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Impact of Cover Crop Usage on Soilborne Diseases in Field Nursery Production

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Abstract: Soilborne pathogens are a significant economic problem for nursery production in the Southeastern United States. The goal of this study was to determine the impact of cover crops on soilborne disease suppressiveness in such systems. Soils from red maple (*Acer rubrum* L.) plantation fields grown with and without cover crops were sampled, either while the cover crops were growing (pre-disked) or post-season, following cover crop incorporation into the soil (post-disked). Greenhouse bioassays were conducted using red maple seeds on inoculated (with *Rhizoctonia solani* (J.G. Kühn) or *Phytophthora nicotianae* (Breda de Haan)) and non-inoculated field soils. The damping-off, root rot disease severity, percent recovery of *Rhizoctonia* and *Phytophthora*, and pseudomonad population were examined during the two years of the experiment. Results showed that cover crop incorporation was beneficial for inducing disease supressiveness characteristics of soil. Cover crop incorporation into the soil significantly or numerically reduced disease severity and pathogen recovery in infested soil compared to the bare soil treatment. Cover crop incorporation was found to be partially associated with the reduction of seedling damping-off. The pseudomonad microbial population was greater when cover crop was present, and is thought to be antagonist to soilborne pathogens. Therefore, cover crops can be integrated in field nursery production systems to suppress soilborne pathogens.

Keywords: soilborne pathogens; *Phytophthora nicotianae*; *Rhizoctonia solani*; cover crop; pseudomonad

1. Introduction

Ornamental nursery production is a lucrative segment of the green industry. In 2014, there were more than 8200 nursery producers in the US, and nursery crop sales comprised 31% of all horticultural sales, exceeding $4.2 billion [1]. The ability of nursery producers to provide pristine and blemish-free ornamental plants is greatly impacted by plant diseases [2]. In particular, soilborne diseases are becoming more problematic, as evidenced by the substantial losses to the nursery industry observed in recent years [3,4]. Infections caused by *Phytophthora* spp. are commonly observed in woody ornamental nursery crops [5–7]. These pathogens can live several years in infected plant tissue, plant debris, soil, and water [5]. *Phytophthora* species, belonging to the oomycetes, are easily disseminated by water, and can be spread by irrigation, runoff and flood events in a nursery [5]. One *Phytophthora* species of concern to the nursery industry is *Phytophthora nicotianae*, which can infect 255 genera in 90 families [8] and is one of the most devastating soilborne pathogens in the Southeastern United States [5,7]. Another soilborne pathogen of concern to nursery producers is *Rhizoctonia solani*, which causes diseases in ornamental crops grown in greenhouses, nurseries, and landscapes. The diseases caused by this pathogen include pre- and post-emergence damping-off, stem rot, foliar blight, and web blight [9]. *Rhizoctonia solani* attacks seeds below the soil surface a few days after sowing and can kill very young

seedlings soon after they emerge from the soil. Management of these soilborne pathogens is critical to maintain healthy nursery stock.

Chemical fungicides have been the most reliable line of defense to prevent losses due to soilborne pathogens [10]. For over 40 years, soil fumigation with methyl bromide was the standard pre-plant treatment for management of soilborne pathogens in a wide range of agricultural crops [11]. When methyl bromide was phased out in the early 21st century, metam sodium or sodium N-methyldithiocarbamate fumigants and post-planting fungicides had to be used to manage soilborne diseases [12]. Concerns regarding resistance development by pathogens due to continuous use of chemical fungicides, environmental drawbacks and the negative effects to successive crops has led to the search for more environmentally sustainable management practices.

Cover crops have been investigated widely in other fields of agriculture for management of plant pathogens, but research on the impact of cover crops in perennial woody ornamental production is lacking. Traditionally, cover crops are planted between seasons and incorporated into the field soil as a green manure [13]. This management option makes sense in a seasonal cropping system, but in woody ornamental production, the crop can remain in the field for several years. Cover cropping may take place prior to planting or during production in the rows (between plants) and/or row middles (the aisles between the rows). The cover crop can be mowed or lightly disked into the middles, but deep disking and incorporation is not recommended to avoid possible root damage to the main woody ornamental crop. Incorporating cover crops into soil is important because adding organic matter to soil can increase the competitiveness of beneficial, non-pathogenic microbes [14,15]. These beneficial microbes can subsequently outcompete soilborne plant pathogens, thereby protecting the main crop from disease [16–18]. For example, *Pseudomonas fluorescens* (Flügge) has gained attention as a potential biocontrol agent for many soilborne pathogens [19–21]. *Pseudomonas* species are the largest group of plant growth promoting rhizobacteria involved in biocontrol of plant diseases [22–25]. Pseudomonads grow rapidly in the rhizosphere, produce a wide variety of growth-promoting substances and adapt to new environments readily, making them well suited as biocontrol agents in agricultural systems [26–29].

The main goal of this research was to investigate the impact of cover crops on soilborne disease suppressiveness. The choice of cover crops for a multi-year production cycle was based on research to be able to protect red maple (*Acer rubrum* L.) trunks from arthropod oviposition activity [30–32]. Soils from field experimental plots with and without cover crops were evaluated before (pre-disked) and after lightly disking (post-disked) the cover crop into the row middles adjacent to the red maple planting. In order to determine the effect of cover crops on soilborne disease suppressiveness, the soil was evaluated for its ability to suppress *P. nicotianae* and *R. solani* in greenhouse bioassays using red maple and for pseudononad bacterial populations over a two-year production cycle.

2. Materials and Methods

2.1. Field Experimental Design and Layout

A replicated field experiment was established at Moore Nursery in Irving College, TN, USA (35.583889° N, 85.713056° W) (Warren Co.). Two treatments were evaluated in a randomized complete block design. The treatments included: (1) cover crop and (2) bare rows. The bare rows were maintained using pre- and post-emergent herbicides. The pre-emergent herbicide SureGuard® (flumioxazin 51%, Valent U.S.A. Corp., Walnut Creek, CA, USA) was applied at a rate of 708.8 g product ha^{-1} in November 2015, March 2016, August 2016, and April 2017. The post-emergent herbicide Finale® (glufosinate-ammonium 11.33%, Bayer Environmental Science, Research Triangle Park, NC, USA) with 80–20 (0.5%) surfactant (Ragan and Massey, Inc., Ponchatoula, LA, USA) was applied as a spot treatment to control persistent weeds. Tree rows were spaced 2.1 m apart with 1.8 m within-row spacing between trees, following current recommendations for field planting [33]. Each 11 by 11 m plot had 25 red maple trees. Each plot was randomly assigned to the treatments and replicated four times.

