plants grown under adequate nutrient supply.

Source of variation	df	Root damage		*R. similis*	Biomass: freshweight (fwt) and dryweight (dwt)				Height	Leaves		
		Death	Necrosis		Root fwt	Shoot fwt	Shoot dwt	Total fwt		Number	Length	Width
F-VALUES												
Main effects												
R. similis (Rs)	1	1694.0***	1434.0***	232.0***	109.0***	41.7***	11.5**	64.2***	40.7***	14.9***	44.5***	25.8***
F. oxysporum (Fo)	1	0.1	2.0	0.8	3.6	0.1	0	0	0.4	1.1	0	1.1
Soil sterility (S)	1	63.0***	0.1	24.2***	74.0***	0.4	8.9**	9.2**	39.7***	259.7***	49.7***	114.5***
Two-way interactions												
Rs × *Fo*	1	1.0	0.3	2.5	2.2	2.9	0	2.4	0.7	0.3	1.8	0.2
Rs × S	1	109.3***	6.9**	35.7***	31.5***	10.3**	0.4	17.7***	1.2	12.2**	8.2**	5.6**
Fo × S	1	2.1	0.4	0.3	0.2	0	1.0	0.1	0.1	0	0	0.3
Three-way interactions												
Rs × *Fo* × S	1	0.2	0.4	0	0.8	0.7	0.1	0.5	4.0	0.6	1.2	0.1
c.v.		74.3	73.7	197.5	30.1	28.1	9.7	28.8	33.3	23.6	24.1	26.6
Degrees of freedom	7	1,048	1,044	1,035	1,048	1,048	1,045	1,050	1,049	1,047	1,047	1,047
MEANS												
		percent	percent	count	gram	gram	gram	gram	cm	count	cm	cm
Grand mean		14.3	17.2	4,541	54.2	205.4	21.6	259.2	38.0	5.5	42.8	19.3
Nematode	*R. similis*	**28.4 a**	**33.5 a**	**8,770 a**	**47.7 b**	**194.0 b**	**20.7 b**	**240.8 b**	**36.5 b**	**5.4 b**	**41.6 b**	**18.9 b**
	None	**0.6 b**	**1.1 b**	**385 b**	**60.6 a**	**216.7 a**	**22.5 a**	**277.3 a**	**39.4 a**	**5.6 a**	**43.9 a**	**19.8 a**
Fungus	*F. oxysporum*	14.2 a	16.7 a	4,763 a	53.0 a	206.0 a	21.5 a	259.0 a	37.8 a	5.4 a	42.7 a	19.4 a
	None	14.4 a	17.7 a	4,323 a	55.4 a	204.9 a	21.7 a	259.3 a	38.1 a	5.5 a	42.8 a	19.3 a
Soil	Sterile	**18.9 a**	17.7 a	**5,919 a**	**48.7 b**	204.5 a	**22.3 a**	**252.2 b**	**36.5 b**	**5.0 b**	**41.5 b**	**18.4 b**
	Non-sterile	**9.9 b**	16.8 a	**3,223 b**	**59.6 a**	206.4 a	**20.9 b**	**266.0 a**	**39.4 a**	**5.9 a**	**44.0 a**	**20.2 a**
R. similis	Sterile	**38.1 a**	**34.9 a**	**11,853 a**	**38.6 c**	**187.1 c**	21.4 a	**223.9 c**	34.7 a	**4.8 c**	**39.8 c**	**17.8 c**
	Non-sterile	**19.0 b**	**32.3 b**	**5,816 b**	**56.6 b**	**200.6 b**	20.0 a	**257.2 b**	38.2 a	**5.9 ab**	**43.4 b**	**20.0 a**
No *R. similis*	Sterile	**0.1 c**	**0.7 c**	**79 cd**	**58.6 ab**	**221.4 a**	23.2 a	**280.0 a**	38.2 a	**5.2 b**	**43.2 b**	**19.1 b**
	Non-sterile	**1.1 c**	**1.5 c**	**678 c**	**62.5 a**	**212.0 a**	21.8 a	**274.6 a**	40.5 a	**5.9 a**	**44.6 a**	**20.4 a**

*Asterisks indicate significant effect (***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05); Means for significant effects are indicated in bold font; those with the same letter are not significantly different (p > 0.05).*
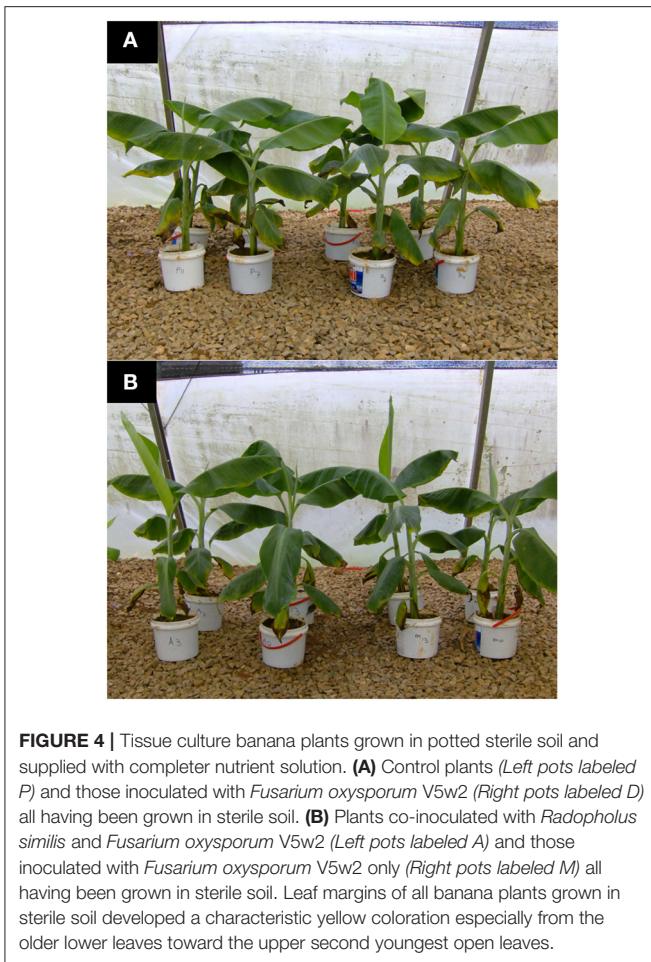
FIGURE 4 | Tissue culture banana plants grown in potted sterile soil and supplied with completer nutrient solution. **(A)** Control plants *(Left pots labeled P)* and those inoculated with *Fusarium oxysporum* V5w2 *(Right pots labeled D)* all having been grown in sterile soil. **(B)** Plants co-inoculated with *Radopholus similis* and *Fusarium oxysporum* V5w2 *(Left pots labeled A)* and those inoculated with *Fusarium oxysporum* V5w2 only *(Right pots labeled M)* all having been grown in sterile soil. Leaf margins of all banana plants grown in sterile soil developed a characteristic yellow coloration especially from the older lower leaves toward the upper second youngest open leaves.

