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RESEARCH

Disentangling Shifts in the Soil Microbiome of Potatoes Infected with *Rhizoctonia solani* Anastomosis Group 3-PT in Search of Potential Biocontrol Agents

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ABSTRACT

Rhizoctonia solani anastomosis group (AG) 3-PT is a devastating pathogenic fungus that causes several diseases in potatoes both in South Africa and globally. The removal of various fungicides from the market and strict regulations on the use of synthetic chemicals make disease management difficult. Therefore, alternative, environmentally safe control measures are being considered, such as the use of biological control agents (BCAs). BCAs are an attractive alternative for improving plant and soil health of economically important crops. To identify key microbial indicators of disease suppression against *R. solani* AG 3-PT, a greenhouse pot trial experiment was conducted using soil from a potato-growing region in KwaZulu-Natal, South Africa. High-throughput sequencing of fungal internal transcribed spacer and bacterial 16S ribosomal RNA was used to characterize the respective fungal and bacterial community composition in the soil with and without artificial inoculation with *R. solani* AG 3-PT. Results indicated that the pathogen caused dysbiosis in the potato soil microbiome, leading to a shift in fungal and bacterial community composition. Differentially abundant microbial taxa in *R. solani* AG 3-PT inoculated soils suggest a promising potential for disease-suppressive activity. Network analysis also confirmed the presence of key taxa involved in the microbial community shifts, which could support their role in the suppression of *R. solani* AG 3-PT. The identification of key microbial indicators against *Rhizoctonia* diseases can contribute to the development of environmentally sustainable potato production systems, which are particularly important considering the implementation of the European Green Deal.

Keywords: biocontrol, disease management, potato, *Rhizoctonia solani* AG 3-PT, soil microbiome

Potato (*Solanum tuberosum* L.) is the most consumed and economically important vegetable crop in the world (Food and Agriculture Organization Statistics 2017). Nutritionally, potato is ideal for human consumption and provides a balanced source of starch, high-quality proteins, vitamins, trace elements, and dietary fiber (Black 2008). This makes it a reliable crop to feed many communities and contribute to food security.

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To increase sustainable and economically feasible potato production, numerous resources have been invested in disease control, soil health, good farm management practices, seed quality, and the breeding of high-yielding varieties (Veeman and Veeman 2004). However, the potato crop, as with many other agricultural crops, is susceptible to devastation by various diseases (Fiers et al. 2012). Potato diseases reduce the yield and quality of fresh produce and therefore pose a threat to global food security (Black 2008). Soilborne diseases are persistent and recurrent in potato production (Larkin 2016; Tsror et al. 2001). These diseases are difficult to control, and management practices such as chemical seed treatments, crop rotation, promotion of rapid emergence, and early harvest of tubers are not always effective or practical (Larkin and Halloran 2014; Stevenson et al. 2001).

Some of the most important soilborne diseases affecting potatoes include black scurf, tuber malformation, and stem canker, which are caused by several anastomosis groups (AGs) of *Rhizoctonia solani*

Kühn. *R. solani* is a soil- and tuber-borne pathogen and is therefore difficult to manage (Muzhinji et al. 2015). The organism is taxonomically complex, leading to the identification of 13 distinct AGs based on their hyphal interactions. These AGs differ both morphologically and ecologically as well as in pathogenicity and host range (Sharon et al. 2006; Sneh et al. 1991). *R. solani* AG 3 subgroup PT is the main AG infecting potatoes globally and is commonly isolated from tubers showing symptoms of black scurf (Banville 1989; Truter and Wehner 2004; Woodhall et al. 2008). In addition to *R. solani* AG 3-PT, AG 2-2IIIB (black scurf, stem, and stolon canker), AG 4HG-I (stem and stolon canker), AG 4HG-III (stem canker), AG A and AG R (black scurf and stem canker) and AG 5 (black scurf) have been isolated from different *Rhizoctonia* disease symptoms in potatoes in South Africa, albeit at lower frequencies (Gush et al. 2019; Muzhinji et al. 2015).

Black scurf is characterized by the presence of dark sclerotia on the tuber surface (Banville et al. 1996). The appearance of these sclerotia decreases the marketable yield of tubers. Furthermore, sclerotia present on seed tubers provide a long-distance dispersal mechanism for the pathogen (Vilgalys and Cubeta 1994). To prevent the development of black scurf, various cultural measures are practiced, such as planting pathogen-free-certified seed tubers with no visible signs of sclerotia; planting in relatively warm, dry, pathogen-free soil; crop rotation with nonhosts; tillage; and early harvesting after haulm destruction (Carling and Leiner 1990; Dijst 1990; Larkin and Halloran 2014; Mulder et al. 1992). Even though these practices provide some control, they are not completely effective, and thus *Rhizoctonia* diseases remain a persistent threat to potatoes (Larkin 2016). Some chemicals have been shown to reduce the inoculum potential of *R. solani* in contaminated fields, but many of these, such as pencycuron and penflufen, have adverse environmental effects and are thus being withdrawn from the market in various countries (Clarke 2020; Jones 2020). Therefore, alternative, environmentally safe disease management strategies are needed.

The incorporation of biological control agents (BCAs) in soilborne pathogen management programs may provide ecologically benign and viable approaches for the control of these pathogens (Lahlali and Hijri 2010). As a nonchemical and targeted approach, biocontrol is receiving increased attention, particularly in light of increased fungicide resistance, deregistration of fungicides, and heightened concerns about the use of harsh chemicals in crop production (O'Brien and Milroy 2017). Therefore, biocontrol provides a key solution for the successful implementation of the European Green Deal, as it plays an important role in enhancing biodiversity and the overall health of plants and soil (Tataridas et al. 2022).

The antagonistic mechanisms of BCAs can involve the production of antibiotics by the BCA, the BCA becoming endophytic in the host plant, or competition with the target pathogen for resources (Lahlali and Hijri 2010; Larkin 2016; Larkin and Tavantzis 2013). Antagonistic microorganisms used as effective BCAs for *R. solani* include both fungal and bacterial taxa (Asaka and Shoda 1996; Mao et al. 1998; Szczech and Shoda 2004; Thrane et al. 2001). Bacterial taxa include *Pseudomonas* spp. (Howell and Stipanovic 1979), *Bacillus* spp. (Pleban et al. 1995), *Burkholderia* spp. (Larkin 2016), *Lysobacter* spp. (Das 2013) and *Laetisaria* spp. (Murdoch and Leach 1993). Fungal taxa include *Trichoderma* spp. (Grosch et al. 2006; Tsror et al. 2001), *Gliocladium* spp. (Lewis and Lumsden 2001), *Verticillium* spp. (Van Den Boogert and Velvis 1992), *Rhizoctonia zeae*, *Corticium* spp., and binucleate *Rhizoctonia* spp. (Escande and Echandi 1991). Only a few studies have documented beneficial microorganisms that contribute to the disease suppression of *Rhizoctonia* and their potential use for biological control in potatoes in the greenhouse (Brewer and Larkin 2005) or field (Larkin 2008; Larkin 2016; Larkin and Tavantzis 2013; Mrabet et al. 2013; Wright et al. 2022).

In the search for potential BCAs, most studies have relied on traditional culturing methods. These culture-based methods exclude the vast majority of microbes (Amann et al. 1995; Larkin and Brewer 2020). Next-generation sequencing technologies have provided a deeper understanding of the composition of soil microbial communities (Sabale et al. 2019). However, our knowledge of how to correlate microbial community changes and interactions between beneficial microbes and pathogens in the soil is still very limited. A better understanding of the changes in soil microbial communities and abiotic factors upon pathogen infection of a particular host will open new avenues for the deployment of beneficial microbes in the sustainable management of soilborne diseases (Peixoto et al. 2022).

This study aimed to investigate the differences in fungal and bacterial community composition between uninoculated soil and *R. solani* AG 3-PT inoculated soil as well as to evaluate how microbial enzymatic activity and soil chemical properties differ in *R. solani* AG 3-PT inoculated and uninoculated soils. We predicted that the introduction of *R. solani* AG 3-PT would alter fungal and bacterial community structure and diversity in the soil microbiome of potatoes and that there would be key taxa and/or groups associated with these shifts. Knowledge of changes in the soil microbial community resulting from the addition of *R. solani* AG 3-PT inoculum to the soil could allow the identification of key indicators of disease suppression of the pathogen (Bai et al. 2019). Results from this study may facilitate the development of beneficial microbial inoculants capable of suppressing *R. solani* AG 3-PT.

MATERIALS AND METHODS

Soil collection. A potato field with a history of black scurf was selected to study existing and potentially disease-suppressive soil microbial communities against *R. solani* AG 3-PT. The study site was located on a potato production farm in Howick, KwaZulu-Natal. The region has mild to warm summers with cold winters, summer rainfall, and brown to red clay soils (Potatoes South Africa 2018). After a potato season, soil for the greenhouse pot experiment was collected from the fallow, weed-free field (29°29′18.6″S, 30°08′24.7″E) in March 2021. Soil samples were collected at a depth of 15 to 30 cm using a spade, which was surface-sterilized using 70% ethanol prior to sample collection. A total of 20 soil samples weighing approximately 20 kg each were collected in a random sampling pattern across the 38-ha field. The soil samples were pooled and mixed to make a composite sample with a total weight of approximately 400 kg. Soil samples were stored in woven bags at room temperature before use in the greenhouse pot trial.