2.2. Cover Crop Application

Crimson clover (*Trifolium incarnatum* L.) and winter wheat (*Triticum aestivum* L.) (Adams-Briscoe Seed Company, Jackson, GA, USA) were seeded on 15 October 2015 using a Herd GT77 Spreader (Herd Seeder Co., Inc., Logansport, IN, USA). Crimson clover was chosen with the expectation to supply nitrogen to wheat so that an additional amount of nitrogenous fertilizer was not needed. Wheat was chosen due to its height to block insect oviposition on the red maple tree trunk. Due to the requirement to protect tree trunks from insect pests, cover crops were sown as close as possible to red maple trees. On 7 September 2016, crimson clover and annual ryegrass (*Lolium multiflorum* Lamarck) (Adams-Briscoe Seed Company, Jackson, GA, USA) were sown. Annual ryegrass was chosen because it was appropriate for the insect management portion of the project and, unlike wheat, would germinate on contact with soil. Seeding rates were 16.8, 84.2 and 34 kg ha^{-1} for crimson clover, wheat, and annual ryegrass, respectively. Cover crops were incorporated (disked) lightly (~2.5 cm) into the soil in August 2016 and 2017 using a tractor (John Deere Model 770, John Deere, Moline, IL, USA) and using a 1.2 m wide model disc (Rigsby Manufacturing Co., Walling, TN, USA).

2.3. Soil Moisture and Temperature Measurement

Soil moisture and temperature were measured bi-weekly from March to August in 2016 and monthly from April to August in 2017 (Figure 1; Figure 2). Soil temperature was measured with an infrared temperature meter (Spectrum Technologies, Inc., East Plainfield, IL, USA) in probe mode, while soil moisture was measured with a FieldScout time domain reflectometer (TDR) soil moisture meter (Spectrum Technologies, Inc., East Plainfield, IL, USA) using a 7.6 cm probe length inserted to a depth of ~7.0 cm. Soil moisture was measured in volumetric water content percentage (VWC%) and temperature in Celsius (°C). Soil moistures and temperatures were taken adjacent to three trees in each plot in the first, third and fifth rows along a diagonal. Three readings at each tree were taken at three different locations—within the tree row, at the interface of the row and middle, and in the middle between rows.

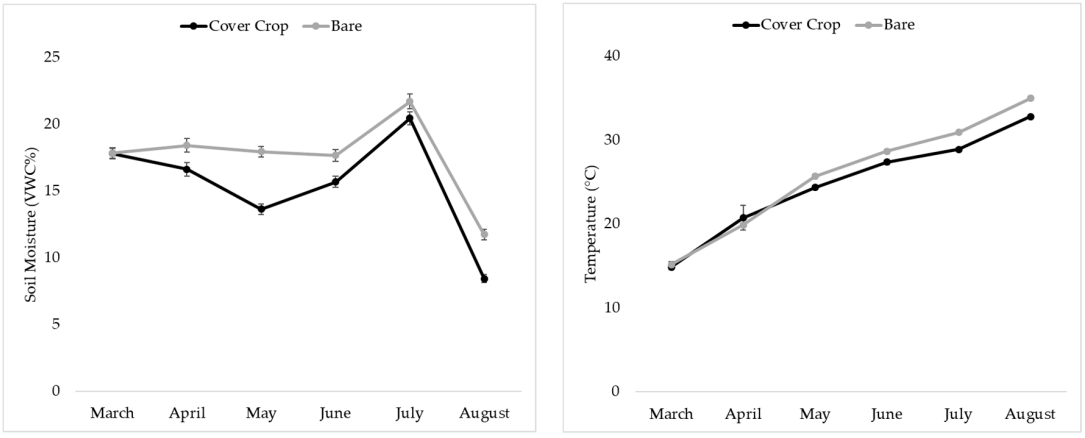

Figure 1. Average soil moisture percentage (VWC%) (±SEM (the standard error of the mean)) from March to August 2016 in cover cropped and bare row treatments measured with a FieldScout time domain reflectometer (TDR) soil moisture meter using a 7.6 cm probe length inserted to a depth of ~7.0 cm (**a**). Average soil temperature (±SEM) from March to August 2016 in cover cropped and bare row plots measured with an infrared temperature meter in probe mode (**b**). Cover crop: crimson clover and winter wheat. Bare: rows were maintained using pre- and post-emergent herbicides.

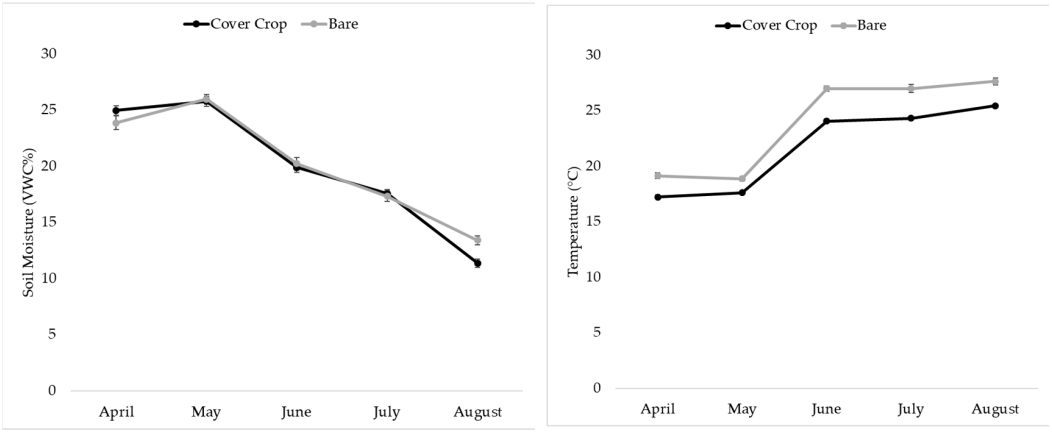

Figure 2. Average soil moisture percentage (VWC%) (±SEM) from April to August 2017 in cover cropped and bare row treatments measured with a FieldScout time domain reflectometer (TDR) soil moisture meter using a 7.6 cm probe length inserted to a depth of ~7.0 cm (**a**). Average soil temperature (±SEM) from April to August 2017 in cover cropped and bare row plots measured with an infrared temperature meter in probe mode (**b**). Cover crop: crimson clover and annual rye grass. Bare: rows were maintained using pre- and post-emergent herbicides.

2.4. Soil Sampling

For greenhouse bioassays to determine soilborne disease suppressiveness, soil samples were collected from cover crop or bare soil treatment both before disking (May 2016 and 2017) and after disking the cover crop into the soil (August 2016 and 2017). Hereafter, the bioassay conducted on soils collected before cover crop incorporation will be referred to as "pre-disked", and soils evaluated after cover crop incorporation will be referred to as "post-disked". Three soil samples were taken from each treatment plot adjacent to the trees evaluated for soil temperature and moisture. Soil was collected from the nearest edge of the tree row where the interface temperature and moisture were evaluated (about 50 cm from the red maple tree to prevent root damage). A 9 kg soil sample was taken from a 30 cm^2 area, with 20 cm in depth, at three sampling points per plot, mixed using a spade in a bucket and then transferred into a plastic bag. Spade, bucket, and hands were cleaned with water and then sterilized with 70% ethanol to prevent contamination between samples. The soil was held in an open plastic bag to allow air circulation. Soil was stored for 1 week at 22 °C in a greenhouse at the Tennessee State University Otis L. Floyd Nursery Research Center in McMinnville, TN, USA (35.680480° N, 85.774580° W) (TSUNRC) before use in tests.