sample. This was conducted in triplicate. Ten grams of each composite sample were mixed with 100 mL distilled water, and macerated using a Waring Laboratory Blender (Christison Scientific, Gateshead, UK). The root extracts were diluted (10×) and their optical density (absorbance) measured at 320 nm using a spectrophotometer (Genesys 10 UV, Thermo Fisher Scientific, USA).

Statistical Analysis

Data were analyzed using SAS 9.1 software (SAS, 2004). Diagnostic check for normality was conducted using Proc Univariate. Proc Transreg was used to find appropriate Box-Cox transformations, which included generation of suitable powers or lambda (λ) for data (Y) that required transformation. Percentage dead roots and percentage necrosis (x) were arcsine$\sqrt{(x/100)}$ transformed. No transformation was done on data for other parameters. Proc GLM was used for three-way factorial analyses of variance (ANOVA) among the treatments. Mean separation was done by *t*-tests (LSD) when there were significant differences among treatment means. Mean separation of optical density of root extracts was done by LS-Means with Bonferroni adjustment for multiple comparisons. Root piece colonization by *Fusarium* was analyzed by Proc Genmod as data having binomial

distribution; Bonferroni-adjusted *p*-values were obtained by Proc Multtest from raw *p*-values in Proc Genmod, and used for pairwise comparisons between treatments. Root coloration data were analyzed by Proc Genmod and pairwise comparisons using contrasts.
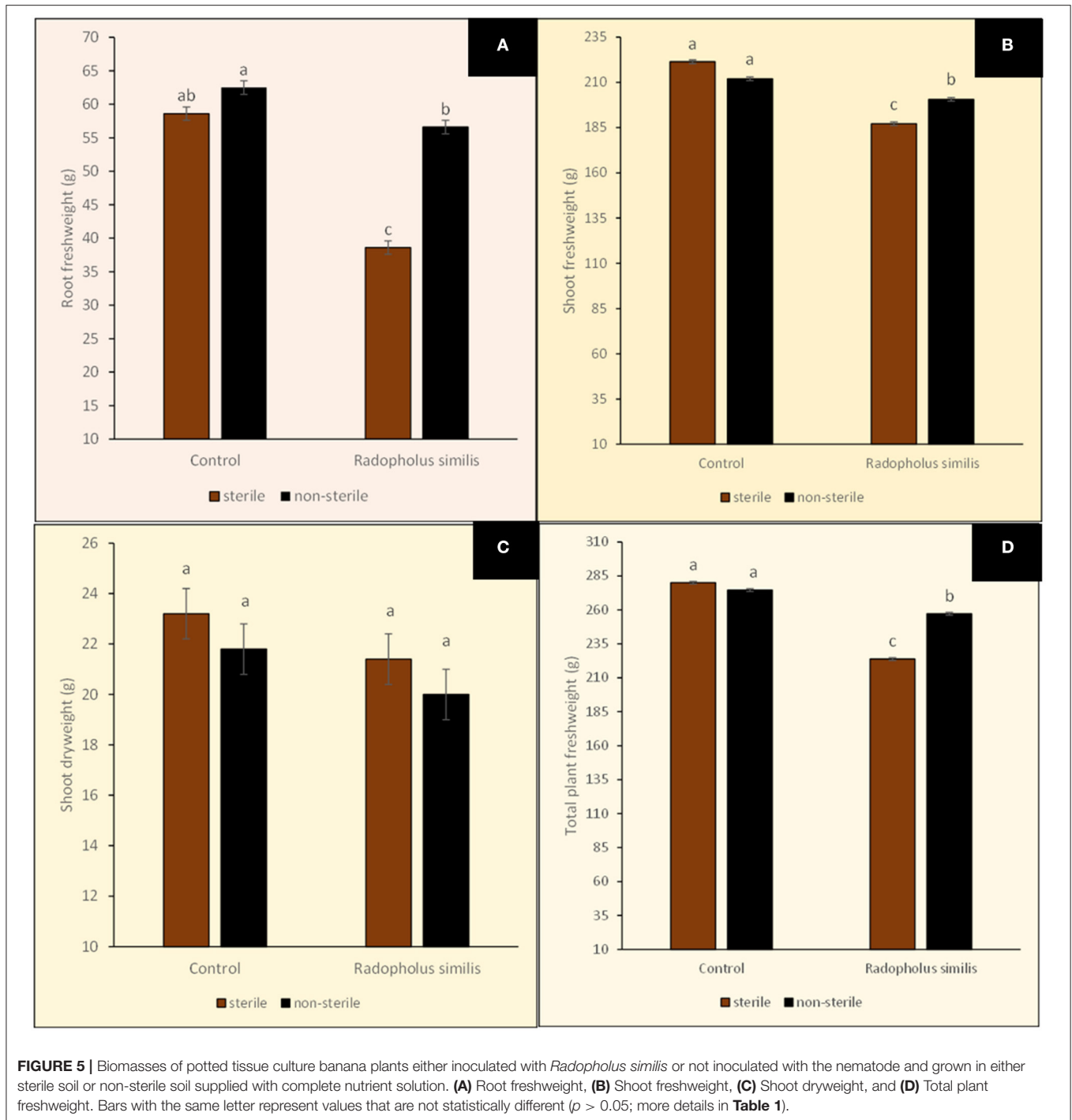
RESULTS

Pre-planting initial plant growth parameters did not vary between the treatments, and they included fresh weight (7.9 ± 0.13 g), plant height (5.5 ± 0.08 cm), number of leaves (3.4 ± 0.02), leaf length (12.2 ± 0.10 cm), leaf width (4.6 ± 0.05 cm), and number of functional roots (3.6 ± 0.04) ($n = 1,084$, mean ± SE, *F*-test, $p > 0.05$).

