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CROP PROTECTION

Agrosilvopastoral system enhances suppressiveness to soybean damping-off caused by *Rhizoctonia solani* and alters *Fusarium* and *Trichoderma* population density

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ABSTRACT. Integrated crop-livestock systems (ICLS) are sustainable options for agricultural diversification, but there have been few studies on the influence of ICLS on soil microorganisms. This work investigated the influence of agropastoral (AP) and agrosilvopastoral (ASP) systems, compared with a non-integrated crop (CO) on the suppression of *Rhizoctonia solani* and on the density of *Fusarium* and *Trichoderma* propagules. In the first assay, soil samples were inoculated with *R. solani* and evaluated for soybean damping-off. After this, the soil was sterilized and re-inoculated with the pathogen for a new evaluation. Finally, 10% of the soil in the pots were substituted by newly soil samples collected from the same plots in the field to evaluate de suppressiveness transference by soil samples. In the second assay, native *Fusarium* and *Trichoderma* propagules were quantified in semi-selective media. Damping-off incidence in ASP was 70% compared to AP and CO. Evaluations with sterilized and transferred soil samples attributed this suppression to biotic factors. *Fusarium* propagules were retrieved in the ASP soil at 70% and 60% the amounts in AP and CO soils, respectively. *Trichoderma* propagules were retrieved in the ASP soil at 150% compared to AP, but similar to CO. The agrosilvopastoral system in the Brazilian subtropics has the potential to reduce pathogens and enhance beneficial microorganisms in the soil.

Keywords: agricultural diversification, integrated crop-livestock system, soil health.

Sistema agrosilvipastoril aumenta supressividade ao tombamento da soja causado por *Rhizoctonia solani* e altera a densidade de populações de *Fusarium* e *Trichoderma*

RESUMO. Sistemas integrados de produção agropecuária (SIPA) são opções sustentáveis para diversificação agrícola, mas existem poucos estudos sobre a influência de SIPA nos microrganismos do solo. Este trabalho investigou a influência de sistemas agropastoril (AP) e agrosilvipastoril (ASP), comparados com lavoura não integrada (CO) na supressão de *Rhizoctonia solani* e na densidade de propágulos de *Fusarium* e *Trichoderma*. No primeiro ensaio, amostras de solo foram inoculadas com *R. solani* e avaliadas para tombamento da soja. Em seguida, o solo foi esterilizado e reinoculado com o patógeno para uma nova avaliação. Finalmente, 10% do solo nos vasos foi substituído por novas amostras de solo coletadas das mesmas parcelas no campo, para avaliar a transferência da supressividade por amostras de solo. No segundo ensaio, propágulos nativos de *Fusarium* e *Trichoderma* foram quantificados em meios semi seletivos. A incidência de tombamento no ASP foi 70% comparada com AP e CO. Avaliações com solo esterilizado e transferido atribuem esta supressão a fatores bióticos. Propágulos de *Fusarium* foram recuperados no solo do ASP a 70% e 60% das quantidades nos solos do AP e CO, respectivamente. Propágulos de *Trichoderma* foram recuperados no solo do ASP 150% comparado com AP, mas semelhante ao CO. O sistema agrosilvipastoril no subtrópico brasileiro tem potencial para reduzir patógenos e aumentar microrganismos benéficos no solo.

Palavras-chave: diversificação agrícola, sistemas integrados de produção agropecuária, saúde do solo.

Introduction

Agricultural diversification including crops and animals grazing in the same area are longstanding production systems and considered the main form of agricultural land use in the world (Bell & Moore 2012). These production systems are included in the definition of Integrated Crop-Livestock System (ICLS) (FAO, 2010). ICLS are planned systems involving temporal and spatial interactions on different scales with animal and crop exploitation within the

same area, simultaneously or disjointedly and in rotation or succession (Moraes et al., 2014). Studies in this area have intensified in recent years due to the benefits these systems bring to the farmer and to the environment when compared to the monoculture. Examples of the benefits of ICLS include reductions in costs and risks, increase in the efficiency of land and machinery use, increased biological diversity, greenhouse gas mitigation, reductions in plant diseases and weed incidence, and increased profitability and incomes (Altieri, 1999; Bell & Moore, 2012).

In the subtropical region of Brazil, ICLSs are characterized by annual rotation of pastures such as black oat (*Avena strigosa*) and annual ryegrass (*Lolium multiflorum*) and crops such as soybean (*Glycine max*), corn (*Zea mays*), bean (*Phaseolus vulgaris*), or rice (*Oryza sativa*) in the same area, under a direct seeding system (Moraes et al., 2014). Planned inclusion of tree species in such production systems is still not common, even though this component is clearly encompassed in the definition of ICLS (Carvalho et al., 2014).

Damping-off are important and complex diseases worldwide, affecting agricultural and forestry crops (Lamichhane et al., 2017). Soybean is a major crop in tropical and subtropical regions, and the four most frequently associated pathogens (*Fusarium*, spp., *Rhizoctonia* spp., *Pythium* spp. and *Phytophthora* spp.) with damping-off in the literature surveyed by the above-mentioned authors affects soybean yield in the main producing countries (Wrather et al., 2010).