2.5. Fungal Culture and Pathogen Inoculum Preparation

Isolate FBG201507 of *P. nicotianae* and isolate FBG201508 of *R. solani* were obtained from the culture collection of Dr. Fulya Baysal-Gurel at the TSUNRC. The *R. solani* specimen was originally isolated from a diseased red maple plant in 2015 and maintained on potato dextrose agar (PDA) medium. The *P. nicotianae* specimen was also originally isolated from a diseased red maple plant in 2015 and was maintained on PARH-V8 medium [34]. Pathogen virulence was confirmed by inoculating red maples with the *R. solani* culture or the *P. nicotianae* culture and, subsequently, re-isolating the pathogens from the infected roots. For the *P. nicotianae* inoculum, the rice grain method, modified after Holmes and Benson [35], was followed. Briefly, 25 g of long grain rice in 18 mL deionized water was autoclaved twice for 30 min, and three 7 mm sized plugs of *P. nicotianae*-colonized V8 juice agar (100 mL of clarified V8 juice (Campbell, Camden, NJ, USA), 15 g of agar (Sigma-Aldrich, St. Louis, MO, USA), and 900 mL of deionized water) were placed in a 250 mL flask and incubated for two weeks at room temperature. The inoculum in the flask was mixed weekly before final use. Soils in square black plastic

pots (10 × 10 × 11 cm) were artificially infested by burying three *P. nicotianae*-colonized rice grains in the soil at a 5 cm soil depth. For the *R. solani* inoculum, an agar slurry was prepared (one Petri dish of a seven-day-old *R. solani* culture blended with 1 L of sterilized distilled water), and each pot was drenched with 100 mL of slurry after seeding [36].

2.6. Red Maple Seed Collection and Planting

Three thousand 'Franksred' red maple (*Acer rubrum* L.) seeds were collected in April 2016 and 2017 and stored in a refrigerator until use. Red maple seeds were sown into the pots filled with soil collected from the field experiment in May 2016 and 2017, and in August 2016 and 2017. Ten red maple seeds were sown in each pot in the 2016 bioassay, while only five seeds were sown in the 2017 bioassay.

2.7. Greenhouse Bioassays

The greenhouse bioassays were conducted at the TSUNRC. The soil sample from each field plot treatment (cover crop or bare row) and replication was divided into individual square black plastic pots (10 × 10 × 11 cm). Those soils were then used as either inoculated (with *R. solani* or *P. nicotianae*) or non-inoculated. For each bioassay, twelve single-pot replications per treatment were arranged in a completely randomized design. Overhead irrigation was set up for 1 min twice per day for the whole experimental period. The pre-disked bioassay was initiated in June 2016 and terminated in October 2016, while the post-disked bioassay was initiated in September 2016 and terminated in January 2017 for the first year. Similarly, pre-disked and post-disked bioassays were conducted between June and October 2017 and between September 2017 and January 2018, respectively, for the second year. The greenhouse was maintained at 27 and 21 ± 2 °C, day and night, respectively, with a 14 h day length and 85% relative humidity.

2.7.1. Evaluation of Red Maple Crop Health

After complete germination of maple seeds, stand data were recorded in the greenhouse bioassays. Dead plants were marked with sterilized toothpicks. Toothpicks were inserted into the soil near dead plants, and the total numbers of dead plants were reported for the damping-off percentage. The remaining plants were removed from pots to evaluate root health. Plants were rinsed for 45 min under running tap water and soil attached to the roots was removed. A visual assessment was conducted to identify root rot severity. Seedlings were evaluated for disease severity using a scale of 0%−100% of the total root system affected at the end of the bioassays. After root assessment, ten randomly selected root samples (~1 cm long root tip, four replicates per treatment) were plated on *Rhizoctonia* selective medium [37] and PARPH-V8 selective medium, respectively. Plates were incubated at 25 °C in the dark (VWR incubator, Radnor, PA, USA). The number of root pieces showing *Rhizoctonia* growth was counted after 2 days, while *Phytophthora* growth was counted after 5 days. The pathogen recovery percentage was calculated by dividing the root pieces showing pathogen growth by the total root pieces plated and multiplying by 100 for each pathogen.

2.7.2. Pseudomonad Colonies

Pseudomonas selective medium (S1 medium) was prepared following the method developed by Gould and his colleagues [38]. A 1 g soil sample from each treatment pot was transferred into a 15 mL tube (Thermo Fisher Scientific Inc., Waltham, MA, USA) containing 10 mL of sterilized water. Serial dilution up to 10^{-6} was performed for each sample, which was then mixed properly using a vortex. After the settlement of soil particles, 100 µL of supernatant was spread-plated using glass beads (3 mm solid glass beads, Walter Stern, Inc., Manorhaven, NY, USA). Plates were incubated at 25 °C for 3 days. The number of colony forming units (CFUs) per gram of soil sample was calculated from the plate counts, the dilution factor and the plated volume.

2.8. Statistical Analysis

Damping-off percentage, disease severity percentage, pathogen recovery percentage, and pseudomonad CFUs were analyzed among cover crop soil and bare soil by year using a generalized linear model (PROC GENMOD) fitted to a normal distribution. The interaction between the soil management strategy (cover crop or bare soil) and disking (pre-disked and post-disked) was analyzed using the same model, and Least Square (LS) means were separated by Tukey's Multiple Comparison Test at $\alpha = 0.05$ (SAS 9.4, SAS Institute, Inc., Cary, NC, USA). The pseudomonad CFUs were log transformed for analysis purposes, but original mean values are presented in the figures. All analyses were performed for both pathogen-infested soil and non-infested soil.

3. Results

3.1. Greenhouse Bioassay without the Addition of Pathogen Inoculum

In the greenhouse bioassays with no pathogen inoculum introduction, damping-off levels of red maple seedlings were low in the soil collected from the cover crop treatment and the bare row treatment, with no significant differences among treatments (2016: $\chi^2_{(1)} = 0.81$, $p = 0.367$; 2017: $\chi^2_{(1)} = 1.69$, $p = 0.194$) (Table 1). There was interaction between management strategy (cover crop treatment or bare soil treatment) and disking (pre-disked or post-disked) in terms of levels of damping-off ($\chi^2_{(1)} = 4.39$, $p = 0.036$) in 2016 (Table 2). However, there were no significant differences between management strategy (cover crop treatment or bare soil treatment) and disking (pre-disked or post-disked) in terms of levels of damping-off ($\chi^2_{(1)} = 0.08$, $p = 0.867$) in 2017 (Table 2). The roots from both treatments were cultured for pathogen recovery. In the two-year experiment, there were no significant differences in *R. solani* or *P. nicotianae* pathogen recovery percentage between the cover crop and bare row treatments (*R. solani*: 2016: $\chi^2_{(1)} = 0.06$, $p = 0.799$; 2017: $\chi^2_{(1)} = 0.06$, $p = 0.806$; *P. nicotianae*: 2016: $\chi^2_{(1)} = 0.73$, $p = 0.393$; 2017: $\chi^2_{(1)} = 0.90$, $p = 0.342$) (Table 1). Similarly, there were no differences in pathogen recovery between pre-disked and post-disked bioassays conducted with cover crop soil or bare soil in 2016 and 2017 (*R. solani*: 2016: $\chi^2_{(1)} = 1.04$, $p = 0.308$; 2017: $\chi^2_{(1)} = 0.24$, $p = 0.623$; *P. nicotianae*: 2016: $\chi^2_{(1)} = 1.14$, $p = 0.286$; 2017: $\chi^2_{(1)} = 0.00$, $p = 1.000$) (Table 2).