At the end of the experiment, rhizosphere populations of *Radopholus similis* in pots of nematode-inoculated plants were significantly higher in sterile soil than in non-sterile soil (**Figure 2**). Dead roots and those having necrotic lesions were mainly found in *R. similis*-inoculated plants (**Figure 3A**). Percentage dead roots in sterile soil was significantly higher than in non-sterile soil (**Table 1**). Percentage root necrosis was significantly higher in *R. similis*-inoculated plants than in plants not inoculated with the nematode (**Table 1**).

R. similis were mainly found in plants inoculated with the nematode (**Figure 3B**), and their density in roots from sterile soil was higher than in roots from non-sterile soil (**Table 1**). Thirteen other nematode species, identified mainly in non-sterile soil among the soil samples, included; the plant parasitic *Helicotylenchus multicintus*, *Meloidogyne* spp., *Pratylenchus goodeyi*, *Xiphinema* spp., *Trophorus* spp., *Scutellonema* spp., *Rotylenchus* spp., *Tylenchus* spp., *Hemicycliophora* spp., *Paratrichodorus* spp., and *Ditylenchus* spp. Saprophytic genera included *Aphelenchus* spp. and *Rhabditis* spp. among numerous others that could not be identified. The first three nematode species were frequently recorded in root samples from non-sterile soil.

Banana plants in the different treatments showed no visually clear differences in their size (**Figure 4**). However, leaf margins of all banana plants grown in sterile soil developed a characteristic yellow coloration especially from the older lower leaves toward the upper second youngest open leaves (**Figure 4**). This discoloration of leaf margins did not occur in banana plants grown in non-sterile soil. *R. similis*-inoculated plants from non-sterile soil had more biomass (root freshweight, shoot freshweight, total freshweight) than those from sterile soil (**Figure 5**; **Table 1**). *R. similis*-inoculated plants had lower shoot dry weights than those not inoculated with the nematode (**Figure 5**; **Table 1**). Plants from non-sterile soil had lower shoot dry weights than those from sterile soil (**Figure 5**; **Table 1**).

Leaves of *R. similis*-inoculated plants from sterile soil were fewer, shorter and narrower than for plants from non-sterile soil (**Figure 6**; **Table 1**). The *R. similis*-inoculated plants were shorter than plants that were not inoculated with the nematode (**Figure 6**; **Table 1**). Plants in non-sterile soil were taller than plants from sterile soil (**Figure 6**; **Table 1**). *Fusarium oxysporum* V5w2 inoculant did not induce significant effects on all the
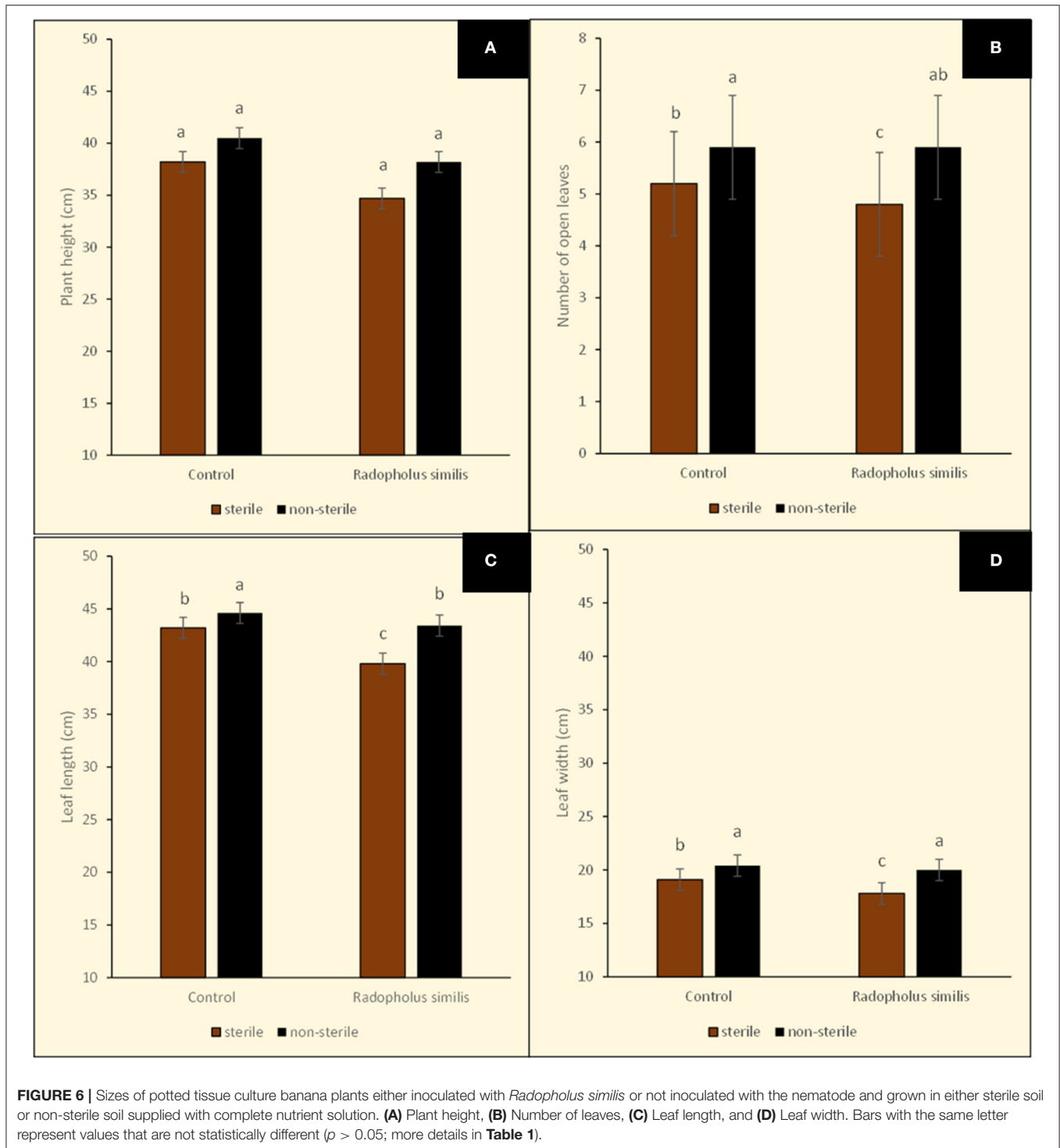
FIGURE 5 | Biomasses of potted tissue culture banana plants either inoculated with *Radopholus similis* or not inoculated with the nematode and grown in either sterile soil or non-sterile soil supplied with complete nutrient solution. **(A)** Root freshweight, **(B)** Shoot freshweight, **(C)** Shoot dryweight, and **(D)** Total plant freshweight. Bars with the same letter represent values that are not statistically different ($p > 0.05$; more details in **Table 1**).