Rhizoctonia solani Kühn [teleomorph: *Thanatephorus cucumeris* (A. B. Frank) Donk] is found in soils worldwide and is considered an important pathogen with a broad host range. It causes diseases on several agronomic crops, ornamentals, and forestry species (González García, Portal Onco, & Rubio Susan, 2006). This pathogen causes reduction in soybean yields worldwide, at quantities up to 11.5 and 26.1 thousand metric tonnes in northern of United States and in Canada, respectively (Wrather et al., 2010; Wrather & Koenning, 2006). In Brazil, this pathogen is responsible for the pre and post-emergence damping-off and also for aerial blight of soybean (Fenille, Souza, & Kuramae, 2002). The availability of resistant cultivars is limited.

Alternative methods to chemical means of disease control can decrease dependence on fungicides, reduce costs, and mitigate damage to the environment, especially in the absence of resistant cultivars (Godfray et al., 2010; Lamichhane, Dachbrodt-Saaydeh, Kudsk, & Messéan, 2016; Tripathi & Dubey, 2004). And damping-off control requires the implementation of an integrated disease management approach combining both preventive and curative tactics and strategies (Lamichhane et al., 2017; Tsror, 2010). Although ICLS promote changes in the environment, little is known about the influence of different arrangements of these production systems in the occurrence of plant disease, particularly when the forestry component is added. The system can lead to environmental modifications, which can increase or decrease plant diseases, depending on the particular requirement of the pathogens (Matson, 1997). Beside this, nitrogen (N) input in the soil can influence microorganisms since they play critical roles regard to N fixation and transformation (Stockdale & Brookes, 2006).

N supplementation has been found to affect also the pathogens and antagonists. For example, high doses of N supplied as urea stimulated *Sclerotinia sclerotiorum* attacking mustard, and this result may be related to the prolonged vegetative period of the plants (Gupta, Awasthi, & Kolte, 2004). Crescent doses of N increased in length and quantity the injuries caused by *R. solani* in rice (Lenz et al., 2009), but reduced disease caused by this pathogen in beans (Rodrigues, Carvalho, & Vale, 2002). Damping-off incidence caused by *R. solani* in spring wheat increased as N stress was progressively reduced (Wall, Neate, Graham, Reuter, & Rovira, 1994); and amendment of sources of N to organic substrates resulted in greater growth and conidia production by *Trichoderma pseudokoningii* on selected substrates (Rajput, Khanzada, & Shahzad, 2014).

Most microorganisms present in a particular soil are not well known and have been underexplored in agriculture, even though they are responsible for the function of agricultural and natural ecosystems (Van der Heijden & Wagg, 2013). An exception is the genus *Trichoderma*, reported as an antagonist of several pathogens by different mechanisms of action, such as volatile and non-volatile compounds and direct hyphae interaction, and also involved in promoting plant growth and stress resistance (Lorito, Woo, Harman, & Monte, 2010).

Saprophytic and pathogenic populations of the genus *Fusarium* are widespread in the world. Even containing many non-pathogenic species, pathogens in this genus are responsible for several plant diseases, as vascular wilt diseases either in temperate and tropical regions (Finlay, 2007). *Fusarium* is frequently reported causing soybean diseases and yield reduction in the major soybean producers countries (Wrather et al., 2010), with emphasis on sudden death (Aoki, O'Donnell, Homma, & Lattanzi, 2003; Farias Neto, Dianese, Santin, & do Couto, 2013; Westphal, Li, Xing, McKay, & Malvick, 2014). The genus *Fusarium* is also one of the most important pathogens associated with wheat (Zhang et al., 2012), corn and rice (Kim et al., 2012).

Current knowledge on soil suppressiveness to plant pathogens and its origin (biotic or abiotic), and

microbial density in the soil do not include systems that integrate grain production, livestock, and forestry. This indicates a lack of knowledge on how different arrangements of ICLSs influence the soil microbiota, as well as the occurrence of major diseases in the cultivated plants. Studies in this regard can provide insights into better management practices in diversified agricultural systems.

The objectives of this work were to assess the influence of two ICLS arrangements and two N doses on a) the soil suppressiveness to soybean damping-off caused by *R. solani*, b) the biotic influence on this suppressiveness, and c) *Fusarium* and *Trichoderma* propagule density, as biological indicators, in the ICLS soil. The integration between crop and livestock (agropastoral system - AP) and integration of crop, livestock, and forestry (agrosilvopastoral system - ASP), with a control system (non-integrated crop - CO) were compared in a long-term field experiment installed in Ponta Grossa, Paraná State, Brazil.

Material and methods

Rhizoctonia solani inoculum

An isolate of *R. solani* anastomosis group 4 was obtained from the plant pathology laboratory of Embrapa Agropecuária Oeste. The inoculum was produced in black oat grains as described in Kinsbursky and Weinhold (1988) and ground to 1 mm in a Wiley-type knife mill. The inoculum was placed in plastic bags and kept at 4ºC until use. Based on a preliminary test (unpublished data), a dose of 0.6 g inoculum per pot containing 1.5 liters of soil was chosen, as at this dose approximately 80% of the plants showed symptoms 35 days after sowing.