Table 1. Average percentages (±SEM) of damping-off of red maple seedlings and pathogen recovery of *Rhizoctonia solani* and *Phytophthora nicotianae* in non-inoculated cover crop soil and bare soil in 2016 and 2017.

Treatment *	2016			2017		
	Damping-off (%)	**Pathogen Recovery of *Rhizoctonia solani* (%)**	**Pathogen Recovery of *Phytophthora nicotianae* (%)**	**Damping-off (%)**	**Pathogen Recovery of *Rhizoctonia solani* (%)**	**Pathogen Recovery of *Phytophthora nicotianae* (%)**
Cover crop	10.1 ± 3.3 a **	12.0 ± 3.0 a	9.5 ± 2.8 a	5.3 ± 2.1 a	10.0 ± 2.5 a	7.0 ± 2.4 a
Bare	15.1 ± 4.2 a	13.0 ± 2.5 a	13.5 ± 3.7 a	11.4 ± 4.6 a	9.0 ± 3.1 a	11.0 ± 3.3 a
p-value	0.367	0.799	0.393	0.194	0.806	0.342

* Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. ** Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$.

Table 2. Average percentages (±SEM) of damping-off of red maple seedlings and pathogen recovery of *Rhizoctonia solani* and *Phytophthora nicotianae* in pre-disked (before cover crop incorporation into the soil) and post-disked (after cover crop incorporation into the soil) bioassays.

Treatment *	2016			2017		
	Damping-off (%)	Pathogen Recovery of *Rhizoctonia solani* (%)	Pathogen Recovery of *Phytophthora nicotianae* (%)	Damping-off (%)	Pathogen Recovery of *Rhizoctonia solani* (%)	Pathogen Recovery of *Phytophthora nicotianae* (%)
Pre-disked cover crop	16.7 ± 6.0 a **	13.0 ± 4.5 a	12.0 ± 4.7 a	5.4 ± 3.2 a	9.0 ± 4.6 a	7.0 ± 4.0 a
Post-disked cover crop	3.8 ± 2.7 b	11.0 ± 4.1 a	7.0 ± 3.0 a	5.1 ± 2.8 a	11.0 ± 2.3 a	7.0 ± 3.0 a
Pre-disked bare	10.5 ± 4.6 ab	16.0 ± 4.7 a	16.0 ± 6.3 a	7.9 ± 3.7 a	8.0 ± 5.1 a	11.0 ± 6.1 a
Post-disked bare	19.3 ± 6.7 a	10.0 ± 2.6 a	11.0 ± 4.1 a	15.5 ± 9.1 a	10.0 ± 3.7 a	11.0 ± 2.3 a
p-value	0.036	0.308	0.286	0.867	0.623	1.000

* The pre-disked bioassay was conducted from June to October, and the post-disked bioassay was conducted from September to January. The soil was not inoculated with either of the pathogens in both years (2016 and 2017). Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: weed or cover crop free. ** Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$.

In 2016, the mean pseudomonad counts were similar in both the cover crop treatment and the bare row treatment ($\chi^2_{(1)} = 1.22$, $p = 0.269$) (Figure 3), but there was a difference detected between pre-disked and post-disked bioassays conducted with cover crop soil compared to bare soil ($\chi^2_{(1)} = 19.04$, $p < 0.001$) (Figure 3). The mean pseudomonad counts increased in the cover crop treatment compared to the bare row treatment ($\chi^2_{(1)} = 45.90$, $p < 0.001$), and the cover crop soil in the post-disked bioassay had significantly higher pseudomonad counts compared to the pre-disked bioassay in 2017 ($\chi^2_{(1)} = 17.79$, $p < 0.001$) (Figure 3). There was interaction between management strategy and disking in terms of pseudomonad counts in 2016 and 2017.

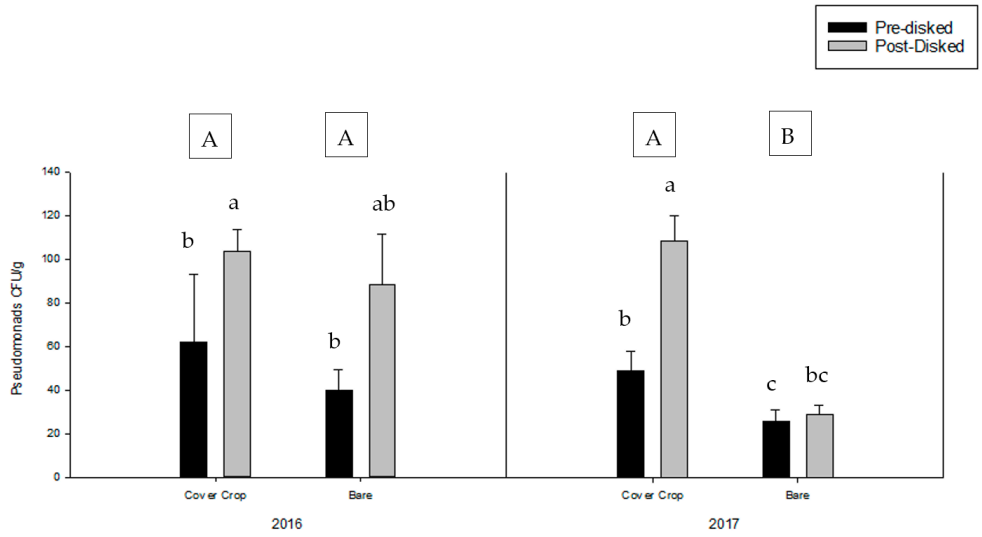

Figure 3. Mean pseudomonad colony forming units (CFUs/g of soil) (±SEM). The pre-disked bioassay (before cover crop incorporation into the soil) was conducted from June to October, and the post-disked bioassay (after cover crop incorporation into the soil) was conducted from September to January. Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$. Values were log transformed for analysis purposes but original mean values are presented in the figure. Capital letters indicate the effect between the cover crop and bare row treatments, and small letters indicate interactions between the pre-disked, cover crop and bare row treatments and post-disked, cover crop and bare row treatments.

3.2. Greenhouse Bioassay with *Rhizoctonia solani*

There were no differences between the cover crop treatment and the bare row treatment in the damping-off percentage when *R. solani* inoculum was added during the two-year experiment (2016: $\chi^2_{(1)} = 0.01$, $p = 0.907$; 2017: $\chi^2_{(1)} = 0.04$, $p = 0.849$) (Figure 4). Similarly, there were no differences in the damping-off percentage between pre-disked and post-disked bioassays in 2016 and 2017 (2016: $\chi^2_{(1)} = 0.72$, $p = 0.397$; 2017: $\chi^2_{(1)} = 1.01$, $p = 0.314$) (Figure 4). A similar effect on *Rhizoctonia* root rot disease severity was recorded when red maple roots were evaluated between the cover crop and bare row treatments ($\chi^2_{(1)} = 0.28$, $p = 0.095$) (Figure 5), and between pre-disked and post-disked bioassays (2016: $\chi^2_{(1)} = 0.22$, $p = 0.637$) in 2016 (Figure 5). However, the cover crop treatment reduced the level of root rot disease severity on red maple seedlings compared to the bare row treatment in 2017 ($\chi^2_{(1)} = 9.16$, $p = 0.003$). The cover crop soil in the post-disked bioassay had significantly lower root rot disease severity compared to the cover crop soil in the pre-disked bioassay ($\chi^2_{(1)} = 2.6$, $p = 0.007$) in 2017 (Figure 5). Similarly, the pathogen recovery percentage was higher in the bare row treatment when pathogen inoculum was added compared to the cover crop treatment during the two years of the experiment (2016: $\chi^2_{(1)} = 10.15$, $p = 0.001$; 2017: $\chi^2_{(1)} = 11.60$, $p = 0.001$) (Figure 6). No differences were recorded when cover crop soil was evaluated for post-disked bioassays compared to pre-disked bioassays (2016: $\chi^2_{(1)} = 2.9$, $p = 0.088$; 2017: $\chi^2_{(1)} = 0.02$, $p = 0.896$) (Figure 6).