assessed plant growth-related parameters, *R. similis* density and root damage by the nematode (**Table 1**).

In the main treatment effects and interactions, leaf tissue nutrient analysis showed that plants grown in non-sterile soil had higher concentrations of N, P, K, Ca and Zn but lower Mg when compared to those grown in sterile soil (**Table 2**). In the main treatment effects, the concentrations of N, K, Ca, and Mg were higher in *R. similis*-inoculated plants while P and Zn were

lower when compared to those not inoculated with the nematode (**Table 2**). In the main treatment effects, the concentrations of N, P, K and Zn were higher in *F. oxysporum* V5w2-inoculated plants while Ca and Mg were lower when compared to those not inoculated with the fungus (**Table 2**).

Fusarium spp. were recorded in roots from all the eight treatments including the controls (**Figure 7**). The proportion of root pieces that yielded *Fusarium* spp. was lower than 30%;

FIGURE 6 | Sizes of potted tissue culture banana plants either inoculated with *Radopholus similis* or not inoculated with the nematode and grown in either sterile soil or non-sterile soil supplied with complete nutrient solution. **(A)** Plant height, **(B)** Number of leaves, **(C)** Leaf length, and **(D)** Leaf width. Bars with the same letter represent values that are not statistically different ($p > 0.05$; more details in **Table 1**).

percentage root piece colonization by *Fusarium* spp. was lowest in the control in sterile soil and highest in the *R. similis* + *F. oxysporum* V5w2 treatment in sterile soil, the other six treatments were not different (**Figure 7**).

Fusarium oxysporum V5w2 could not be visually distinguished from other *Fusarium* spp. that grew from

root pieces that had been plated in $KClO_3$-enriched PDA (**Figure 8**). Twenty-five other endophytic fungi were isolated from the banana root pieces (**Figure 8**), 11 genera among them were identified and included; *Fusarium* spp., *Rhizoctonia* spp., *Trichoderma* spp., *Sclerotium* spp., *Bipolaris* spp., *Curvularia* spp., *Mucor* spp., *Rhizopus* spp., *Papulaspora*

TABLE 2 | Nutrient content in banana leave tissues from plants under independent and combined inoculation with *Radopholus similis* and *Fusarium oxysporum* V5w2 having grown in sterile or non-sterile soil and supplied with complete nutrient solution.

Treatments		N %	P %	K %	Ca %	Mg %	Zn (ppm)
	Grand mean	2.93	0.14	3.86	1.47	0.64	6.16
Nematode	*R. similis* (Rs)	2.96	0.14	3.94	1.58	0.64	5.96
	None	2.90	0.15	3.77	1.37	0.63	6.37
Fungus	*F. oxysporum* V5w2 (Fo)	3.01	0.15	3.94	1.46	0.63	6.20
	None	2.86	0.14	3.77	1.49	0.64	6.13
Soil	Sterile	2.84	0.12	3.74	1.47	0.68	5.36
	Non-sterile	3.02	0.16	3.97	1.48	0.59	6.97
Rs × Fo × Soil	Sterile	2.97	0.13	3.76	1.13	0.65	5.61
	Non-sterile	2.86	0.16	3.79	1.66	0.61	7.21
	Sterile + Rs	2.75	0.11	3.55	1.53	0.68	4.40
	Non-sterile + Rs	2.87	0.17	3.98	1.62	0.62	7.29
	Sterile + Fo	2.95	0.13	3.71	1.48	0.70	5.66
	Non-sterile + Fo	2.85	0.16	3.84	1.22	0.56	7.01
	Sterile + Rs + Fo	2.71	0.11	3.96	1.72	0.71	5.77
	Non-sterile + Rs + Fo	3.51	0.17	4.27	1.44	0.56	6.36

spp., *Pleurophragmium* spp., and *Trichocladium* spp. At least 14 of the microbes that included *Rhizoctonia* spp., *Trichoderma* spp., *Sclerotium* spp., *Curvularia* spp., *Rhizopus* spp., *Papulaspora* spp., *Pleurophragmium* spp., and *Trichocladium* spp. inhibited *F. oxysporum* V5w2 *in vitro*; seven were inhibited by *F. oxysporum* V5w2 among them *Sclerotium* sp. *Bipolaris* and *Mucor* among other unidentified microbes showed no negative interactions with *F. oxysporum* V5w2. Among the *Fusarium* spp. that were identified, some inhibited *F. oxysporum* V5w2, others were inhibited while the remaining ones did not interact antagonistically with the inoculated fungus.

The four groups of *F. oxysporum* V5w2-inoculated plants varied significantly in frequency of externally light-brown colored roots, with absence of light-brown roots in plants that were not inoculated with the fungus (**Figure 9**). In both sterile and non-sterile soil, *F. oxysporum* V5w2-inoculated plants had higher frequency of light-brown roots as compared to those inoculated with *R. similis* + *F. oxysporum* V5w2. The frequency of light-brown roots was higher in sterile than in non-sterile soil in both *F. oxysporum* V5w2 and *R. similis* + *F. oxysporum* V5w2 treatments.