Description of the evaluated agricultural systems and soil sampling

The field experiment evaluated consisted of an ICLS long-term experiment put in place in 2006 in Ponta Grossa county, Paraná State, Brazil. The climate of the study site is mesothermal humid subtropical. The experiment had treatments arranged in an incomplete factorial in randomized blocks with 3 replications. Experimental factor one was production system with three levels: agropastoral (AP) system, agrosilvopastoral (ASP) system, and a control (CO) with a non-integrated crop. Experimental factor two was the amount of N applied as urea in the winter pasture, with two levels: 90 kg ha^{-1} (in all production systems) and 180 kg ha^{-1} (in AP and ASP systems).

All production systems had summer crops of soybean and corn on alternated crop seasons. The winter grazing in all production systems consists of black oat and annual ryegrass intercropped. Cattle grazing occurred for an uninterrupted period of 90 to 120 days every winter in AP and ASP systems, and stocking rate was managed to maintain the pasture with 20 cm height. Summer crops and winter pastures were established by direct seeding (no-tillage). The tree component of the ASP system was composed of eucalyptus (*Eucalyptus dunnii*) and silver oak (*Grevillea robusta*) alternated in single rows, with 4.5 m between trees and 14 m between rows. Each plot had an area of about one hectare, except CO plots, which were established within the AP plots in 2010 and sized at 100 m^2.

Four representative 1.5 L soil samples from zero to 15 cm depth were collected randomly from each plot. In total, 60 soil samples were collected. This corresponds to the five combinations of production system and amount of N, distributed in 3 blocks, where 4 samples per plot were collected. Samples were taken with the aid of a shovel, taking care to collect a uniform width block of soil from the surface to 15 cm depth. The soil was packed in plastic bags to retain moisture and transferred to aluminum pots of 1.5 L capacity on the following day and analyzed separately. For further evaluation of *Fusarium* and *Trichoderma* population density, an aliquot of approximately 100 g was taken from each soil sample, sieved through an 1 mm opening sieve, and stored at 4ºC in capped acrylic pots.

Soil samples were collected twice, on August (winter) and November (spring) 2014. To evaluate the capacity of suppressiveness transference, soil samples were collected again on February 2015 (summer) and used to replace 10% of the volume of the soil in each pot. The experiments for evaluating the suppression to *R. solani* started one day after collecting soil samples. The measurements of the density of *Fusarium* and *Trichoderma* were carried out only with the soil samples collected in August and November and started three weeks after each sampling.

The production systems were characterized for soil temperature at layer 0-13 cm from July 2014 to June 2015. Temperature measurements were taken once a month in the morning (8:00 - 9:00 a.m.) and afternoon (2:00 - 3:00 p.m.), with 4 portable skewer type thermometers. The average temperature of the soil under ASP was 19.2 ± 0.07°C, similar to the CO treatment (19.3 ± 0.11°C). Both temperatures were lower than that observed for AP (19.9 ± 0.07°C), although differences were not observed along all the year (Figure 1).