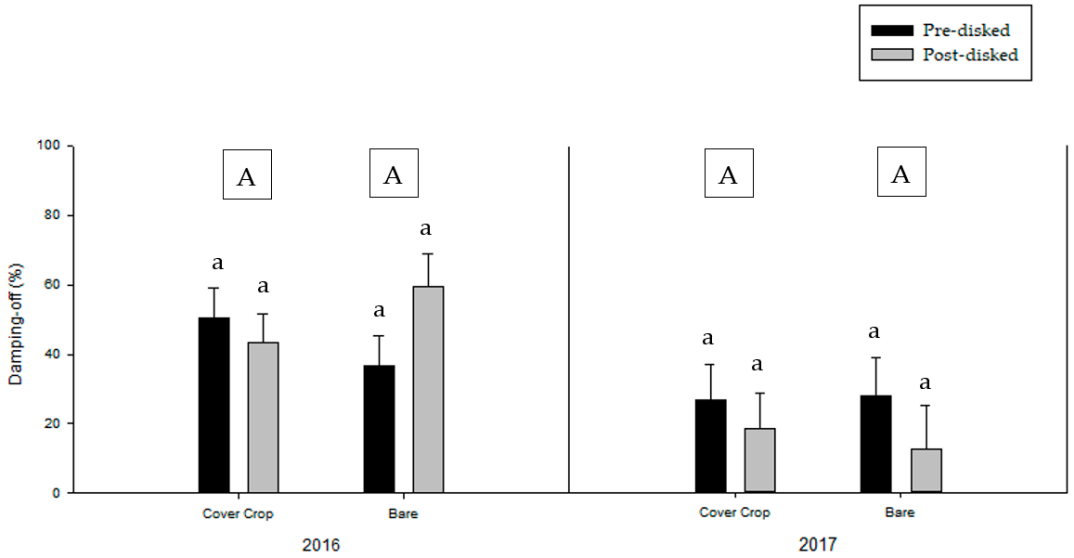

Figure 4. Average percentage of damping-off (±SEM) of red maple seedlings when *Rhizoctonia solani* was inoculated into the soil in a greenhouse bioassay. The pre-disked bioassay (before cover crop incorporation into the soil) was conducted from June to October, and the post-disked bioassay (after cover crop incorporation into the soil) was conducted from September to January. Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$. Capital letters indicate the effect between the cover crop and bare row treatments, and small letters indicate interactions between the pre-disked, cover crop and bare row treatments and post-disked, cover crop and bare row treatments.

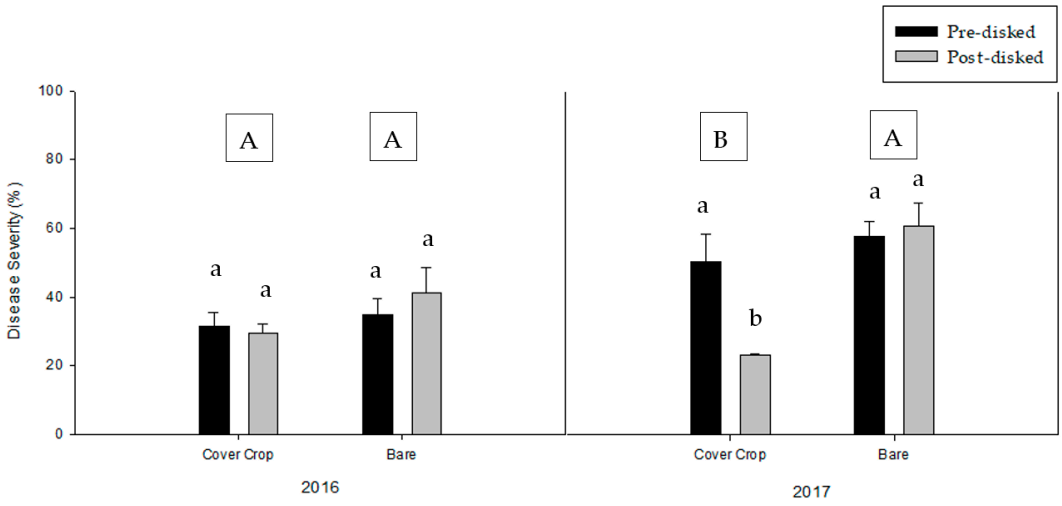

Figure 5. Average percentage of disease severity (±SEM) of red maple seedlings when *Rhizoctonia solani* was inoculated into the soil in a greenhouse bioassay. Disease severity was assessed using 0%–100% root infection by pathogens. The pre-disked bioassay (before cover crop incorporation into the soil) was conducted from June to October, and the post-disked bioassay (after cover crop incorporation into the soil) was conducted from September to January. Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$. Capital letters indicate the effect between the cover crop and bare row treatments, and small letters indicate interactions between the pre-disked, cover crop and bare row treatments and post-disked, cover crop and bare row treatments.

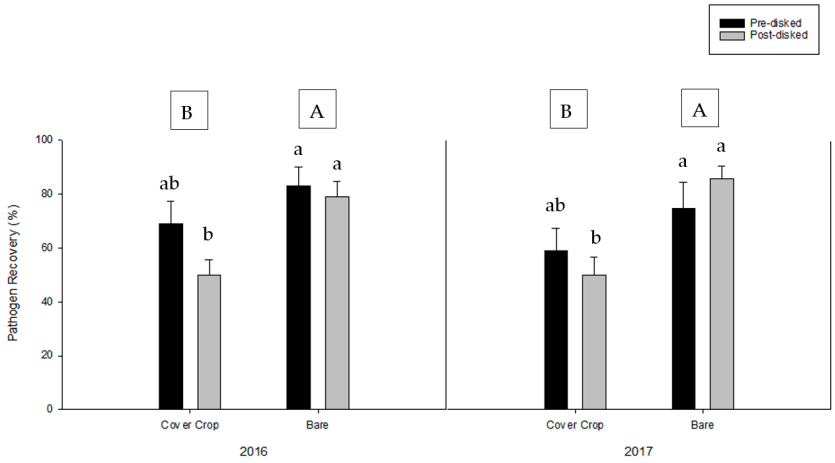

Figure 6. Average pathogen recovery percentage (±SEM) from red maple seedling roots when *Rhizoctonia solani* was inoculated into the soil in a greenhouse bioassay. Ten 1 cm length roots were plated in *R. solani* selective media, and the number of roots with *R. solani* recovered was counted. The pre-disked bioassay (before cover crop incorporation into the soil) was conducted from June to October, and the post-disked bioassay (after cover crop incorporation into the soil) was conducted from September to January. Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$. Capital letters indicate the effect between the cover crop and bare row treatments, and small letters indicate interactions between the pre-disked, cover crop and bare row treatments and post-disked, cover crop and bare row treatments.