Optical density of root extracts from plants that were treated with the *F. oxysporum* V5w2 or with *R. similis* was lower than for control plants (**Figure 10**). The combination of *R. similis* and *F. oxysporum* V5w2 treatment resulted in a similar optical density as any of the two treatments alone. Soil sterility did not affect the optical density of root extracts.

DISCUSSION

Under the conditions of the current study, increased necrosis and percentage dead roots were apparent in all banana plants that were inoculated with *R. similis* resulting in a lower living root biomass. This confirms the pest potential of *R. similis*

(Huang et al., 2019). Density of *R. similis* and percentage dead roots were lower in banana plants that were grown in non-sterile soil than in sterile soil. This is an indication of the presence of nematode-suppressive biota in the non-sterile soil (Topalović et al., 2020; Jayaraman et al., 2021). It is likely that soil biota such as the root-invading microbes that were identified in samples from non-sterile soil help suppress *R. similis* and other nematodes. Further suppression of soil populations of *R. similis* in the present experiment may have been a result of competition by the other identified plant parasitic nematodes namely; *H. multicintus*, *Meloidogyne* spp., and *P. goodeyi*. Competitive interaction between *R. similis* and other plant parasitic nematodes has been reported (Moens et al., 2006), with *R. similis* appearing to suppress the population of *Meloidogyne* spp. in roots of field-grown banana plants (Ochieno, 2010).

Banana plants grown in non-sterile soil exhibited better growth, had heavier roots and more leaves compared to those planted in sterile soil. This plant growth promotion in non-sterile soil has been attributed to root infection by beneficial microbes such as vesicular arbuscular mycorrhizal fungi (AMF) that symbiotically enhance the acquisition of nutrients, and especially phosphorus (Gough et al., 2020). Evidence from the present study shows that leaves from plants grown in non-sterile soil accumulated high concentrations of N, P, K, Ca, and Zn. Such effects on leaf nutrient content were also partly evident in plants inoculated with *R. similis* or *F. oxysporum* V5w2, considering that these organisms also are part of the functional biota in the non-sterile soil rhizosphere. The provision of complete nutrient solution may have shifted the function of rhizosphere biota toward improved nutrient acquisition (Ochieno, 2010). However, poor growth of banana plants in steam-sterilized soil may have been due to modification of nutrient compounds to less available forms (Evan et al., 2003; De Deyn et al., 2004; Dietrich et al., 2020). Soil sterilization therefore
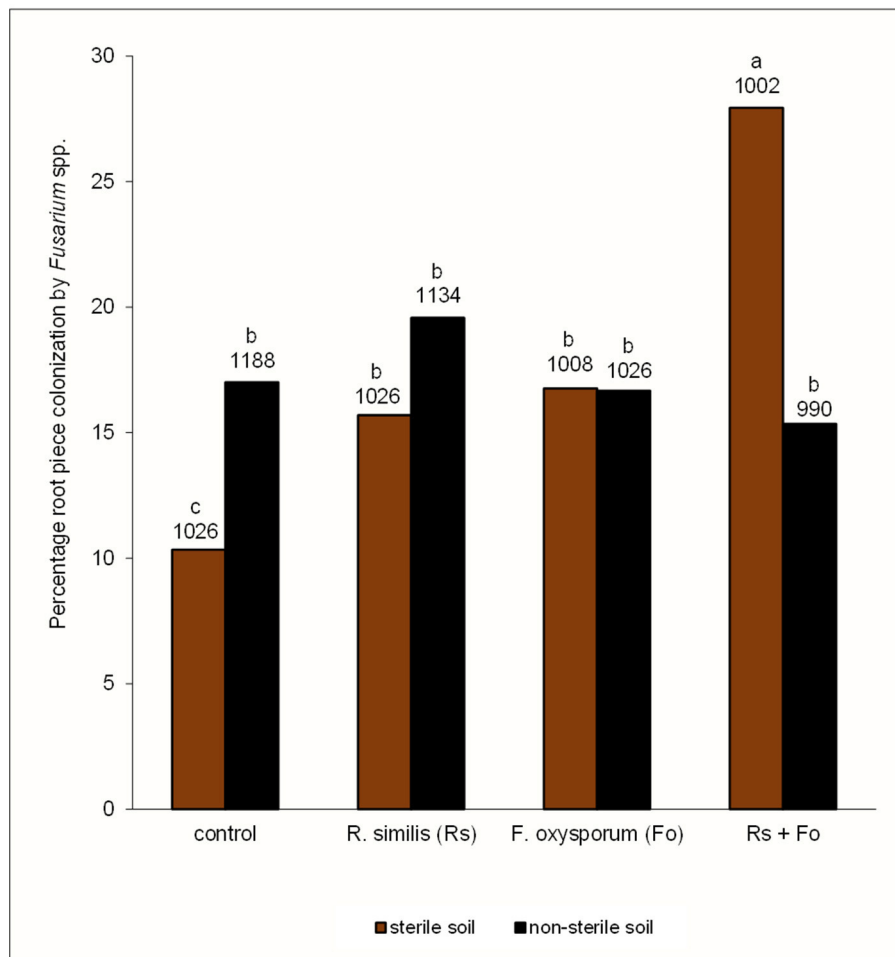
FIGURE 7 | Percentage root piece colonization by *Fusarium* spp. in potted tissue culture banana plants under independent or combined inoculation with *Fusarium oxysporum* V5w2 and *Radopholus similis*, grown in either sterile soil or non-sterile soil supplied with complete nutrient solution. Bars with the same letter represent values that are not statistically different ($p > 0.05$), with the sample sizes indicated below the letters (df $= 7$, $\chi^2 = 118.21$, $p < 0.0001$).

eliminates the rhizosphere ecosystem services associated with nutrient acquisition and cycling by beneficial microbes (Jacoby et al., 2017; Li et al., 2019).