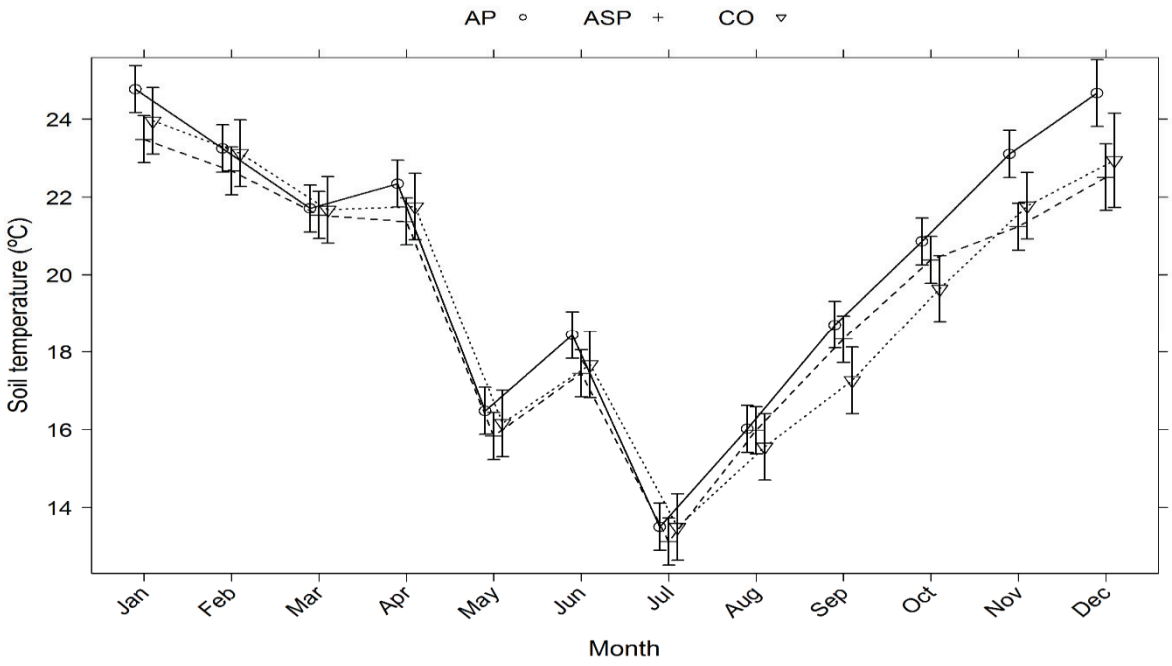

Figure 1. Soil temperature in the 0-13 cm layer, in three production systems along a year, from July 2014 to June 2015. AP: agropastoral system; ASP: agrosilvopastoral system; CO: control with non-integrated crop. Bars show the 95% confidence interval. Symbols in the bar indicate estimated average. Measured with four portable skewer type thermometers in the morning and afternoon, once a month.

Soil suppressiveness to *Rhizoctonia solani*

A pot culture study was carried out with similar procedures as described in Samavat, Heydari, Zamanizadeh, Rezaee, and Aliabadi (2014). Ten seeds of soybean cultivar NS 5909 RR were sown in orifices 1 cm in diameter and 2 cm depth in each pot containing 1.5 liters of soil. Before closing the orifices, 0.6 g of *R. solani* inoculum was distributed on the soil surface of each pot. The pots were arranged randomly in a glasshouse of the Universidade Federal do Paraná, in Curitiba, Paraná State, Brazil.

A sample of each combination of production system and amount of N was used as a control without infestation to check for possible natural occurrence *R. solani* in the soil. There was no incidence of symptoms in these pots in any evaluation, so they were not included in statistical analysis. The lot of seeds used in the experiments was collected in March 2014 and tested negative for the presence of *R. solani* using a blotter test without disinfecting the seeds' surface, indicating there was no influence from uncontrolled occurrences of the pathogen in the soil or seeds.

Seeds were sown for three consecutive times every 35 days without new infestation of the soil. The number of germinated plants and plants showing symptoms caused by *R. solani* on the hypocotyl or main root was counted. Disease incidence per pot was calculated by the sum of the number of plants with symptoms divided by the number of germinated plants.

The effect of the pre-incubation of *R. solani* inoculum in the soil on the incidence of damping-off may differ between the three evaluations resulting from the three consecutive sowings. In particular, different results may be found for the first evaluation (T1) because no re-infestation was done before the second (T2) and third (T3) evaluation times. For this reason, fitted models were used: Ma) without time effects, Mb) individual effects for T1, T2, and T3, and Mc) one effect for T1 and a common effect for T2 and T3. Maximized log-likelihood values, number of parameters, and the Akaike Information Criteria guided the decision on whether and how the analysis of the three consecutive evaluations should be considered. The models that consider the effect of evaluation times (models Mb and Mc) had similar fitting averages and were clearly better than model Ma, which does not consider evaluation times. These results confirm the stable conditions for the second and third evaluation. Therefore, analysis was concentrated on the data from the T2 and T3, which were considered replications.

After the third evaluation, the pots with soil were autoclaved for one hour at 120°C in two consecutive days and re-infested with the pathogen seven days

later. Three consecutive soybeans sowing were then carried out with the same protocol described before. The experiments were performed twice, one with the soil samples from winter (first experiment) and other with soil samples from spring (second experiment), as described above.

After these evaluations, 10% of the volume of the soil in each pot of the first experiment was replaced by newly soil samples collected from the same plots in the field. The protocol was repeated in order to assess the capacity of damping-off suppressiveness transference.

Fusarium and *Trichoderma* density

Fusarium and *Trichoderma* were used in this work as biological indicators. *Fusarium* as a genus of several pathogens and *Trichoderma* representing biological control agents (BCA). Population levels of *Fusarium* spp. and *Trichoderma* spp. in each soil sample were estimated by dilution plating on agar (Pérez-Brandán et al., 2014). An aliquot of 10 g of soil was diluted in 90 mL of sterile distilled water and stirred for 90 minutes at 170 rpm on an orbital flask shaker. Suspensions were diluted to a concentration of 10^{-3}, and 0.5 mL was then spread on Petri dishes containing two different types of culture media with four plates per sample.

The Nash and Snyder (1962) medium was used to quantify *Fusarium* spp., while weak potato dextrose agar (PDA) (7,8 g PDA Himedia® + 16 g agar-agar + 0.8 mL L^{-1} lactic acid) was used for *Trichoderma* spp. quantification. The plates were incubated in the dark at 22 to 26°C and evaluated after 5 days by visual quantification of colony forming units per plate. Light microscope was used when necessary to identify *Fusarium* and *Trichoderma* colony forming units per plate by morphological characteristics. The experiment was performed twice, with two different soil samples of experimental field (winter and spring times), as described above. As the results were similar between samples time, fitted models were used to compare the time effect. Maximized log-likelihood values, number of parameters, and the Akaike Information Criteria indicated the two experiments with soil samples collected in the winter and spring could be analyzed together.