In 2016 and 2017, the mean pseudomonad counts increased in the cover crop treatment compared to the bare row treatment (2016: $\chi^2_{(1)} = 18.12$, $p < 0.001$; 2017: $\chi^2_{(1)} = 19.17$, $p < 0.001$), and the cover crop soil in the post-disked bioassay had significantly higher pseudomonad counts compared to other treatments (2016: $\chi^2_{(1)} = 15.08$, $p < 0.001$; 2017: $\chi^2_{(1)} = 17.83$, $p < 0.001$) (Figure 7).

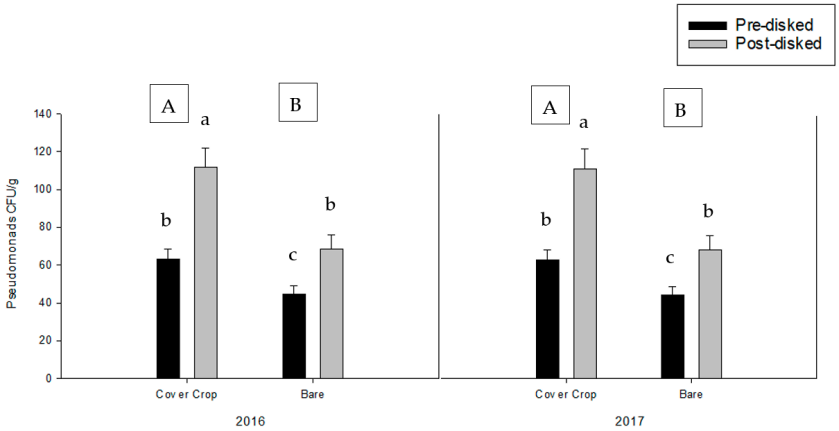

Figure 7. Mean pseudomonad counts (CFUs/g of soil) (±SEM) when *Rhizoctonia solani* was inoculated into the soil. The pre-disked bioassay (before cover crop incorporation into the soil) was conducted from June to October, and the post-disked bioassay (after cover crop incorporation into the soil) was conducted from September to January. Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$. Values were log transformed for analysis purposes but true mean values are presented in the figure. Capital letters indicate the effect between the cover crop and bare row treatments, and small letters indicate interactions between the pre-disked, cover crop and bare row treatments and post-disked, cover crop and bare row treatments.

3.3. Greenhouse Bioassay with *Phytophthora nicotianae*

The damping-off percentage did not differ between the cover crop and bare row treatments when *Phytophthora nicotianae* inoculum was added in 2017 ($\chi^2_{(1)} = 0.82$, $p = 0.366$), but the presence of cover crops significantly lowered the occurrence of damping-off compared to bare soil during 2016 ($\chi^2_{(1)} = 6.61$, $p = 0.01$) (Figure 8). The damping-off percentage was similar in both pre-disked or post-disked bioassays during the two years of the experiment (2016: $\chi^2_{(1)} = 3.62$, $p = 0.057$; 2017: $\chi^2_{(1)} = 0.85$, $p = 0.358$). The cover crop treatment reduced *Phytophthora* root rot disease severity compared to the bare row treatment in the two years of the experiment (2016: $\chi^2_{(1)} = 10.22$, $p = 0.001$; 2017: $\chi^2_{(1)} = 5.55$, $p = 0.018$ (Figure 9). However, *Phytophthora* root rot severity did not differ with the post-disked incorporation of the cover crop into the soil (2016: $\chi^2_{(1)} = 2.88$, $p = 0.089$; 2017: $\chi^2_{(1)} = 2.69$, $p = 0.104$) (Figure 9). The *Phytophthora* pathogen recovery percentage was higher in the bare row treatment when pathogen inoculum was added compared to the cover crop treatment in 2016 ($\chi^2_{(1)} = 5.23$, $p = 0.021$) (Figure 10), but no differences were detected in 2017 ($\chi^2_{(1)} = 1.87$, $p = 0.171$) (Figure 10). *Phytophthora* pathogen recovery was lower in cover crop soil for the post-disked bioassay compared to the pre-disked bioassay in 2017 (2016: $\chi^2_{(1)} = 6.89$, $p = 0.009$; 2017: $\chi^2_{(1)} = 10.20$, $p = 0.001$) (Figure 10).

In both years, the mean pseudomonad counts were higher in the post-disked bioassays compared to pre-disked bioassays for cover crop soil (2016: $\chi^2_{(1)} = 8.68$, $p = 0.003$; 2017: $\chi^2_{(1)} = 24.05$, $p < 0.001$) (Figure 11). Similarly, the mean pseudomonad count increased in the cover crop treatment compared to the bare row treatment in 2017 (2016: $\chi^2_{(1)} = 0.30$, $p = 0.583$; 2017: $\chi^2_{(1)} = 139.45$, $p < 0.001$) (Figure 11). There was interaction between management strategy (cover crop treatment or bare row treatment) and disking (pre-disked or post-disked) in terms of pseudomonad counts in 2016 ($\chi^2_{(1)} = 4.59$, $p = 0.032$).

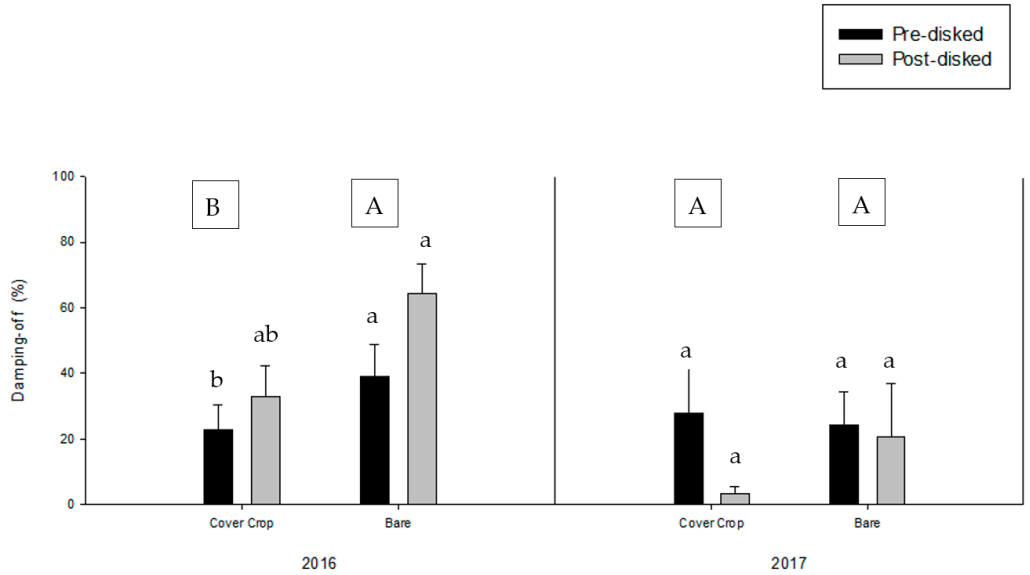

Figure 8. Average percentage of damping-off (±SEM) of red maple seedlings when *Phytophthora nicotianae* was inoculated into the soil in a greenhouse bioassay. The pre-disked bioassay (before cover crop incorporation into the soil) was conducted from June to October, and the post-disked bioassay (after cover crop incorporation into the soil) was conducted from September to January. Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$. Capital letters indicate the effect between the cover crop and bare row treatments, and small letters indicate interactions between the pre-disked, cover crop and bare row treatments and post-disked, cover crop and bare row treatments.