Interestingly, banana plants grown in sterile soil had higher shoot dryweight than those from non-sterile soil. Although this increased shoot dryweight may be interpreted as better plant growth in sterile soil, the yellowing of banana leaf margins may point to an onset of a nutrient stress possibly related to magnesium deficiency (He et al., 2020). Banana plants growing under Mg deficiency exhibit the accumulation of sugars like sucrose and amylose in the leaves that may have contributed to the increase in shoot dryweight (He et al., 2020; Ogura et al., 2020). However, in the present study, leaf tissues of banana plants grown in sterile soil had higher Mg content than those from non-sterile soil, raising questions on whether these plants were actually Mg deficient. It is possible that the banana plants mobilized Mg^{2+} from lower older leaves toward the upper younger ones in response to the nutrient

deficiency (Ogura et al., 2020). This may partly explain the higher Mg content in the sampled youngest open leaves of banana plants grown in sterile soil. There are varied explanations on the manifestation of Mg deficiency in plants (Hauer-Jákli and Tränkner, 2019; Chaudhry et al., 2021), considering that the associated symptoms may even be expressed in plants growing in soils with high contents of this very important nutrient (Jezek et al., 2015). For instance, Mg deficiency may be induced through antagonistic effects of K^+ or Ca^{2+} ions or decline in soil pH that limit the uptake and utilization of Mg^{2+} (He et al., 2020; Wang et al., 2020; Chaudhry et al., 2021). Although soil sterilization increases the availability of soil nutrients (De Deyn et al., 2004), this may also create nutrient imbalances that may affect the uptake and utilization of Mg^{2+} by plants (Raghupathi et al., 2002; Wairegi and van Asten, 2011). This may have been the case in the present study, considering that the banana plants grown in sterile soil kept receiving 100 mL of complete nutrient solution through
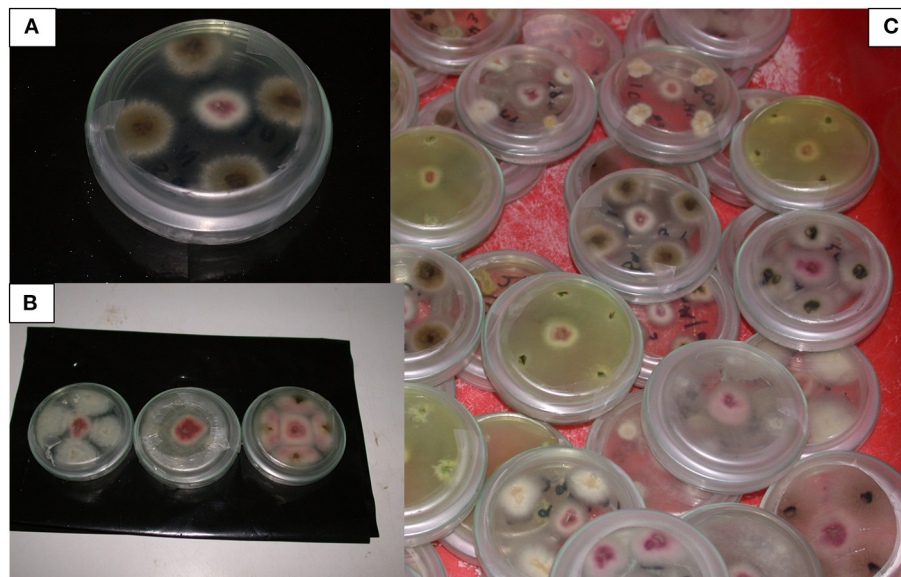
FIGURE 8 | *Fusarium* spp. and other endophytic microbes isolated from potted tissue culture banana plants under independent or combined inoculation with *Fusarium oxysporum* V5w2 and *Radopholus similis*, and grown in either sterile soil or non-sterile soil supplied with complete nutrient solution. **(A)** *F. oxysporum* V5w2 can be seen with pinkish-white mycelia at the central spot surrounded by an endophytic fungus with brownish mycelia isolated from the banana roots in a Petri dish containing potato dextrose agar (PDA). **(B)** *F. oxysporum* V5w2 at the central spot surrounded by *Fusarium* spp. isolated from non-inoculated plants *(third right)*. **(C)** Diversity of endophytic microbes isolated from roots of tissue culture banana plants especially those grown in non-sterile soil.

a microbe-deficient rhizosphere. Rhizosphere microbes in the non-sterile rhizospheres may have shared the supplied nutrients with banana roots while engaging in nutrient cycling activities (Shen et al., 2015). The growing banana plants may also have exhibited an increasing demand for nutrients, which was not properly addressed through the regular supply of 100 mL of complete nutrient solution without considering the size or age of the plants.

Radopholus similis density, root damage and plant growth parameters were not different between plants inoculated with the nematode and those co-inoculated with the nematode and *F. oxysporum* V5w2. These results are contrary to previous reports that have linked the suppression of *R. similis* by *F. oxysporum* V5w2 to its successful *in planta* establishment and induced resistance activities in banana roots (Niere et al., 1999; Vu et al., 2006; Paparu et al., 2009b; Waweru et al., 2013, 2014). The low optical density of root extracts from *F. oxysporum* V5w2-inoculated plants, and the light-brown coloration of their roots provide circumstantial evidence on the interaction between banana roots with the fungal pathogen. These observations on color change in roots and their extracts tended toward corroborating Sikora et al. (2008) "we noticed a distinct lack of root-rotting in excised banana roots following dual inoculation of non-pathogenic *F. oxysporum* and a lesion nematode *Pratylenchus goodeyi* in roots that we expected to be necrotic in this combination." The light-brown root color and the low optical density of their extracts could have been subtle browning reactions, probably due to consumption of organic nitrogen from the roots by *F.*

oxysporum V5w2 (Divon et al., 2005, 2006), resulting in higher concentration of unreacted phenolic compounds that are not dark colored (Bittner, 2006). Furthermore, the percentage of root pieces infected with *Fusarium* spp. was higher when *F. oxysporum* V5w2 and *R. similis* were both applied in sterile soil. *Fusarium oxysporum* V5w2 although not discernible from other *Fusarium* spp., may have exhibited better colonization of banana roots through wounds and necrotic tissues inflicted by *R. similis* (Ochieno, 2020). The *in vitro* tests indicated the presence of *Fusarium* spp. that did not interact inhibitively with *F. oxysporum* V5w2, and may be considered related to the inoculated fungus. Therefore, enhanced root piece colonization by *Fusarium* in roots of co-inoculated plants with *F. oxysporum* V5w2 and *R. similis* is an indicator of disease interactions between the two pathogens and other *Fusarium* spp.