Inoculum preparation. Inoculum of *R. solani* AG 3-PT isolate Rh13 (culture collection number CMW 40583, Forestry and Agricultural Biotechnology Institute, GenBank accession number KJ777561) (Muzhinji et al. 2015) was prepared following the protocol of Muzhinji et al. (2014). Isolate Rh13 was cultured on potato dextrose agar (PDA) and incubated at 25°C in the dark for 2 weeks. Five PDA plugs (10 mm in diameter) were added to 10 g of barley grains in 13 conical flasks sterilized by autoclaving at 121°C for 20 min. The grains and *R. solani* were incubated for 14 days, shaking at 2-day intervals, until barley grains were completely colonized by visible fungal mycelia.

Pot trial. Visually disease-free sprouted mini-tubers (cultivar Mondial) were planted in 5-liter pots filled with soil from the KwaZulu-Natal study site and placed in a greenhouse compartment. Each pot was planted with a single seed tuber at a depth of

10 cm. Plants were watered every second day with approximately 400 ml of tap water to field capacity. The experiment was laid out in a randomized complete block design with two different soil treatments: *R. solani* AG 3-PT inoculated and uninoculated. For the *R. solani* AG 3-PT inoculated treatment, *R. solani* AG 3-PT isolate Rh13 inoculum at a density of 1.42×10^3 colony-forming units per gram of soil was spread evenly on the soil layer above the tuber and then covered with moistened soil to ensure a mini-tuber planting depth of 10 cm (Muzhinji et al. 2015). Sterile barley grains (10 g per pot) were added to the soil for the uninoculated treatment. Each treatment consisted of 13 replicates, resulting in a total of 26 pots. Replicates one to nine were used for the greenhouse soil sampling for next-generation sequencing. Potato tubers from replicates 7 to 13 were used for the disease assessment. Plants were grown for 92 days in the greenhouse at 25 ± 2°C with 12 h of light per day. Plants were fertilized with 2 g Multifeed nutrient solution (Nulandis) (3:1:6) per pot once at planting and again 2 months after emergence.

Greenhouse soil sampling for next-generation sequencing. Destructive sampling was conducted three times throughout the growing season; namely, 1 week after emergence (replicates one to three), at flowering (replicates four to six), and at harvest (replicates seven to nine). For each treatment at each time point, three plants were harvested for bulk and rhizosphere soil samples. For the bulk soil, approximately 3 g of soil around the outermost area of the pot, where there were no visible roots, was collected per sample. For the rhizosphere soil, the whole plant was carefully uprooted together with the soil adhering to the roots. The excess soil was gently shaken off and approximately 3 g of soil loosely attached to the roots of each plant was collected (Zimudzi et al. 2018). After sampling, the soil samples were stored at −80°C until further analysis.

Disease assessment. To confirm the virulence of *R. solani* AG 3-PT isolate Rh13, black scurf symptoms were assessed on potato tubers (replicates 7 to 13) harvested at the end of the greenhouse pot experiment. Tuber count and yield were measured for every pot, and the incidence and severity of blemishes on harvested progeny tubers were evaluated 92 days after planting. Re-isolations were made from diseased progeny tubers as described previously to confirm pathogen identity and thus Koch's postulates (Gush et al. 2019). Disease severity was estimated on a 0 to 5 scale using a modification of the scheme described by Carling and Leiner (1990), with 0 = no blemishes observed, 1 = less than 1% of the tuber surface covered with blemishes, 2 = between 1 and 10% of the tuber surface covered with blemishes, 3 = between 11 and 20% of the tuber surface covered with blemishes, 4 = between 21 and 50% of the tuber surface covered with blemishes, and 5 = 51% or more of the tuber surface covered with blemishes. To calculate the disease index, the following formula was used: $\mathrm{DIn} = \Sigma[0(n_0) + 0.25(n_1) + 0.5(n_2) + 0.75(n_3) + 1(n_4)] \times 100/(N_{\mathrm{total}})$, where n_x is the number of tubers in the x rating class and N is total number of tubers in each category.

Statistical analysis of pathogenicity trial. Data were analyzed using the statistical analysis software ARM (version 2019.8). The treatment means were separated using the least significant difference (LSD) test at 5% level of significance.

Soil chemical analysis. Soil chemical analysis was done on the KwaZulu-Natal soil samples according to standard protocols at Agri Technovation (MicroLife Research Centre, Wellington, South Africa). The pH was determined using 1 M potassium chloride and electrical resistance was determined in a saturated soil-water paste. Total phosphorus, calcium, sodium, potassium, and magnesium were determined by inductively coupled plasma (ICP) analysis with 1% citric acid extraction. Zinc, manganese, and copper concentrations were determined by ICP in 0.02 M diammonium ethylenediaminetetraacetic acid soil extracts. Sulfur was determined in calcium phosphate soil extracts and boron in hot water soil extracts. The soil organic carbon and total nitrogen were determined by the Walkley-Black and Dumas methods, respectively (Habig et al. 2018).

Soil microbial enzymatic activity. The ability of the soil microbial population to mineralize carbon, phosphorus, and nitrogen was assayed by measuring the respective β-glucosidase, alkaline phosphatase, and urease activity in the soil. β-Glucosidase and alkaline phosphatase activity was calculated spectrophotometrically at a wavelength of 410 nm by determining the rate of hydrolysis of *p*-nitrophenyl glucoside and *p*-nitrophenyl phosphate, respectively (Dick et al. 1997). Urease activity was determined by ammonia release from urea, quantified at 690 nm after 2-h incubations using the colorimetric sodium nitroprusside method as described by Kandeler and Gerber (1988). The urea content was calculated with reference to a standard calibration graph derived from urea standards (Kandeler and Gerber 1988).

Data on soil microbial enzymatic activity were subjected to nonparametric statistical analyses using Statistica 13 (StatSoft, Inc.). Microbial enzymatic activity was statistically analyzed by cluster analyses (vertical hierarchical tree plots) and constructed using Ward's clustering algorithm and the Euclidean distance measure (i.e., geometric distance between variables in a multidimensional space). Homogeneous grouping with Fisher's LSD was calculated at $P < 0.05$ for soil microbial enzymatic activity (Habig and Swanepoel 2015).

DNA extraction and sequencing. From storage, thawed soil samples were first pretreated with ethidium monoazide bromide to remove extracellular DNA before DNA extraction following a previously validated protocol (Carini et al. 2017; Wagner et al. 2015). Subsequently, 0.5 g of soil from each of the 36 soil samples (two treatments, two soil types, three sampling times, and three replicates) was subjected to genomic DNA extraction using DNeasy PowerSoil Kits (Qiagen) according to the manufacturer's protocol. The DNA was quantified using a Qubit 2.0 fluorometer (Invitrogen, Life Technologies). The internal transcribed spacer (ITS) region of ribosomal RNA was amplified using the fungal-specific primers ITS1 BITS (5′-ACCTGCGGARGGATCA-3′), B58S3 (5′-GAGATCCRTTGYTRAAAGTT-3′), ITS2 gITS7f (5′-GTGAATCATCGARTCTTTG-3′), and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), targeting the ribosomal ITS1-ITS2 region (Bokulich and Mills 2013). Bacterial 16S ribosomal RNA gene regions were amplified using primers 515F (5′-GTGYCAGCMGCCGCGGRA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) targeting the V4 region (Castillo et al. 2017). Paired-end 2× 250-bp sequencing was performed on a MiSeq instrument (Illumina Inc.) at Admera Health.

Amplicon sequencing analysis. Fungal and bacterial sequence reads were processed using QIIME 2 (version 2021.11) (Bolyen et al. 2019) and filtered using the DADA2 pipeline (Callahan et al. 2016). Quality trimming was performed for all sequences greater than 220 bp for fungal reads and 300 bp for bacterial reads. Fungal and bacterial sequences containing more than two ambiguous base calls, quality scores of less than 25, or more than one mismatch to the sample-specific barcode or the primer sequences were excluded from further downstream analysis. Resulting amplicon sequence variants (ASVs) were assigned to taxonomies using the Ribosomal Database Project naive Bayesian classifier with the UNITE fungal database (version 8) (https://unite.ut.ee/) (with 99% similarity cutoff) for fungal species and the SILVA database (version 132) (Quast et al. 2013) (with 99% similarity cutoff) for bacterial species. Fungal and bacterial rarefaction curves were generated to assess the sequencing depth of each sample using the 'vegan' package

(Oksanen et al. 2007) in RStudio. A total of 36 fungal and 36 bacterial samples were used for downstream analysis.

Statistical analysis. Alpha diversity metrics (richness, Shannon and Simpson's diversity indices), beta diversity metrics, and ordination were calculated using 'phyloseq' (McMurdie and Holmes 2013) and the 'vegan' package (Oksanen et al. 2007) in RStudio. The relative abundances and alpha diversity indices of the different soil types (rhizosphere or bulk) and states (uninoculated or *R. solani* AG 3-PT inoculated) were tested using the Shapiro-Wilk normality test (Royston 1982). Significant differences in relative abundances at the genus level were calculated using analysis of variance (ANOVA) for normally distributed data (Chambers et al. 1992) and the Kruskal-Wallis test for non-normally distributed data (McKnight and Najab 2010). The rarefied absolute abundance tables were $\log(x + 1)$ transformed for beta diversity analysis. Beta diversity indices between groups were calculated using the Bray-Curtis dissimilarity metric (Lozupone et al. 2011) and visualized in principal coordinate analysis plots (Jolliffe and Cadima 2016). Significant differences in beta diversity between soil types, states, and sampling times were calculated using a permutational multivariate ANOVA (PERMANOVA) (Anderson and Walsh 2013) with 1,000 permutations using the 'adonis' function of the 'vegan' package. Variation within the different soil types, states, and sampling times was tested using analysis of multivariate homogeneity of group dispersions ('betadisper'), and similarity was tested with analysis of similarities (Anderson 2006) using the same number of permutations as the PERMANOVA test.