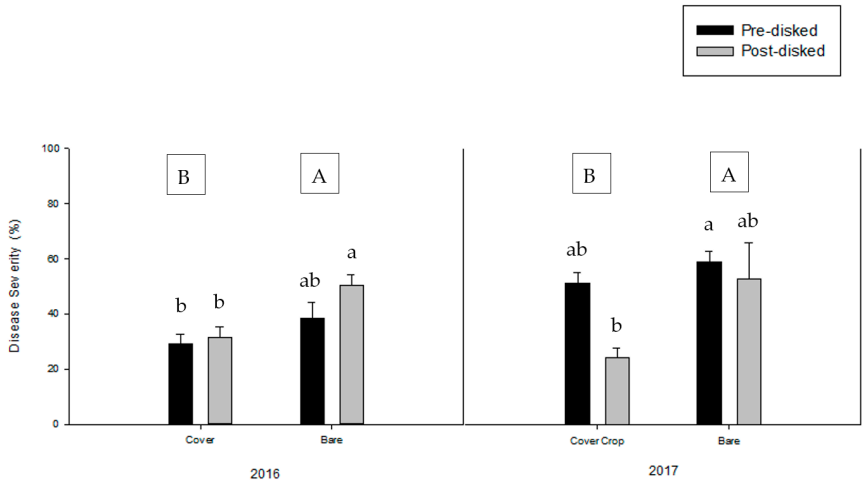

Figure 9. Average percentage of disease severity (±SEM) of red maple seedlings when *Phytophthora nicotianae* was inoculated into the soil in a greenhouse bioassay. Disease severity was assessed using 0%–100% root infection by pathogens. The pre-disked bioassay (before cover crop incorporation into the soil) was conducted from June to October, and the post-disked bioassay (after cover crop incorporation into the soil) was conducted from September to January. Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$. Capital letters indicate the effect between the cover crop and bare row treatments, and small letters indicate interactions between the pre-disked, cover crop and bare row treatments and post-disked, cover crop and bare row treatments.

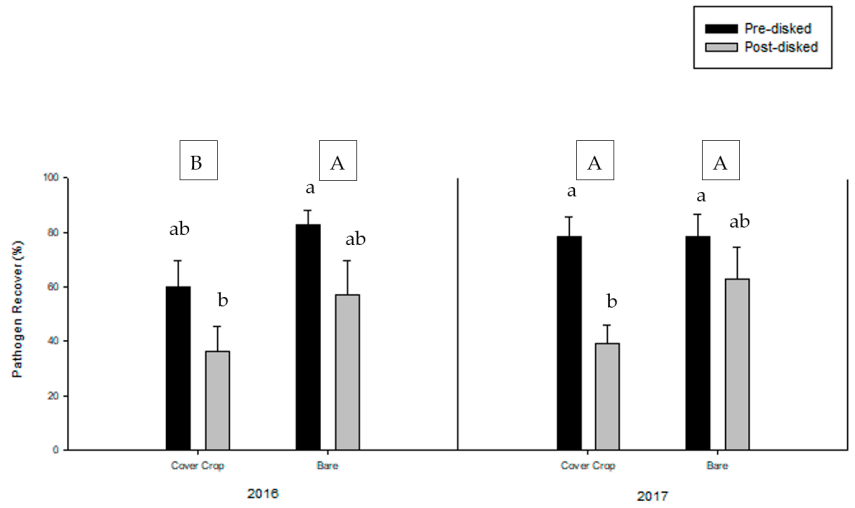

Figure 10. Average pathogen recovery percentage (±SEM) from red maple seedling roots when *Phytophthora nicotianae* was inoculated into the soil in a greenhouse bioassay. Ten 1 cm length roots were plated in *P. nicotianae* selective media, and the number of roots with *P. nicotianae* recovered was counted. The pre-disked bioassay (before cover crop incorporation into the soil) was conducted from June to October, and the post-disked bioassay (after cover crop incorporation into the soil) was conducted from September to January. Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$. Capital letters indicate the effect between the cover crop and bare row treatments, and small letters indicate interactions between the pre-disked, cover crop and bare row treatments and post-disked, cover crop and bare row treatments.

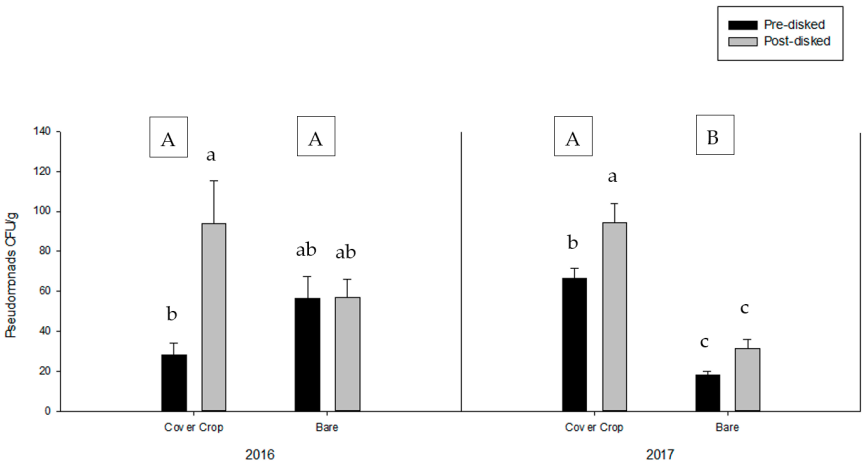

Figure 11. Mean pseudomonad counts (CFUs/g of soil) (±SEM) when *Phytophthora nicotianae* was inoculated into the soil. The pre-disked bioassay (before cover crop incorporation into the soil) was conducted from June to October, and the post-disked bioassay (after cover crop incorporation into the soil) was conducted from September to January. Cover crop: crimson clover and winter wheat (2016), and crimson clover and annual rye grass (2017). Bare: rows were maintained using pre- and post-emergent herbicides. Means that do not share a letter are significantly different, according to Tukey's Multiple Comparison Test at $p < 0.05$. Values were log transformed for analysis purposes but original mean values are presented in the figure. Capital letters indicate the effect between the cover crop and bare row treatments, and small letters indicate interactions between the pre-disked, cover crop and bare row treatments and post-disked, cover crop and bare row treatments.