It was evident that banana plants from non-sterile soil exhibited lower percentage root piece colonization by *Fusarium* spp. than those grown in sterile soil, and particularly those co-inoculated with *R. similis* and *F. oxysporum* V5w2. This still indicates the presence of pest-inhibitive biota in non-sterile soil (Kaushal et al., 2020; Zhang et al., 2020), which may have included *Trichoderma* spp., *Rhizoctonia* spp., and other *Fusarium* species that inhibited *F. oxysporum* V5w2 *in vitro*, as well as fungivore invertebrates including the saprophytic nematodes that were not identified in the present study. Inoculation of banana plants with *F. oxysporum* V5w2 has been found to result in wilt disease symptoms, especially when nutrient supply is limited or the plants are older (Ochieno, 2010, 2020, 2021).
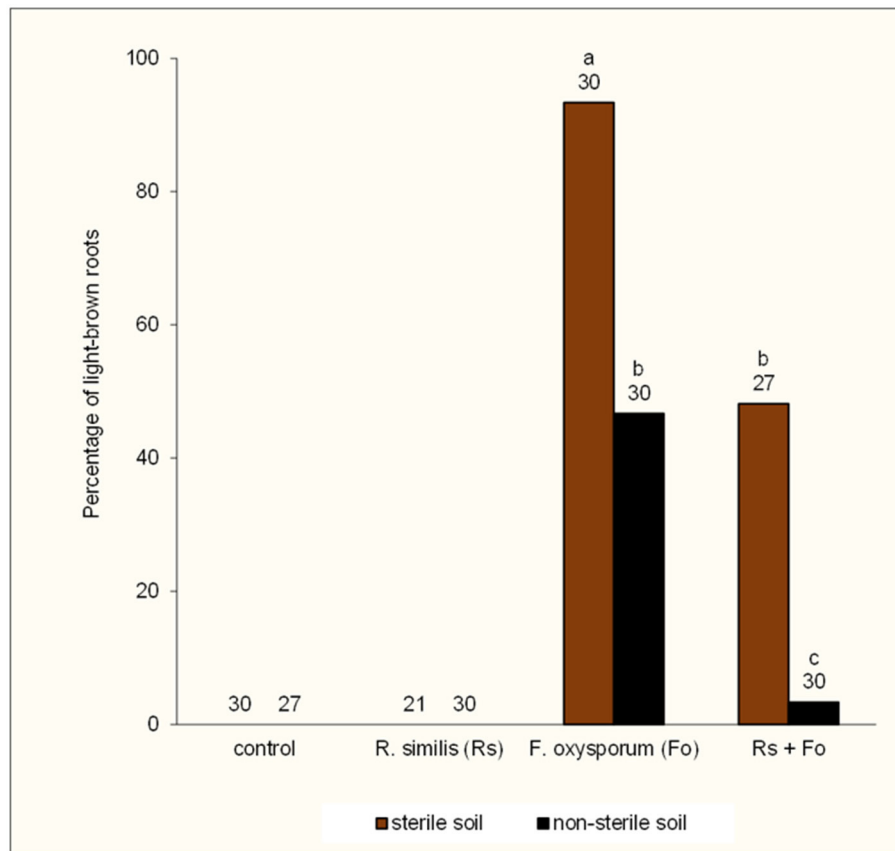
FIGURE 9 | Percentage of light-brown roots in potted tissue culture banana plants under independent or combined inoculation with *Fusarium oxysporum* V5w2 and *Radopholus similis*, and grown in either sterile soil or non-sterile soil supplied with complete nutrient solution. Bars with the same letter represent values that are not statistically different ($p > 0.05$), with the sample sizes indicated below the letters (df $= 3$, $\chi^2 = 59.67$, $p < 0.0001$).

This was not the case in the present study, probably due to the fact that the banana plants had been maintained under adequate nutrient supply while the experimental period was relatively short.

Results from the current study present contrasting aspects in the improvement of banana plant genetic materials through tissue culture technology. It is well-known that the aseptic tissue culture techniques intended to remove invertebrate pests and microbial pathogens from banana tissues (Van den Houwe et al., 2020), also do eliminate beneficial endophytic biota (Liu et al., 2019). However, in some cases, this view has been held with little attention on the removal of beneficial rhizosphere biota through soil sterilization as part of production of tissue culture banana plantlets (Ochieno, 2010, 2020). This is because soil sterilization is intended to eliminate microbial pathogens and plant parasitic nematodes among other pests (Li et al., 2019). However, the fact that *Fusarium* spp. were recovered from plants not inoculated with *F. oxysporum* V5w2 and grown in non-sterile soil points to the risk of spreading the fungus to pathogen-free areas. This is because cross-contamination and recolonization of tissue culture plants and sterilized soil with *Fusarium* spp. among

other microbial agents is a common problem (Kabuye et al., 2011; Warman and Aitken, 2018; Ochieno, 2020).