Data analysis

Statistical analysis of soil suppressiveness to *R. solani* was performed by fitting a generalized linear mixed model with binomial sampling distribution, logistic link, and a random effect assigned to each of the experimental units. The response variable was the number of symptomatic plants among the germinated ones, which numbers were also informed in the binomial model. The random effect accounts for possible dependence between plants within pots, leading to binomial over dispersion. Factors were the production system (with tree levels - AP, ASP, and CO) and N (two levels, 90 and 180 kg ha^{-1}) arranged in an incomplete factorial design since the combination CO*180 was not available. The full model considered all factors and the random effect.

The statistical significance of experimental factors was assessed by likelihood ratio tests on a sequence of nested models defined by sequentially adding terms for (I) single intercept, (II) N rates, (III) production system, and (IV) interaction between N rates and production system. Treatment comparisons were performed by contrasting the effects of production system for each N level, with family-wise error rates of 5%. A correlation between seed germination and proportion of plants with symptoms was computed to assess the influence of the *R. solani* inoculum.

A similar generalized linear mixed model structure was used for *Fusarium* and *Trichoderma* density analysis, with a Poisson distribution for the response variable and adding a random effect to account for measurements (Petri dishes) taken at the same experimental unit (soil sample). Statistical analyses were performed using *R* software (R Core Team, 2015) with models fitted by the function *glmer* from the add-on packages *lme4* (Bates, Maechler, Bolker, & Walker, 2014), and *glht* from the package *multcomp* (Hothorn, Bretz, & Westfall, 2008). Functions of the package *do By* (Hojsgaard & Halekoh, 2014) were used for organizing data for the analysis and plots.

Results and discussion

The average proportion of germinated plants was 0.79 in the first experiment and 0.76 in the second experiment. Moreover, the correlation coefficients between germination and proportion of plants with symptoms for the first and second experiment were -0.17 and -0.18, respectively. These values indicate negligible effects of the inoculum on the germination of the soybean seeds.

Likelihood ratio tests indicated the model (IV) with the interaction between N and production system effects, as the model of choice for the first (winter) experiment (Figure 2A). The ASP treatment with 90 kg ha^{-1} of N resulted in at least one-half lower incidence of symptoms caused by *R. solani* than the other interactions between treatments.

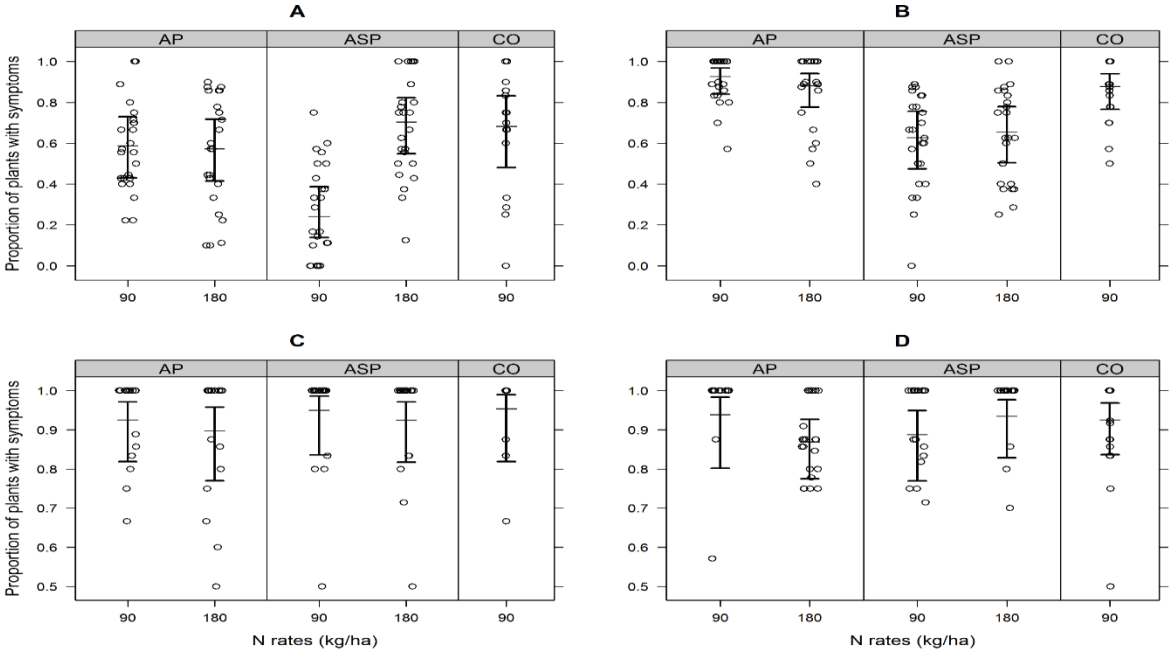

Figure 2. Proportion of soybean plants with symptoms caused by *Rhizoctonia solani* 35 days after sowing, depending on the production system and amount of nitrogen, in artificially infested soil collected in August (A) and November (B) 2014; and in sterilized and artificially infested soil collected in August (C) and November (D) 2014. AP: agropastoral system; ASP: agrosilvopastoral system; CO: control with non-integrated crop. Bars show the 95% confidence interval. Horizontal line across the bar indicates estimated average. Mean values of two consecutive assessments (sowings) in four samples per plot for each combination of production system and N rate, in three blocks. Points are the observed proportions in the experimental units.