3.4. Soil Moisture and Temperature

Bare soil had higher soil moisture in all recorded months in 2016, with the exception of April, and higher moisture in 2017, with the exceptions of April and July. Bare soil had higher soil temperature in all recorded months in both years, with the exception of April 2016. The lowest moisture difference was 0.02 VWC% in March and 0.22 VWC% in July between cover crop soil and bare soil for 2016 and 2017, respectively. The highest moisture difference was 4.32 VWC% in May and 2.06 VWC% in August between cover crop soil and bare soil for 2016 and 2017, respectively. The temperature difference ranged from 0.33 °C in March 2016 to 2.19 °C in August 2016, and from 1.27 °C in May 2017 to 2.92 °C in June 2017, between cover crop soil and bare soil.

4. Discussion

Cover crops are known to prevent soil erosion [39,40] and effect weed suppression [41,42], and are capable of changing soil microbial populations [14]. These characteristics are often related to increased soil fertility in agricultural and ornamental nursery production systems [43,44]. The possible mechanisms of soilborne pathogen suppression by cover crop introduction may include: (1) the addition of green manure into the soil, which ultimately becomes organic matter, (2) introduction of or increased beneficial microorganisms [14], (3) reduced dispersal of pathogens [45–47], and (4) toxins/chemicals produced by cover crops that are harmful to pathogens [48]. The few possible mechanisms found in our systems are discussed below.

In a greenhouse bioassay with *Rhizoctonia solani* added, while the incorporation of the cover crop had no effect on the reduction of damping-off and *Rhizoctonia* root rot disease severity in 2016, the incorporation of cover crops into the soil reduced *Rhizoctonia* root rot disease severity in 2017. Our results show that cover crop usage reduced *Rhizoctonia* pathogen recovery during the two years of the experiment, suggesting that when *Rhizoctonia* root rot disease is severe, cover crop usage can reduce disease severity. Wen and colleagues evaluated a cereal rye cover crop for effectiveness at suppressing *R. solani* and found that short-term cereal rye cover cropping lowered the severity of *Rhizoctonia* root rot on soybean in *R. solani*-inoculated soils [49]. They also highlighted that a longer period of cover cropping may be required to see the effect of the cover crop on pathogen suppression. These results could be related to the addition of soil organic amendments and mineral nutrients. Baysal-Gurel and her colleagues also found suppression of *R. solani* in woody ornamental production by use of cover crops as a source of green manure [50]. Amending soil with plant residues has been proposed to enhance organic matter and soil fertility and reduce the severity and incidence of diseases caused by soilborne pathogens [51]. Another reason for soilborne disease suppressiveness with the usage of a cover crop can be soil moisture and/or soil temperature. In our red maple production field, the soil temperature (7 cm below the soil surface) was monitored, and it was found that cover crop treatment had lowered the soil temperature compared to the bare treatment [32]. Similarly, lower soil moisture was recorded during the cover crop growing period in cover crop soil compared to bare soil. The level of moisture in the soil has been found to be associated with soil microorganism activity in many studies [52–57]. Less moisture reduces the opportunity for pathogen development [52], and this could explain how the higher moisture and temperature in bare soil facilitated pathogen development in our system. The increased concentration of pseudomonads in the soil can explain the effectiveness of cover crop incorporation.

A similar reduction in disease was seen in a greenhouse bioassay with *Phytophthora nicotianae* added in the soil. The incorporated cover crop may reduce the opportunity for dissemination of *P. nicotianae* spores in soil. Ristaino and Johnston found that wheat or rye cover crop introduction to a bell pepper field reduced the splash dispersal of *Phytophthora* pathogens [46]. Similarly, the bare field treatment had the maximum disease incidence and higher spread across the rows of the bell pepper field. In a no-till system, more diverse soil microorganisms were observed [58], which could be in the form of soilborne pathogens. The addition of organic amendments to the soil also has been associated with the reduction of soilborne *Phytophthora* in many systems [51,59–62]. In our

study, cover crop incorporation by lightly disking into the soil was efficient at reducing *Phytophthora* root rot disease severity compared to bare soil. Incorporation of the cover crop into the soil, which led to increased organic amendments, reduced moisture and temperature, and caused increased pseudomonad populations, which most likely played a role in *Phytophthora* disease suppression.

The introduction of fluorescent pseudomonads has been associated with decreased soilborne pathogen populations in cover crops and other systems [63–67]. The main properties of pseudomonads are their ability to grow rapidly in the rhizosphere, their production of a wide variety of growth-promoting substances, and their ability to adapt to new environments. The level of pseudomonad count in our cover crop treated soils increased noticeably in the second year of the production cycle in *R. solani*-inoculated soil, which could be due to the two years of cumulative effects from cover crop residues. Interestingly, cover crops were able to enhance beneficial microorganism levels in the soil, which likely had antagonistic activity to suppress soilborne pathogens. Pathogen suppression has also been observed in wheat fields, where the cultivation of wheat cultivars enhanced the production of fluorescent pseudomonads, which had antagonistic activities against *Rhizoctonia* root rot of apples and other ornamental pathogens [68,69]. Large populations of *Pseudomonas* spp. were also found with cover crop use in carrot cultivation [70] and cover crops of oat and spring vetch in *Scorzonera* cultivation [71].

Suppressive soil can be defined as soil with the capacity to develop a very low level of disease even in the presence of virulent pathogens [69]. It has been found that both biotic and abiotic components in soil are equally important to suppress pathogen levels. Therefore, properties such as chemical and physical properties, conditions such as organic amendments, microbes such as pseudomonads, and abiotic factors such as temperature and moisture are important attributes to consider for pathogen suppressiveness [67]. Similarly, making certain manipulations to the soil, such as the introduction of cover crops in the production system, can induce the suppressiveness characteristics. The properties were enhanced either by the presence of a cover crop as a standing crop or as organic amendments added beneath the soil, changing the damping-off, disease severity, pathogen recovery, and soil microbial status in our system. Some of the microbial status changes were more pronounced depending on the type of pathogen. However, the general finding was that cover crops were able to reduce the levels of the two pathogens introduced into our system by inducing disease supressiveness characteristics of the soil.

We found evidence that the cover crop reduced the pathogen pressure in the red maple nursery production system. The incorporation of cover crops into the soil and the associated biotic and abiotic factors were able to increase soilborne disease suppressiveness. The reduced levels of damping-off, disease severity and pathogen recovery in the presence of a cover crop suggest that the use of cover crops can be beneficial in a woody ornamental production system. Cover crops can aid the primary crop by reducing the need for synthetic crop protection materials, such as fungicides. By incorporating these cover crops into production, it is possible to develop strategies for pest and disease management that reduce our reliance on agrochemicals, leave fewer pesticide residues, and prevent pest and pathogen resistance to pesticide chemistries.

Thus, we recommend more long-term studies to evaluate the relationship between population changes in pseudomonads and soil resiliency against *R. solani* and *P. nicotianae* in different cover cropping situations.

Author Contributions: S.D., F.B.-G., K.M.A. and J.B.O. conceived of the experiments. S.D., F.B.-G. and K.M.A. designed the experiments. S.D., F.B.-G. and T.S. performed the experiments; S.D. analyzed the data. S.D. and F.B.-G. wrote the paper; F.B.-G. revised the paper; F.B.-G and K.M.A. received the funding.

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Conflicts of Interest: The authors declare no conflict of interest.

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