Co-occurrence network analysis (rhizosphere and bulk) was performed using the nonparametric Spearman's correlation test with a correlation threshold between −0.6 and 0.6 and *P* value threshold of 0.01 (Barberán et al. 2012). The connections between ASVs (edges) correspond to a significant positive or negative correlation between ASVs (nodes) (Dini-Andreote et al. 2014). The networks were plotted using Gephi (Bastian et al. 2009). A NetShift analysis was performed to identify community shifts (rhizosphere and bulk) by potential keystone driver taxa based on their different states and network associations (https://web.rniapps.net/netshift/) (Kuntal et al. 2019).

Taxa that were significantly overrepresented in the different soil types or states were identified as microbial markers using a linear discriminant analysis effect size (LEfSe) analysis (Segata et al. 2011). The ASV abundance counts were converted to relative abundances for the LEfSe analysis. Significant differences in abundance at species level were calculated using the Kruskal-Wallis test with a cutoff of 99% ($P < 0.01$). A distance-based redundancy analysis (dbRDA) was performed using the 'vegan' package to investigate the effects of soil chemical properties and extracellular enzyme activities on the fungal and bacterial community beta diversity distribution (Oksanen 2010). Soil chemistry and enzymatic activity variables were standardized with the function decostand(). The dbRDA was performed using capscale(). The function ordiR2step() was used to check for co-linearity and to select the variables to use in the redundancy analysis model. These variables were checked for significance, which led to the removal of variables with a co-linearity score above 10. Thereafter, a forward stepwise model with 1,000 permutations was performed to select the best set of variables that could explain the variational effects on fungal and bacterial community composition. The final dbRDA models were calculated using ANOVA with an adjusted *P* value threshold of 0.001.

RESULTS

Disease assessment. At harvest of the greenhouse pot experiment, progeny tubers from plants in *R. solani* AG 3-PT inoculated treatments had a significantly ($P < 0.05$) higher disease incidence and index compared with those from uninoculated treatments (Table 1). No disease symptoms were observed in progeny tubers from uninoculated treatments. According to the univariate procedure and normality plots, the data were normally distributed with homogeneous treatment variance. When comparing the *R. solani* AG 3-PT inoculated and uninoculated treatments, there were no significant differences between treatments in terms of tuber yield ($P = 0.05$).

Changes in soil microbial community diversity of potatoes upon *R. solani* AG 3-PT infection. Amplicon sequencing analysis was done to compare fungal and bacterial community compositional differences between the *R. solani* AG 3-PT inoculated and uninoculated rhizosphere and bulk soil samples at three different sampling times (seedling, flowering, and at harvest) from the potato pot trial. No significant differences were observed in the different sampling times (rhizosphere and bulk) (Supplementary Fig. S1); however, specific trends were observed in alpha and beta diversity in the different sampling times. The alpha diversity of samples (rhizosphere and bulk) revealed that fungal diversity decreased from seedling to flowering and then increased from flowering to at harvest (Supplementary Fig. S1A). The opposite trend was observed for bacterial samples, in which the diversity increased from seedling to flowering and then decreased from flowering to at harvest (Supplementary Fig. S1B). Although no significant effects were observed in beta diversity for both fungal (Supplementary Fig. S2A) and bacterial (Supplementary Fig. S2B) samples, the Bray-Curtis beta diversity dissimilarity analysis showed that bacterial communities clustered into more distinct communities during flowering compared with seedling and at harvest. For this reason, further analysis focused on comparing fungal and bacterial community compositional differences between the *R. solani* AG 3-PT inoculated and uninoculated rhizosphere and bulk soil samples from the potato pot trial.

A genus-clustered comparison between the different soil treatments revealed that there were 78 (20.9%) and 202 (27.8%) shared fungal and bacterial ASVs, respectively, across all treatments (Fig. 1). Comparisons between treatments in the rhizosphere soil revealed that more unique ASVs were observed in the uninoculated treatment compared with the *R. solani* AG 3-PT inoculated treatment for both fungi (8 versus 3.8%) and bacteria (6.9 versus 6.1%). The same trend was observed for bacterial ASVs in the bulk soil, in which the uninoculated samples exhibited a higher percentage (5.1 versus 4.1%) of unique ASVs. By contrast, more unique fungal ASVs were observed in the *R. solani* AG 3-PT inoculated (7.8%) bulk soil treatment compared with the uninoculated (5.9%) bulk soil.

The alpha diversity of both *R. solani* AG 3-PT inoculated and uninoculated samples also revealed that the fungal diversity in the rhizosphere soil was significantly lower ($P < 0.00002$) compared

TABLE 1
Tuber yield, disease incidence, and index means of the greenhouse pot trial with potato tubers inoculated with *Rhizoctonia solani* anastomosis group 3-PT in KwaZulu-Natal soil

Treatment	Mean tuber yield, g	Mean disease incidence, DI/tt[a]	Mean disease index, DIn/tt[b]
Inoculated	103.64	100.00[c]	742.14[c]
Uninoculated	105.43	0	0

[a] Disease incidence per total number of tubers.
[b] Disease index (disease severity × incidence) per total number of tubers.
[c] Values are significantly different ($P < 0.05$).

with the bulk soil (Fig. 2A). Similarly, fungal diversity in the *R. solani* AG 3-PT rhizosphere inoculated treatments was lower compared with the uninoculated treatments, although not significantly. By comparison, no difference in diversity was observed for bacterial communities with the introduction of the pathogen (Fig. 2B). The Bray-Curtis beta diversity dissimilarity analysis showed that fungal communities were significantly different, albeit to a small degree, between the *R. solani* AG 3-PT inoculated and uninoculated treatments ($P < 0.002$, $R^2 = 0.036$) as well as between the rhizosphere and the bulk soil ($P < 0.0009$, $R^2 = 0.037$) (Fig. 3A). Similarly, bacterial community composition clustered into more distinct communities in the *R. solani* AG 3-PT inoculated compared with uninoculated treatments ($P < 0.0009$, $R^2 = 0.053$) as well as in the rhizosphere and bulk soil ($P < 0.002$, $R^2 = 0.046$) (Fig. 3B). A comparison of the rhizosphere soil further revealed distinct community clustering between the *R. solani* AG 3-PT inoculated and uninoculated treatments in fungal ($P < 0.0009$, $R^2 = 0.086$) and bacterial ($P < 0.0009$, $R^2 = 0.135$) samples (Supplementary Fig. S3). Although not significant, the bulk soil revealed more distinct clustering between the *R. solani* AG 3-PT inoculated and uninoculated treatments in bacterial communities ($P < 0.28$, $R^2 = 0.064$) compared with fungal communities ($P < 0.89$, $R^2 = 0.054$) (Supplementary Fig. S4).

Microbial enzymatic activity of potatoes upon *R. solani* AG 3-PT inoculation. Extracellular enzyme activity assays were conducted to assess the impact of *R. solani* AG 3-PT on soil microbial productivity. β-Glucosidase, alkaline phosphatase, and urease activity was used as an indicator of microbial conversion and degradation of organic substrates into plant-available nutrients. The results from these assays showed that soil microbial communities associated with *R. solani* AG 3-PT inoculated and uninoculated treatments differed in their ability to mineralize carbon (β-glucosidase), nitrogen (urease), and phosphorus (alkaline phosphatase) under high pH conditions.

β-Glucosidase and alkaline phosphatase activity was significantly ($P < 0.05$) higher in *R. solani* AG 3-PT inoculated treatments compared with uninoculated treatments (Fig. 4). The same trend was observed between rhizosphere and bulk soils in the

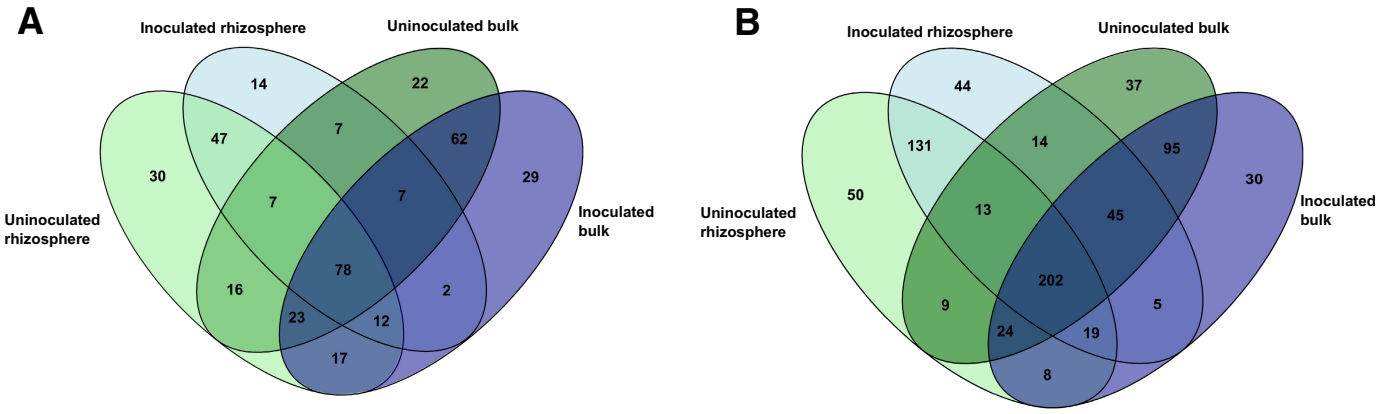

Fig. 1. Unique and shared **A,** fungal and **B,** bacterial genus-clustered amplicon sequence variants between the *Rhizoctonia solani* anastomosis group 3-PT inoculated and uninoculated rhizosphere and bulk soil samples from the potato pot trial. The shared taxa are highlighted in bold.