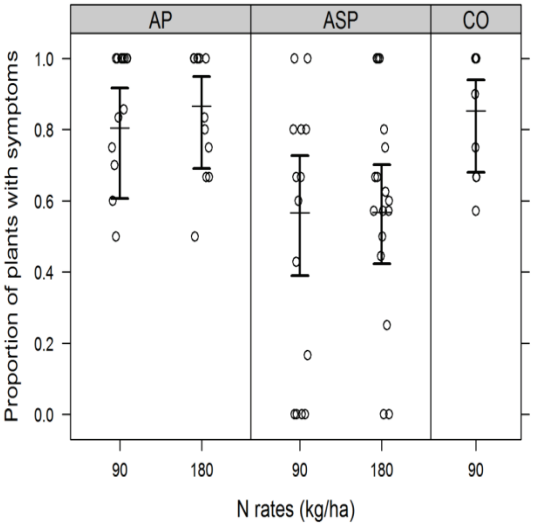

Figure 3. Proportion of soybean plants with symptoms caused by *Rhizoctonia solani* 35 days after sowing, depending on the production system and amount of nitrogen, in sterilized and artificially infested soil collected in August 2014 after substituting 10% volume of soil in each pot with newly soil samples collected on the same field plots in February 2015. AP: agropastoral system; ASP: agrosilvopastoral system; CO: control with non-integrated crop. Bars show the 95% confidence interval. Horizontal line across the bar indicates estimated average. Mean value of two consecutive assessments (sowings) in four samples per plot for each combination of production system and N rate, in three blocks. Points are the observed proportions in the experimental units.

In the second (spring) experiment disease incidence in ASP was approximately 70% of the other systems, independent of N rate (Figure 2B). There was no difference in the incidence of symptoms caused by *R. solani* among treatments in both experiments when the sterilized soil was evaluated (Figure 2C and D). After substituting 10% of the volume of the infested soil with new soil samples collected in the summer from the same plots in the field, only production system effects were observed, with the lowest proportion of symptomatic plants in the ASP treatment (Figure 3), similar to what was observed for the second experiment with non-sterilized soil (Figure 2B).

As noted by Tsror (2010), effective management of *R. solani* requires the implementation of an integrated disease management approach, and within this approach, cultural practices that decrease inoculum density in the soil are the most important measures. In the first experiment (winter), a lower incidence of symptoms caused by *R. solani* was observed in the lower N rate in ASP system (Figure 2A). N supplementation can affect pathogens and antagonistic to pathogens, resulting in both positive and negative results because of the complex interaction between microorganisms, nutrients, and plants (Lenz et al., 2009; Rodrigues et al., 2002).

As observed by Pontes et al. (2017) in the same field experiment of this work, the 90 kg ha^{-1} N dose showed lower sward height and herbage mass compared to the one with 180 kg ha^{-1} N. The lower disease incidence observed in areas where less herbage mass was produced is in accordance with Kühn et al. (2009), who noticed *R. solani* hyphae growth in fresh organic matter during the saprophytic phase of the pathogen, emphasizing the influence of soil organic matter on the pathogen growth.

Fresh or immature composts not only serve as a food base for biological control agents but can also result in negative effects. Composts support the saprophytic growth of plant pathogens and increase disease risk (Bonanomi, Antignani, Capodilupo, & Scala, 2010). The higher amounts of fresh organic matter produced in the 180 kg ha^{-1} dose of N of ASP may have supported the development of *R. solani* during the saprophytic phase (winter). The difference between disease incidences in the two N doses was only observed in the first experiment with soil samples collected during the pastoral phase in the winter. Interaction with different N rates was not detected in the second experiment with soil collected in the spring (Figure 2B), nor in the experiment with 10% substituted soil collected in the summer (Figure 3), confirming the suppressiveness of the ASP soil to this pathogen.

The absence of differences in disease incidence between treatments after soil sterilization (Figure 2C and D) shows that biotic factors may be involved in suppressiveness to *R. solani*. Biological factors such as microbial biomass and activity, and the abundance of specific microbial groups have been frequently associated with suppressiveness to several pathogens (Bonilla, Gutiérrez-Barranquero, Vicente, & Cazorla, 2012), although the main factor involved in soil suppressiveness may vary according to the pathogen (Bonanomi et al., 2010). It is difficult to distinguish between primary and secondary factors that are responsible for suppressiveness, what makes difficult the extrapolation or generalizations of the results obtained when only one situation is studied (Höper & Alabouvette, 1996). Soil suppressiveness result from biotic and abiotic factors, in a diverse and complex set of mechanisms, and the contributing factors normally work interactively, requiring a holistic approach to studying it (Ghini & Morandi, 2006).