In the context of ecosystem services, tissue culture technology should be developed toward the multiplication of healthy mother plants without destruction of the beneficial microbes within the endosphere microbiome. This is because the highly stringent aseptic tissue culture techniques eliminate beneficial endophytic microbes (Bubici et al., 2019). The micro-propagated plants multiplied without elimination of beneficial microbes should be grown in healthy soils without destruction of the beneficial rhizosphere microbes. This is because soil sterilization is currently regarded as an unpopular technique because it kills all soil life (Li et al., 2019). Such a proposal would even make more sense with advancement in methods of profiling biota within endophytic and rhizosphere microbiomes of certified tissue culture mother plants. Probably, the concept of "Tissue Culture Microbiome Conservation Technology" could emerge as a new area of focus. Micropropagation techniques utilizing non-sterile explants have already been developed for conservation of endangered plant species (Bowes, 1990, 1999; Bowes and Curtis, 1991). This would link well with the concepts of natural biological
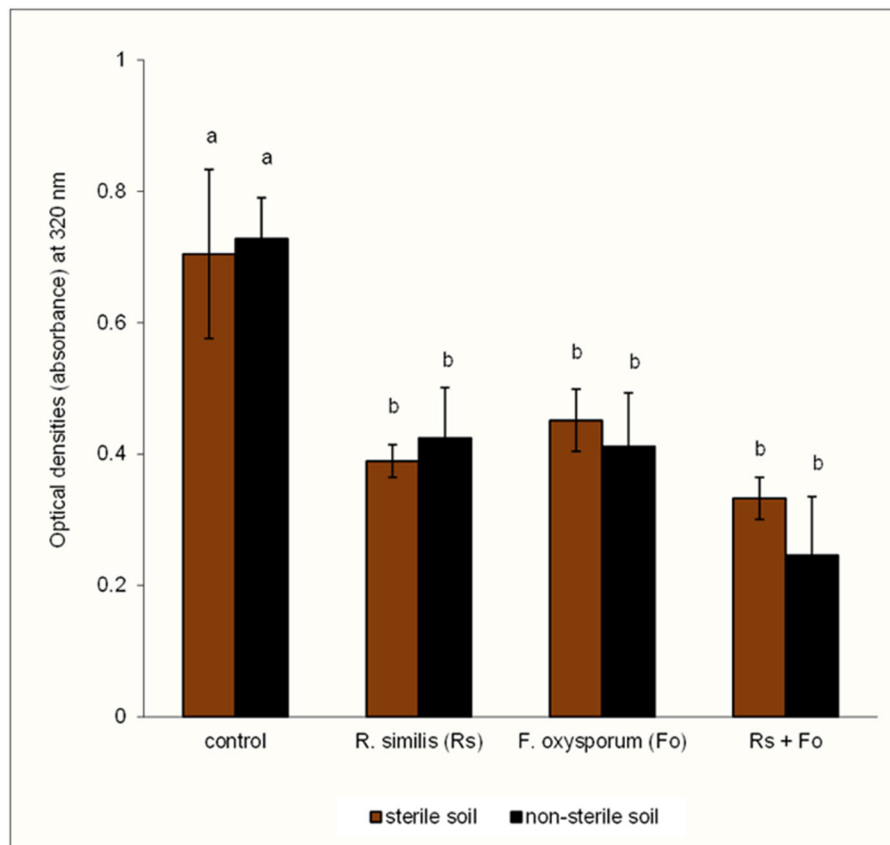
FIGURE 10 | Optical density of root extracts from potted tissue culture banana plants under independent or combined inoculation with *Fusarium oxysporum* V5w2 and *Radopholus similis*, and grown in either sterile soil or non-sterile soil supplied with complete nutrient solution. Bars with the same letter represent values that are not statistically different [$F_{(7,16)} = 14.79$, $p < 0.0001$].

control and conservation biological control (van Lenteren et al., 2018), especially as Conservation Agriculture (CA) among other agroecological approaches are increasingly gaining importance within Sustainable Food Systems (Lal, 2020; Nyanga et al., 2020; Page et al., 2020; Ochieno et al., 2021).

On the other hand, the highly stringent aseptic tissue culture techniques including soil sterilization would make more sense if the planting material is propagated from sick mother plants using unhealthy soil substrates. This could be more of a rescue plan for plants with desirable traits, especially those under the threat of extinction posed by invertebrate pest and microbial pathogen infestations. In this case, the rich biodiversity of beneficial endophytic and rhizosphere microbes could be re-introduced through plant and rhizosphere inoculation to restore the ecosystem services (Bubici et al., 2019). Classical biological control or augmentative biological control through inundation and inoculation could be implemented (van Lenteren et al., 2018). This could be done while putting into perspective concepts and technologies aimed at unraveling the composition and function of endophytic and rhizosphere microbiomes for sustainable food systems in agroecosystems (Ahkami et al., 2017; Fernández-González et al., 2019; Berg et al., 2020; Kaushal et al., 2020).

In summary, the present study reveals a root-rot disease complex of banana involving the root parasitic nematode *R. similis* and pathogenic *F. oxysporum* V5w2. Tissue culture banana plants exhibit uptake of biota from the rhizosphere to replenish the endophytic microbiomes. Soil sterilization eliminated the plant growth promoting effect of rhizosphere microbes and their natural suppressiveness toward the *R. similis-F. oxysporum* disease complex of banana plants. In-depth studies are still necessary to determine the actual identity of *F. oxysporum* V5w2 and conditions under which this fungus affects banana plants. Such information would contribute toward determination of the pending registration of *F. oxysporum* V5w2 as a biopesticide in line with the International Standards for Phytosanitary Measures and the Laws of Kenya (Wabule et al., 2003; Avedi et al., 2014; KEPHIS, 2014; FAO-ISPM, 2021). It is concluded that, soil sterilization eliminates beneficial microbes that provide natural pest suppression ecosystem services against *Radopholus similis* and *Fusarium oxysporum* in the endosphere and rhizosphere of tissue culture banana plants. From analysis of these endosphere and rhizosphere ecosystem services, I recommend the integration of microbiome conservation into tissue culture technology through the proposed "Tissue Culture Microbiome Conservation Technology."

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the data has remained in thesis form as per the conflict related to consensus over the outcomes (Ochieno, 2020). Requests to access the datasets should be directed to dwochieno@gmail.com.

AUTHOR CONTRIBUTIONS

The author confirms not being the sole contributor of this work and has approved it for publication in line with the conflict of interest.

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Conflict of Interest: This work has been disassociated from studies that propose *Fusarium oxysporum* V5w2 as an Endophytic Biological Control Agent and the matter has already been openly declared (https://doi.org/10.1016/j.sciaf.2020.e00605). Prof. Marcel Dicke, Prof. Arnold van Huis, Dr. Thomas Dubois, Dr. Danny Coyne, and Dr. Piet J. A. van Asten have renounced their co-authorship due to difference of opinion about the interpretation of the data and the conclusions of this study. Masinde Muliro University of Science and Technology (MMUST) with its Centre for African Medicinal Flora and Fauna (CAMNFF) was not involved in the research described in this article. The Scientific Integrity Committee of Wageningen University & Research (CWI) has already advised on these issues.

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