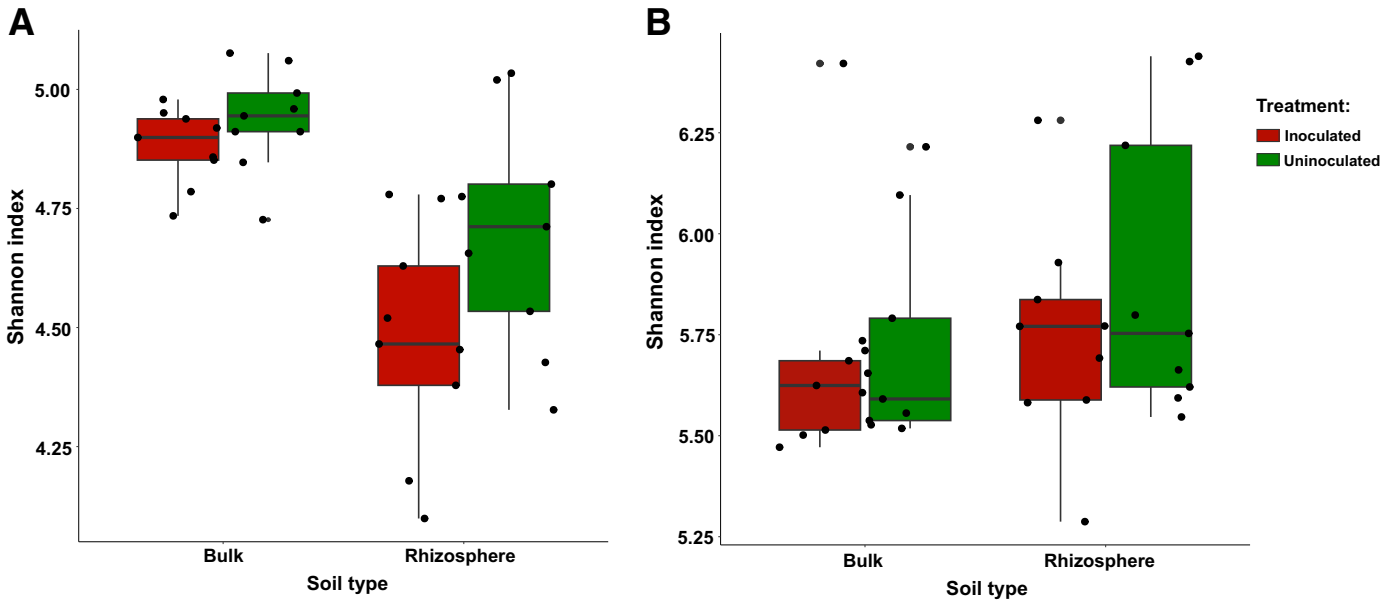

Fig. 2. Alpha diversity changes of **A,** fungal ($P < 0.00002$) and **B,** bacterial ($P < 0.01$) communities in the soil microbiome of potato plants. Shannon index box plots show differences in diversity in the rhizosphere and bulk soils as well as the *Rhizoctonia solani* anastomosis group 3-PT inoculated and uninoculated treatments.

R. solani AG 3-PT inoculated treatments, with the former exhibiting significantly ($P < 0.05$) higher enzymatic activity. Urease activity was significantly ($P < 0.05$) higher in rhizosphere soils compared with bulk soils. However, microbial activity did not differ significantly between *R. solani* AG 3-PT inoculated and uninoculated treatments in rhizosphere soils.

A dbRDA of soil chemical properties and microbial enzymatic activities was conducted to further explore the possible effects on the microbial composition of both *R. solani* AG 3-PT inoculated and uninoculated soil communities. β-Glucosidase activity ($P < 0.001$, $R^2 = 0.021$) and phosphorus activity ($P < 0.001$, $R^2 = 0.012$) explained 2 and 1%, respectively, of the beta diversity distribution for the fungal communities (Fig. 5A). Soil pH also significantly contributed to both fungal ($P < 0.001$, $R^2 = 0.01$) (Fig. 5A) and bacterial ($P < 0.001$, $R^2 = 0.04$) (Fig. 5B) community shifts. Magnesium was identified as the major variable driving bacterial community composition ($P < 0.001$, $R^2 = 0.072$), explaining 7% of the beta diversity distribution of bacterial communities.

Impact of *R. solani* AG 3-PT on soil microbial communities of potatoes. To identify specific taxa involved in the microbial shifts observed with the introduction of *R. solani* AG 3-PT into the soil, a LEfSe analysis was conducted between the different treatments and soil sample types. As expected, the biggest difference in microbial shifts between the *R. solani* AG 3-PT inoculated and uninoculated treatments was observed in the rhizosphere soil. More differentially abundant fungal taxa were identified in the rhizosphere soil (23 taxa at the highest identified rank) compared with the bulk soil (two taxa at the highest identified rank). There were 74 differentially abundant bacterial taxa identified in the rhizosphere soil and none in the bulk soil.

In the rhizosphere soil, there were more overrepresented fungal taxa associated with the uninoculated treatments compared with the *R. solani* AG 3-PT inoculated treatments (Fig. 6A). The most notable overrepresented (linear discriminant analysis [LDA] scores >4 and high abundances) fungal taxa associated with the *R. solani* AG 3-PT inoculated treatments included *Talaromyces*

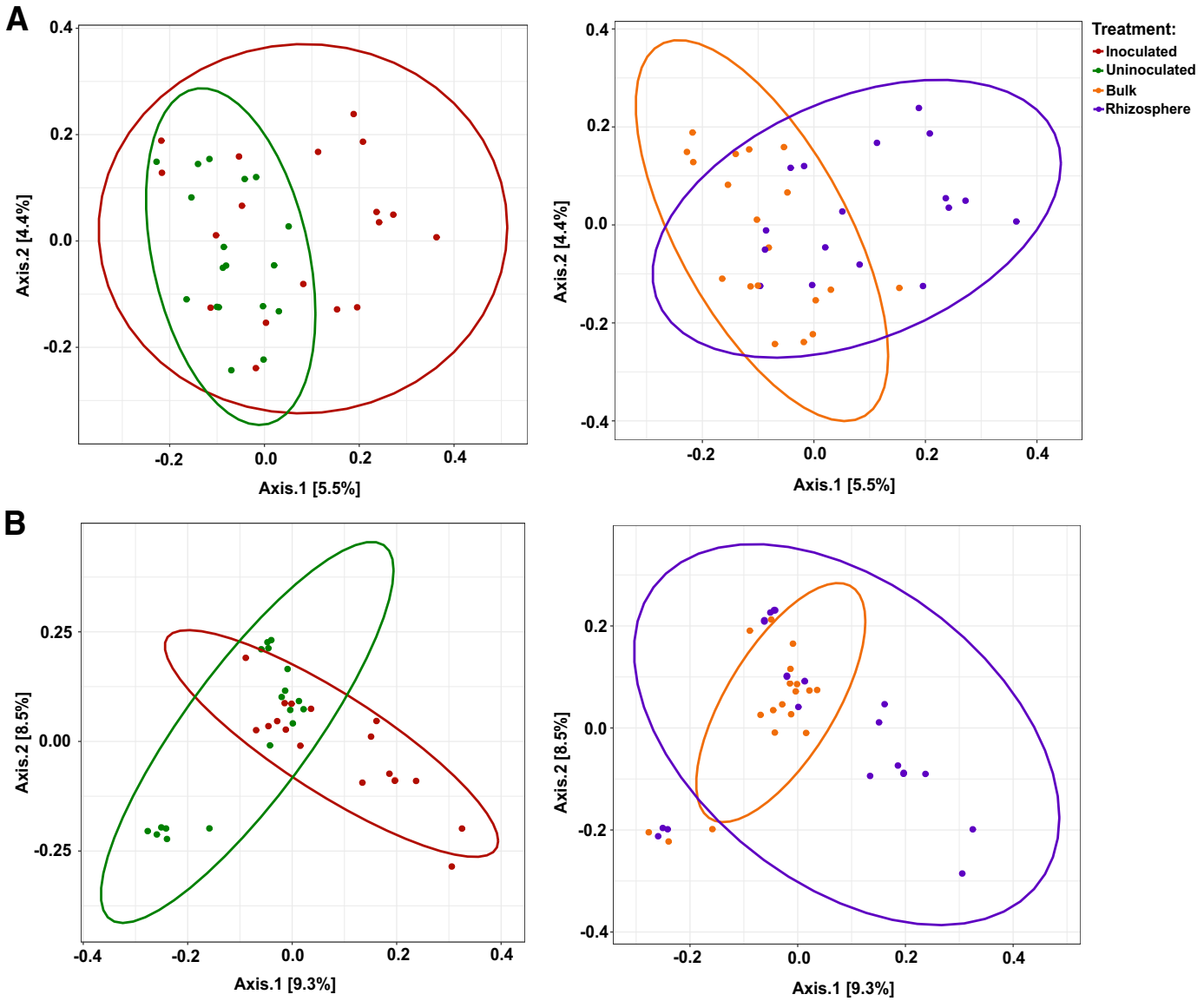

Fig. 3. Dissimilarities in **A,** fungal and **B,** bacterial communities in the soil microbiome of potatoes. Principal coordinate analysis plots display the Bray-Curtis dissimilarity matrices between fungal communities in the two different treatments ($P < 0.002$, $R^2 = 0.036$) and soil types ($P < 0.0009$, $R^2 = 0.037$) and between bacterial communities in the two different treatments ($P < 0.0009$, $R^2 = 0.053$) and soil types ($P < 0.002$, $R^2 = 0.046$).