When 10% of the sterilized and re-infested soil in the pots was substituted by newly collected soil samples, differences among treatments were observed again (Figure 3), showing the capacity of suppression transference by soil samples. These results are in accordance with Wiseman, Neate, Keller, and Smith (1996), who succeeded in transferring suppression to *R. solani* from one soil to another. Besides this, as the samples used to substitute in the pots were collected after and independently of the previous samplings, this configures it as a new (third) assessment of the soil suppressiveness to *R. solani.*

The transfer of suppressiveness to *R. solani* by soil samples indicates that the suppressiveness is based on individual or select groups of microorganisms (Weller, Raaijmakers, Gardener, & Thomashow, 2002). *Trichoderma* spp. have been reported as antagonists of different pathogens (Lorito et al., 2010). The highest density of this microorganism observed in the ASP treatment compared to the AP treatment (Figure 4B) contributes to explain the suppressiveness to *R. solani*, in the same way as observed by Liu & Baker (1980). The antagonism of *Trichoderma* spp. to *R. solani* is well documented (Grosch, Scherwinski, Lottmann, & Berg, 2006). However, we cannot attribute this suppressiveness only to *Trichoderma*, since the CO plots had similar levels of this microorganism and did not express suppressiveness.

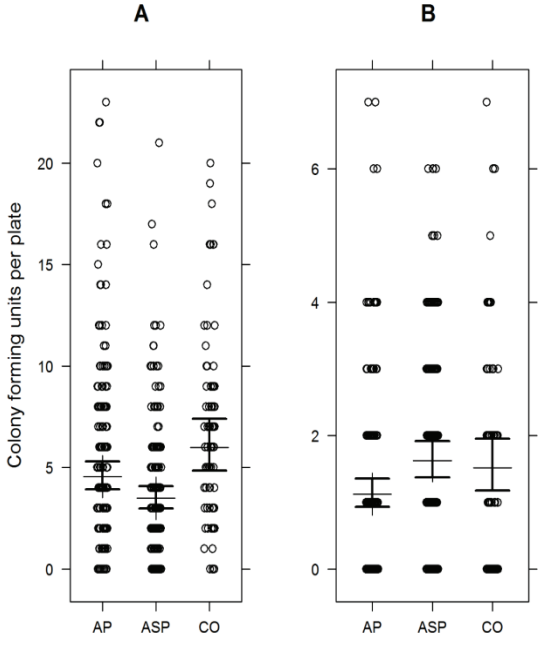

Figure 4. Colony forming units of *Fusarium* spp. (A) and *Trichoderma* spp. (B) in soil samples from different production systems. AP: agropastoral system; ASP: agrosilvopastoral system; CO: control with non-integrated crop. Bars show the 95% confidence interval and the horizontal line across the bar indicate the estimated average. Mean values of two experiments with soil collected in August and November 2014; with three replicates (Petri dishes) in four samples per plot, in three blocks. Points are the observed proportions in the experimental units.

Two genera, one representing pathogens and another representing BCA, were investigated in the soil samples. As there was no incidence of *R. solani* in non-infested pots in any experiment for soil suppressiveness, the genus *Fusarium* was chosen representing pathogens. *Trichoderma* was chosen as a BCA.

The experiments with the two independent soil samplings (first on winter and second on spring), when analyzed separately, showed similar results for the population density of *Fusarium* spp. and *Trichoderma* spp. in the soil, so the two soil samplings were considered as replicates and the results analyzed together (Figure 4). N rates were not significant in any of the experiments for any microorganism. *Fusarium* propagules retrieved in the ASP soil was approximately 70% and 60% of the amounts compared to AP and CO soils, respectively. *Trichoderma* propagules retrieved in the ASP soil was at approximately 150% the amounts compared to AP, but at similar amounts compared to CO. PDA plates were included to evaluate total fungi cultivable in this media. The results on PDA (data not shown) were similar to *Fusarium* spp. Microorganisms in the soil can be in active, potentially active (ready to start utilization of available substrates and grow) and dormant phases. Plate-count techniques are dynamic approaches and efficient to access the active/potentially active phases of cultivable microorganisms (Blagodatskaya & Kuzyakov, 2013).

As previously suggested (Pérez-Brandán et al., 2014), there is a positive effect of *Trichoderma* on the greater diversity of plant species, confirmed in the present work in the ASP treatment. Soil moisture is also known to be an important factor influencing *Trichoderma* spp. (Jin, Harman, & Taylor, 1991). Although soil moisture was not recorded in the present work, the presence of trees in the ASP treatment and the maintenance of a greater quantity of residues on the soil surface of the CO (not grazed) may have contributed to the maintenance of moisture in these systems. Evidence of this moisture maintenance is the previously established relationship between soil temperature and moisture (Al-Kayssi, Al-Karaghouli, Hasson, & Beker, 1990), and the average soil temperatures recorded in the field experiment, that was lower in ASP and CO than in AP. Although, soil respiration is influenced by both temperature and moisture (Akinremi, McGinn, & McLean, 1999; Li, Yan, Yue, & Wang, 2008).