spp., *Talaromyces funiculosus*, *Aspergillus alabamensis*, and *R. solani*. Overrepresented fungal taxa in the uninoculated treatments included *Thielavia inaequalis* and Sordariomycetes. *T. inaequalis* is also classified in the Chaetomiaceae family, Sordariales order, and Sordariomycetes class. In the bulk soil, the overrepresented fungal taxa included *R. solani* (LDA 3.34) in the inoculated treatments and Chaetomiaceae (LDA 3.92) in the uninoculated treatments (Fig. 6B).

In contrast to fungal communities, there were more overrepresented bacterial taxa associated with the *R. solani* AG 3-PT inoculated treatments compared with the uninoculated treatments (Fig. 6C). The most notable overrepresented (LDA scores >4 and high abundances) bacterial taxa associated with the *R. solani* AG 3-PT inoculated treatments included *Burkholderia-Caballeronia-Paraburkholderia*, *Luteibacter*, *Massilia*, and Micrococcaceae. Overrepresented bacterial taxa in the uninoculated treatments included Vicinamibacterales, KD4-96, Gemmatimonadaceae, Gaiellales, and subgroup 7.

Co-occurrence networks were constructed to observe the positive and negative associations between taxa in *R. solani* AG 3-PT inoculated and uninoculated treatments (Fig. 7). The fungal network of the *R. solani* AG 3-PT inoculated soils was larger, with a higher number of taxa or "nodes" (204 inoculated versus 185 uninoculated), connections, or "edges" (525 inoculated versus 472 uninoculated) and negative connections (13 inoculated versus 1 uninoculated) compared with the network for communities in the uninoculated soil (Fig. 7A). By contrast, the bacterial network of the uninoculated soils was larger, with a higher number of nodes (343 uninoculated versus 322 inoculated) and edges (1,100 uninoculated versus 913 inoculated), although there were fewer negative connections (0 uninoculated versus 15 inoculated) compared with the network for communities in the *R. solani* AG 3-PT inoculated networks (Fig. 7B). The top five fungal and bacterial taxa with the highest degree and betweenness centrality scores were identified as potential keystones in the uninoculated and *R. solani* AG 3-PT inoculated networks (Table 2).

A NetShift association network analysis between the *R. solani* AG 3-PT inoculated (rhizosphere and bulk) and uninoculated (rhizosphere and bulk) treatments highlighted fungal and bacterial taxa that were potential key "drivers" of the microbial shifts that

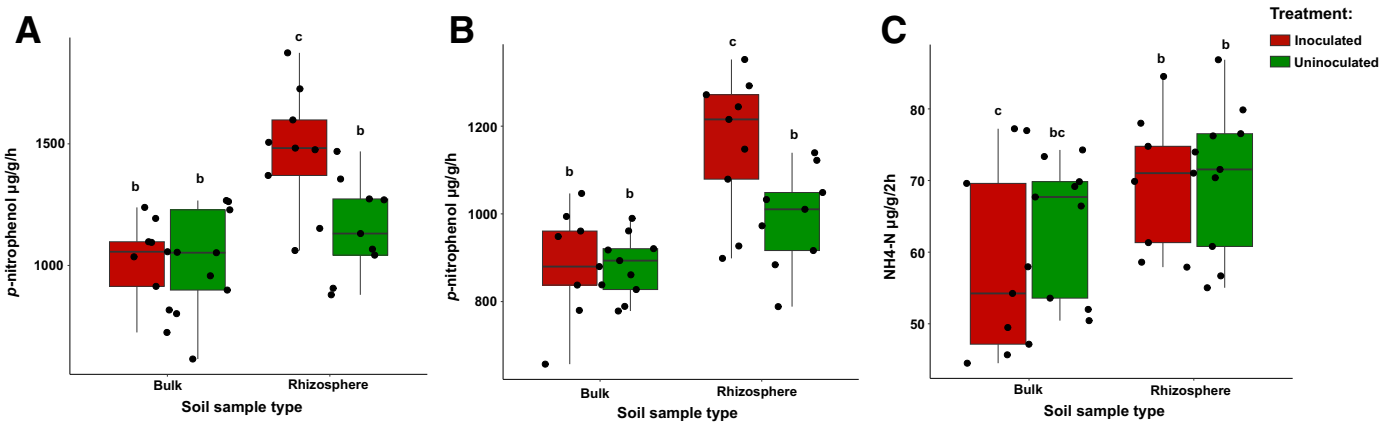

Fig. 4. Soil microbial **A,** β-glucosidase, **B,** alkaline phosphatase, and **C,** urease activity between *Rhizoctonia solani* anastomosis group 3-PT inoculated and uninoculated soil and soil types (rhizosphere and bulk) in a pot trial. Treatments with corresponding letters do not differ significantly ($P > 0.05$).

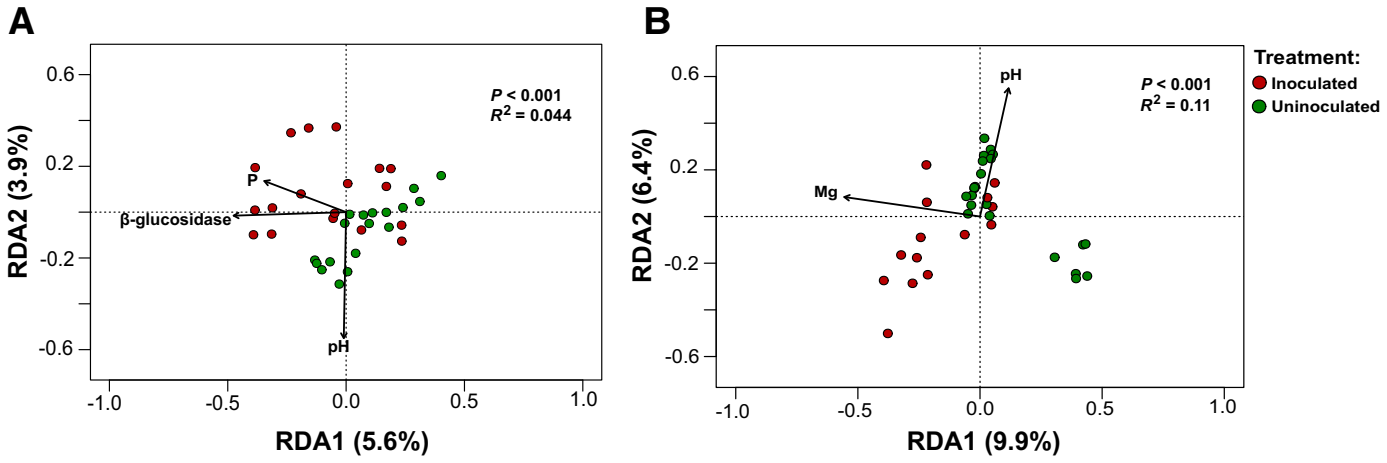

Fig. 5. Distance-based redundancy analysis (dbRDA) plots showing Bray-Curtis distribution of the **A,** fungal and **B,** bacterial communities in the soil significantly (adjusted $P < 0.001$) affected by chemical properties and extracellular enzyme activities (represented by black arrows). Red dots represent communities in the *Rhizoctonia solani* anastomosis group 3-PT inoculated treatments, and green dots represent communities in the uninoculated treatments. Significant variables include β-glucosidase activity (*p*-nitrophenol micrograms per gram per hour), magnesium ammonium acetate (milligrams per kilogram), pH (potassium chloride), and phosphorus Bray1 (milligrams per kilogram) in the soil. RDA1 and RDA2 show the percentage of total variance explained by the first two eigenvalues. The length of the arrows is equal to the multiple correlation of the variable.

occurred with the introduction of the pathogen to the potato soil microbiome. These driver taxa were identified by their increase in importance using the betweenness centrality score from a healthy (i.e., uninoculated) state to a diseased (i.e., *R. solani* AG 3-PT inoculated) state (Kuntal et al. 2019). Unidentified fungal taxa in the order Sordariales were identified as potential key driver taxa of the microbial community shifts with the introduction of *R. solani* AG 3-PT to the potato soil microbiome (Fig. 8A). The taxon Sordariales was also identified as a biomarker in the LEfSe analysis and as a potential keystone taxon in the co-occurrence network (node 443) with a degree of 10 and a betweenness centrality score of 499,55. Various bacterial taxa were identified as potential key drivers (Fig. 8B). By comparison, bacterial driver taxa identified across the different analyses, including *Pedobacter*, *Enterobacteriaceae*, *Pseudoflavitalea*, *Luteibacter*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Sphingopyxis*, *Dyadobacter*, *OLB 12*, *Chitinophaga*, *Luteolibacter*, and Verrucomicrobiaceae, were most overrepresented in the *R. solani* AG 3-PT inoculated treatments. Thermomicrobiaceae, *Piscinibacter*, RB41, and Gaiellales were most overrepresented in the uninoculated treatments (Fig. 6C).

DISCUSSION

The composition and function of soil microbiomes can protect plants from soilborne pathogens, leading to disease suppression (Durán et al. 2018; Wei et al. 2019). With the release of specific disease-induced root exudates, plants recruit microorganisms directly related to the plant host genotype to aid in certain functions, such as directly suppressing soilborne pathogens (Berry and Widder 2014; Lemanceau et al. 2017; Mercado-Blanco and Bakker 2007; Pascale et al. 2020; Reinhold-Hurek et al. 2015). In the present study, shifts in the soil microbial community resulting from the addition of *R. solani* AG 3-PT inoculum to rhizosphere and bulk soils revealed potential fungal and bacterial taxa of *Rhizoctonia* disease suppression.

Soil microbial enzymatic activity indicated an increase in microbial activity in the rhizosphere soil and *R. solani* AG 3-PT inoculated treatments, possibly due to the higher microbial biomass in the rhizosphere (Fig. 4) (Philippot et al. 2013). The increase in the *R. solani* AG 3-PT inoculated treatments can be attributed to the addition of active *R. solani* AG 3-PT cellular biomass, leading to greater production of enzymes. Furthermore, decomposition of diseased plant tissues releases nutrients into the soil, which may have attracted various beneficial microbes to the rhizosphere, consequently increasing microbial enzymatic activity (Garbeva et al. 2006; Tsror 2010). The rhizosphere is a highly competitive environment in which beneficial microbial communities play crucial roles, not only in nutrient acquisition but also in host defense and disease suppression (Bakker et al. 2013; Berendsen et al. 2012; Philippot et al. 2013). Therefore, we propose that the key fungal and bacterial taxa present in the *R. solani* AG 3-PT inoculated treatments could be recruited by the potato crop (cultivar Mondial) through the plant-triggered induced systemic resistance response after infection and nutrient utilization from plant tissue decomposition.

The loss in fungal diversity (Fig. 2A) and scattered community distribution (Fig. 3A; Supplementary Fig. S3A) after pathogen infection suggest that the pathogen caused microbial community dysbiosis (loss of diversity and functional potential), leading to a disruption of fungal composition and function. The increased β-glucosidase activity in the *R. solani* AG 3-PT inoculated treatments suggests that the increase in pathogen biomass and the potential of the fungal community to confer benefits resulted in greater production of β-glucosidase for the utilization of available carbon. Increased available carbon has been demonstrated to raise the incidence of black scurf disease in potato tubers, as the pathogen utilizes cellulose from plant tissue as a carbon source (Garbeva et al. 2006; Tsror 2010). Similar results have been observed in other studies, which concluded that competition for substrate through plant stimulation of beneficial fungal communities reduced pathogen

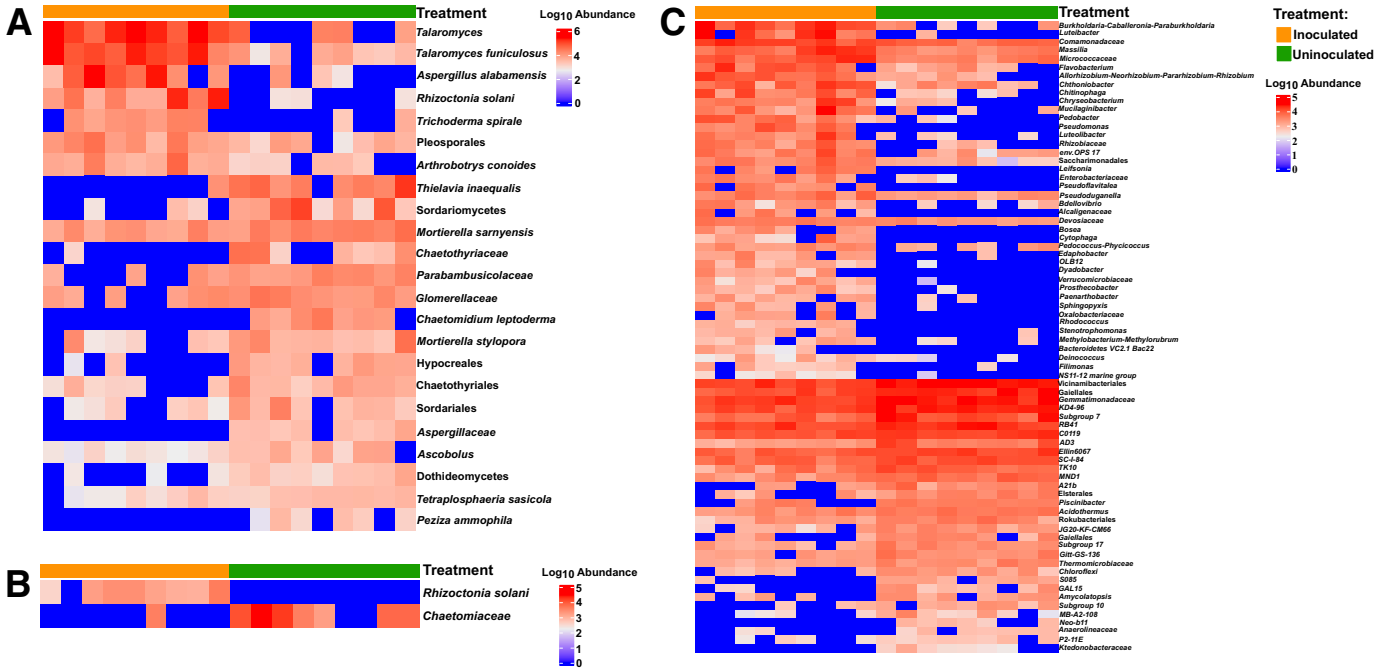

Fig. 6. Linear discriminant analysis effect size (LEfSe) of taxa significantly ($P < 0.01$) overrepresented in *Rhizoctonia solani* anastomosis group 3-PT inoculated compared with uninoculated treatments in soil planted with potatoes. Overrepresentation is indicated as $\log_{10}$ change. Heat maps showing fungal taxa overrepresented in **A,** rhizosphere soil and **B,** bulk soil as well as bacterial taxa overrepresented in **C,** rhizosphere soil. No bacterial biomarkers were identified in the bulk soils.

invasion and enhanced plant defenses (Chiu et al. 2017; McLaren and Callahan 2020; Solanki et al. 2022).

Fewer differentially abundant fungal taxa were identified in the *R. solani* AG 3-PT inoculated rhizosphere and bulk soils compared with the uninoculated treatments, which is indicative of the negative impact of *R. solani* AG 3-PT on the fungal community (Fig. 6A). The most overrepresented fungi in the *R. solani* AG 3-PT inoculated treatments included phosphate-solubilizing fungi, such as *T. funiculosus*, *Trichoderma spirale*, and *A. alabamensis*. Albeit low level of significance, the presence of phosphorus could suggest a better understanding of the shifts in fungal community composition in the *R. solani* AG 3-PT inoculated treatments. This could indicate a high abundance of potential phosphate-solubilizing fungi (Tian et al. 2010). In addition to *R. solani*, these taxa are associated with the ability to solubilize phosphates and could aid in overall potato crop fitness by enhancing phosphate acquisition and plant growth (Go et al. 2023; Hakim et al. 2015; Kucey et al. 1989; Whitelaw 1999). However, these fungal taxa were not identified as key drivers

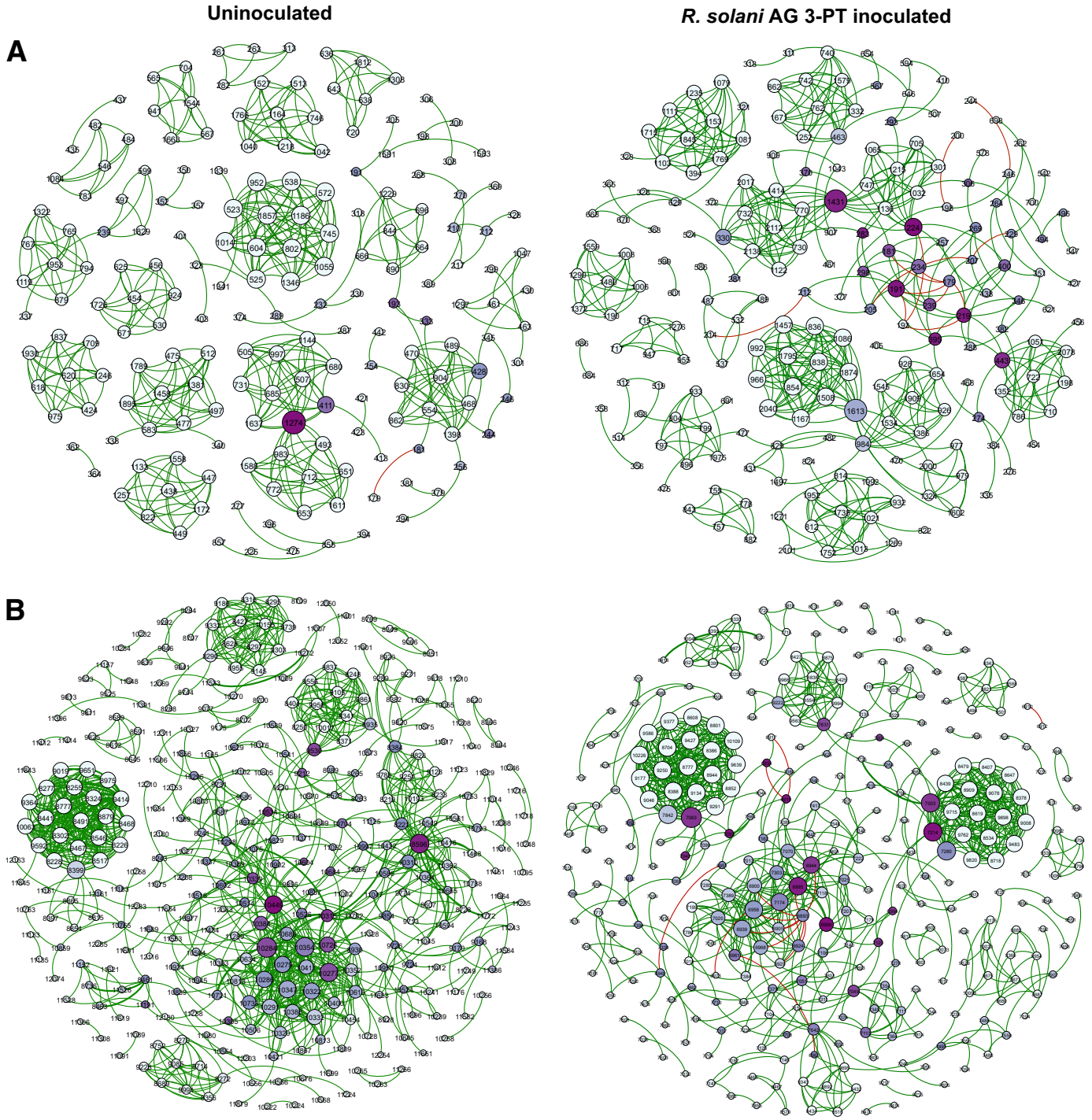

Fig. 7. A, Fungal and **B,** bacterial co-occurrence networks ($P < 0.01$) of potato soil microbiomes (rhizosphere and bulk) associated with uninoculated (left) and *Rhizoctonia solani* anastomosis group (AG) 3-PT inoculated plants (right) in the KwaZulu-Natal region. Node sizes are proportional to their degree distribution and colors represent the betweenness centrality score (from light to dark purple). Edge colors represent the positive (green) and negative (red) interactions between nodes. Numbers within nodes represent taxon identifiers (IDs) (Supplementary Material).

in the NetShift analysis. β-Glucosidase activity in the *R. solani* AG 3-PT inoculated treatments was shown to explain a higher percentage of the beta diversity distribution than phosphorus; thus, we suggest that carbon mineralization played a more important role in the recruited beneficial fungi (Fig. 5A).

The taxonomic group Sordariales was identified as a potential contributor to the shifts observed in the fungal community following *R. solani* AG 3-PT inoculation in the rhizosphere (Fig. 8A). The unidentified Sordariales taxa were differentially abundant in both the *R. solani* AG 3-PT inoculated and uninoculated treatments and exhibited only positive connections in the community co-occurrence networks (Fig. 7A). The most notable overrepresented fungal taxa in the uninoculated treatments included *T. inaequalis* (Chaetomiaceae family, Sordariales order, Sordariomycetes class) and Sordariomycetes (Fig. 6A). Interestingly, no studies have investigated the role of *T. inaequalis* as a potential BCA. In the bulk soil, the overrepresented fungal taxa included *R. solani* in the inoculated treatments and Chaetomiaceae in the uninoculated treatments (Fig. 6B). *Chaetomium* spp. have been found to produce secondary metabolites that exhibit significant antifungal activity against plant pathogenic fungi, including *Botrytis cinerea*, *Alternaria solani*, *Magnaporthe oryzae*, and *Gibberella saubinettii* (Ibrahim et al. 2021; Li et al. 2018). *Chaetomium globosum* and *Chaetomidium leptoderma* have also shown antagonistic effects against *R. solani* (Aggarwal et al. 2014; Di Pietro et al. 1992; Moya et al. 2016; Walther and Gindrat 1988; Yue et al. 2018).

Although there was no loss in bacterial diversity (Fig. 2B), community composition shifts (Fig. 3B) also suggest a change in bacterial communities caused by inoculation of the pathogen. The distinctly clustered bacterial communities suggest that there was recruitment of specific bacteria to the rhizosphere upon *R. solani* AG 3-PT infection (Supplementary Fig. S3B) (Bakker et al. 2013; Berendsen et al. 2012). We propose that root-associated bacterial communities could be derived from the bulk soil with the occurrence of the "rhizosphere effect" in the potato soil microbiome upon introduction of *R. solani* AG 3-PT, as has been shown in similar studies (Hou et al. 2020; Mendes et al. 2013).

The introduction of *R. solani* AG 3-PT demonstrated a negative impact of dysbiosis caused by the pathogen on the bacterial community in the rhizosphere microbiome of potatoes (Petersen and Round 2014). The high number of nodes and positive connections in the bacterial networks indicates a complex, synergistic, and diverse environment (Fig. 7B). High bacterial diversity has been suggested to promote disease suppression and increase resistance to pathogen invasion (Bell et al. 2005; Hu et al. 2016; Kristensen et al. 2016). The *R. solani* AG 3-PT inoculated treatments exhibited more connected components and longer path lengths in the co-occurrence networks (Fig. 7B) as well as more differentially abundant bacterial taxa (Fig. 6C), suggesting a more complex interactive community compared with the uninoculated treatments. A study by Mendes et al. (2011) showed that bacterial taxa associated with *R. solani*-suppressive soils in sugar beet were more abundant than those in uninoculated soils. Similarly, the present study showed an increase in differentially abundant bacterial taxa associated with the *R. solani* AG 3-PT inoculated treatments (Fig. 6C), providing insight into the potato plant's exploitation of soil bacteria consortia upon pathogen attack. The various potential bacterial biomarkers (LEfSe) and keystone (co-occurrence networks) and driver (NetShift) taxa identified in

TABLE 2
Fungal and bacterial taxa of potato soil microbiomes (rhizosphere and bulk) with the highest degree and betweenness centrality scores in uninoculated and *Rhizoctonia solani* anastomosis group 3-PT inoculated treatments[a]

Domain	Treatment	Node	Taxon	Degree	Betweenness centrality
Fungi	Uninoculated	1,274	*Basidioascus*	17	80
Fungi	Uninoculated	193	*Rhizophlyctis rosea*	12	20
Fungi	Uninoculated	411	*Clonostachys*	10	17
Fungi	Uninoculated	333	*Actinomucor elegans*	2	17
Fungi	Uninoculated	244	*Papulaspora funabasensis*	2	15
Fungi	Inoculated	224	Parabambusicolaceae	12	973.25
Fungi	Inoculated	191	Aspergillaceae	11	726.15
Fungi	Inoculated	219	*Chloridium aseptatum*	9	688.72
Fungi	Inoculated	1,431	*Cystobasidium*	17	620
Fungi	Inoculated	395	*Helicoma*	7	618.76
Bacteria	Uninoculated	10,449	*Steroidobacteraceae*	25	7,189.49
Bacteria	Uninoculated	8,596	*Blastocatellaceae*	27	4,423.69
Bacteria	Uninoculated	10,534	*Moraxellaceae*	8	3,396.34
Bacteria	Uninoculated	10,315	*Gemmataceae*	14	3,068.39
Bacteria	Uninoculated	9,530	*Diplorickettsiaceae*	15	2,398.48
Bacteria	Inoculated	6,996	SM2D12	14	7,329.90
Bacteria	Inoculated	7,214	*Caulobacteraceae*	21	5,985.52
Bacteria	Inoculated	7,092	P2-11E	5	5,675.5
Bacteria	Inoculated	6,934	MB-A2-108	4	5,597.48
Bacteria	Inoculated	6,919	*Rhodococcus*	5	5,499.21

[a] The terms node, degree, and betweenness centrality are widely used in microbial co-occurrence networks (Berry and Widder 2014).

this study suggest that the interaction between these identified bacterial groups could play an important role in the rhizosphere of the potato plant upon *R. solani* AG 3-PT infection (Hu et al. 2016; Li et al. 2019; Wei et al. 2015, 2019).

Although the literature is sparse, bacterial taxa found to be antagonistic to *R. solani* include *Pseudomonas* spp. (Howell and Stipanovic 1979), *Bacillus* spp. (Pleban et al. 1995), *Burkholderia* spp. (Larkin 2016), *Lysobacter* spp. (Das 2013), and *Laetisaria* spp. (Murdoch and Leach 1993). Likewise, *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. have been linked to soil suppressiveness of various pathogens (Garbeva et al. 2006; Gómez Expósito et al. 2015; Haas and Défago 2005; Raaijmakers and Mazzola 2012). Only *Burkholderia* spp. and *Pseudomonas* spp. were identified as biomarkers in the present study (Fig. 6C). However, these taxa did not appear as potential key drivers of the bacterial shift between uninoculated and *R. solani* AG 3-PT inoculated treatments. As with the fungal results, several bacterial taxa identified in the present study have been associated with phosphate-solubilizing capabilities. For instance, several *Burkholderia*, *Flavobacterium*, *Enterobacteriaceae*, *Rhizobium*, and *Pseudomonas* spp. are phosphate-solubilizing organisms, providing soluble phosphorus to the plant. These plant growth-promoting rhizobacteria acidify the environment with citrate, succinate, gluconate, or acetate to solubilize phosphorus for plant uptake, which could aid in defense against the pathogen. The metabolism of these taxa could also explain the low pH observed in samples from inoculated treatments (Fig. 5B) (Figueiredo et al. 2011; Spaepen and Vanderleyden 2011; Vessey 2003).

Various nitrogen-fixing bacteria, such as *Pseudomonas*, *Burkholderia*, and *Rhizobium* species, have been found to enhance nitrogen availability in non-leguminous crops (Khaitov 2018; Shata et al. 2007; Shoghi-Kalkhoran et al. 2018). In a study conducted by Chapelle et al. (2016), rhizobacteria taxa, including Burkholderiaceae, Oxalobacteraceae, Sphingomonadaceae, and Sphingobacteriaceae, were identified as differentially more abundant in *R. solani* inoculated suppressive soils and exerted oxidative stress in the rhizobacterial community in sugar beet. Of these rhizobacteria, *Burkholderia-Caballeronia-Paraburkholderia* (family Burkholderiaceae), Oxalobacteraceae, *Sphingopyxis* (family Sphingomonadaceae), and *Chitinophaga* and *Pedobacter* (family Sphingobacteriaceae) were differentially abundant in the *R. solani* AG 3-PT inoculated treatments in the present study (Fig. 6C), which is consistent with the findings of Chapelle et al. (2016). Taxa including *Sphingopyxis*, *Chitinophaga*, and *Pedobacter* were identified as key drivers of the community shift between uninoculated and *R. solani* AG 3-PT inoculated treatments, suggesting that these taxa may play a role in *Rhizoctonia* disease suppression, as suggested by Chapelle et al. (2016) (Fig. 8B). Members of the family Oxalobacteraceae are known for their ability to protect plant roots against fungal infection by inhibiting fungal growth (Johnsen et al. 2010;

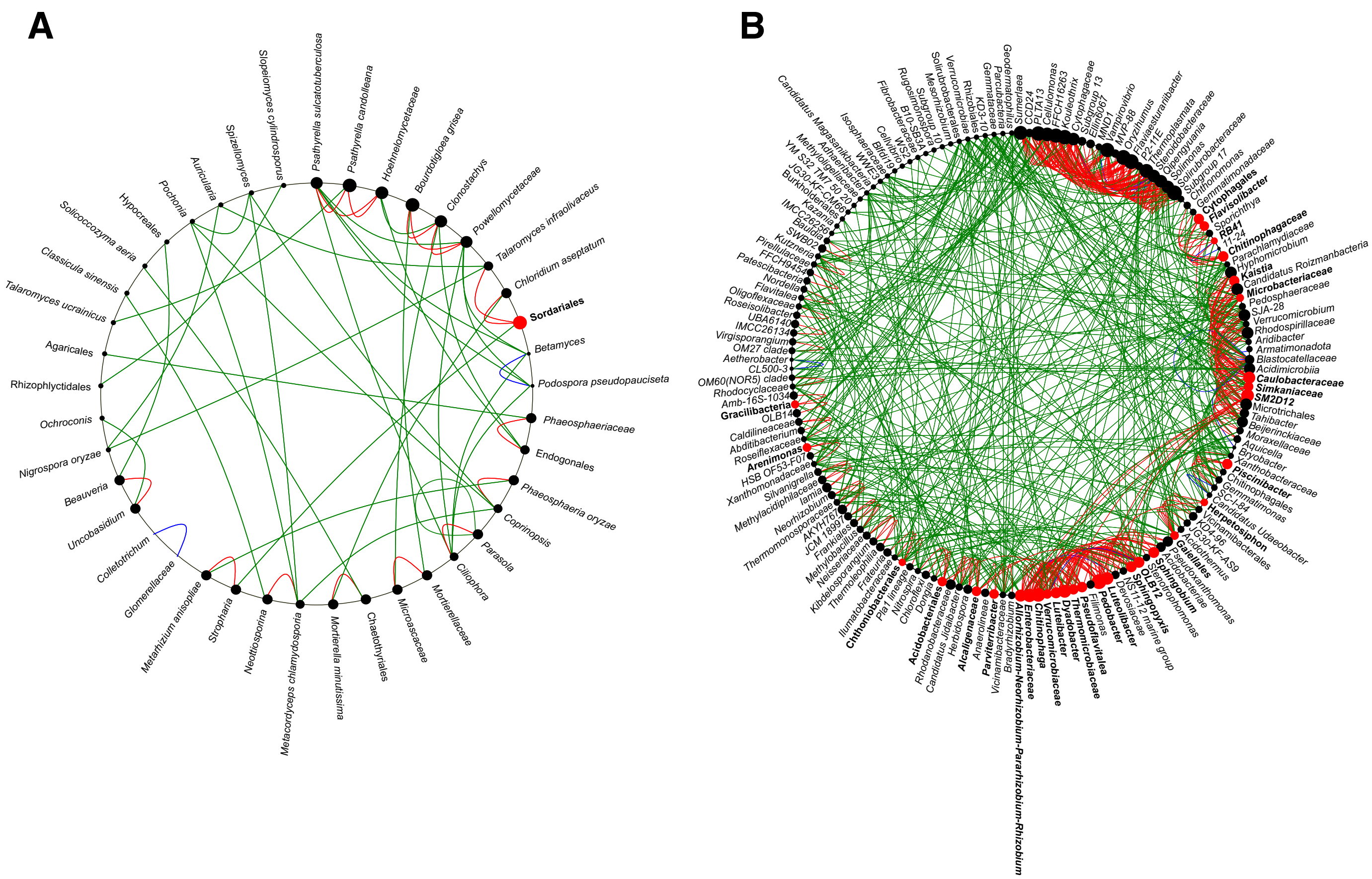

Fig. 8. Potential "driver" taxa based on **A,** fungal and **B,** bacterial NetShift analysis of uninoculated and *Rhizoctonia solani* anastomosis group (AG) 3-PT inoculated treatments ($P < 0.01$) in potato soil microbiomes (rhizosphere and bulk). Green lines (edges) show connections present only in uninoculated treatments, red lines show connections present only in *R. solani* AG 3-PT inoculated treatments, and blue lines show connections present in both uninoculated and inoculated treatments. Node sizes are proportional to their scaled neighbor shift (NESH) score, which indicates important microbial taxa in microbial association networks. Red nodes indicate taxa with an increased betweenness centrality score from an uninoculated treatment to an inoculated treatment. Thus, large red nodes indicate taxa that are potential important "drivers" behind *R. solani* AG 3-PT suppression in the soil microbiome of potatoes.

Leveau et al. 2010). Although *Massilia* (family Oxalobacteraceae) was not identified as a driver taxon in this study, it was found to be differentially abundant in both *R. solani* AG 3-PT inoculated and uninoculated treatments and has also been associated with protective properties against *R. solani* infestation (Fig. 6C) (Yin et al. 2013). Furthermore, *Pseudomonas*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, and Rhizobiaceae were differentially abundant in the *R. solani* AG 3-PT inoculated treatments, whereas *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* was identified as a key driver in the community shift upon *R. solani* AG 3-PT infection (Fig. 8B). We suggest that the potato plant may have selected these various taxa for increased plant growth promotion and, indirectly, pathogen defense by metabolic competition for nitrogen and magnesium (Figs. 4 and 5B) (Chapelle et al. 2016; Moromizato et al. 1991; Pascale et al. 2020).

Conclusions. This study reveals insights into soil microbial community diversity and compositional shifts that occur upon *R. solani* AG 3-PT infection of potatoes. Our results suggest that the introduction of *R. solani* AG 3-PT leads to microbial compositional and functional changes in the rhizosphere soil. We identified various fungal and bacterial taxa that could be putative key players in community changes and interactions upon pathogen infection and could therefore be potential *Rhizoctonia* disease-suppressive agents. Although this study does not necessarily provide the precise identification of specific BCAs, we provide a framework that can facilitate future studies investigating potential biological control agents. Further testing of the identified microbial indicators in vitro, in vivo, and in situ as single inoculants and in different combinations should be done to confirm their disease-suppressive ability against *R. solani* AG 3-PT. Compared with single inoculations, the identified microbial taxa and/or groups will not only benefit the crop as an efficient disease control barrier against *R. solani* AG 3-PT infection but will also enhance the synergistic plant–microbe relationship for a potentially greater physiological effect in potatoes (Li et al. 2019). The results are essential to ensure resilience of the potato industry for agricultural sustainability, long-term productivity, and economic viability.

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