Production systems and the introduction of animals influences the soil microbial community (Silva et al., 2015). In the present study, the density of *Fusarium* spp. propagules was inversely proportional to the density of *Trichoderma* spp. propagules in the ASP treatment. The negative correlation between the population densities of these two genera in the soil has already been reported as an indication of soil health (Innocenti, Roberti, & Piattoni, 2015; Kim & Knudsen, 2013; Sant et al., 2010; Yangui, Rhouma, Triki, Gargouri, & Bouzid, 2008). The lower density of viable *Fusarium* propagules in the AP treatment compared to the CO treatment indicates that manure was more effective than crop residues in reducing *Fusarium* in the soil. The AP system presents lower residues due to grazing, but this activity results in manure distribution over the soil. Manure may have stimulated the microorganisms diversity in the soil, reducing the potentially pathogenic populations, as previously reported (Sun, Zhang, Guo, Wang, & Chu, 2015).

ICLS aim to achieve synergism and emergent properties as a result of interactions among the soil, plants, animals, and the atmosphere (Moraes et al., 2014). Some of these attributes were confirmed in the case study examined in this work. A lower incidence of soybean damping-off caused by *R. solani* (Figures 2A-B and 3), a lower density of *Fusarium* spp. (Figure 4A), and a higher density of *Trichoderma* spp. (Figure 4B) in the soil of the ASP system were detected. These results suggest that the agricultural diversification promoted by the ASP system results in healthy soil, with higher resiliency to disturbances or stresses, in line with discussions in Van Bruggen and Semenov (1999). The biological components of soil are critical to soil health, and suppressiveness is a relevant attribute of this characteristic (Van Bruggen et al., 2015).

This is probably the first investigation addressing crop disease in an ASP system, and future research should investigate the influence of such a complex production system in relation to other diseases.

Conclusion

The diversification promoted by the ASP system in the Brazilian subtropics has the potential to reduce *Rhizoctonia solani* and *Fusarium* spp. and enhance beneficial microorganisms like *Trichoderma* spp. in the soil.

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References

Akinremi, O. O., McGinn, S. M., & McLean, H. D. J. (1999). Effects of soil temperature and moisture on soil respiration in barley and fallow plots. *Canadian Journal of Soil Science*, *79*(1), 5-13.

Al-Kayssi, A. W., Al-Karaghouli, A. A., Hasson, A. M., & Beker, S. A. (1990). Influence of soil moisture content on soil temperature and heat storage under greenhouse conditions. *Journal of Agricultural Engenering Research*, *45*(1), 241-252.

Altieri, M. A. (1999). The ecological role of biodiversity in agroecosystem. *Agriculture, Ecosystems and Environment*, *74*(1), 19-31.

Aoki, T., O'Donnell, K., Homma, Y., & Lattanzi, A. R. (2003). Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex-*F. virguliforme* in North America and *F. tucumaniae* in South America. *Mycologia*, *95*(4), 660-684. https://doi.org/10.2307/3761942

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). *lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-5*. Retrieved on August 25, 2015. from http://cran.r-project.org/package=lme4

Bell, L. W., & Moore, A. D. (2012). Integrated crop-livestock systems in Australian agriculture: Trends, drivers and implications. *Agricultural Systems*, *111*(1), 1-12. https://doi.org/10.1016/j.agsy.2012.04.003

Blagodatskaya, E., & Kuzyakov, Y. (2013). Active microorganisms in soil: Critical review of estimation criteria and approaches. *Soil Biology and Biochemistry*, *67*(1), 192-211. https://doi.org/10.1016/j.soilbio.2013.08.024

Bonanomi, G., Antignani, V., Capodilupo, M., & Scala, F. (2010). Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology and Biochemistry*, *42*(2), 136-144. https://doi.org/ 10.1016/j.soilbio.2009.10.012

Bonilla, N., Gutiérrez-Barranquero, J. A., Vicente, A., & Cazorla, F. M. (2012). Enhancing soil quality and plant health through suppressive organic amendments. *Diversity*, *4*(4), 475-491. https://doi.org/ 10.3390/d4040475

Carvalho, P. C. F., Moraes, A., Pontes, L. S., Anghinoni, I., Sulk, R. M., & Batello, C. (2014). Definições e terminologias para Sistema Integrado de Produção Agropecuária. *Revista Ciencia Agronomica*, *45*(5), 1040-1046.

Farias Neto, A. L., Dianese, A. C., Santin, M. R., & Couto, B. C. (2013). Caracterização de genótipos de soja na região dos Cerrados quanto à reação à podridão vermelha da raiz, causada pelos fungos *Fusarium tucumaniae* e *Fusarium brasiliense*. *Revista Ceres*, *60*(2), 215-220. https://doi.org/10.1590/S0034-737X2013000200009

Fenille, R. C., Souza, N. L., & Kuramae, E. E. (2002). Characterization of *Rhizoctonia solani* associated with soybean in Brazil. *European Journal of Plant Pathology*, *108*(8), 783-792.

Finlay, R. (2007). The fungi in soil. In J. van Elsas, J. Janson, & J. Trevors (Eds.), *Modern Soil Microbiology* (p. 107-146). Boca Raton, FL: CRC Press.

Food and Agriculture Organization of the United Nations [FAO]. (2010). An international consultation on integrated crop-livestock systems for development: The way forward for sustainable production intensification. *Integrated Crop Management*, *13*(1), 1-79.

Ghini, R., & Morandi, M. A. B. (2006). Biotic and abiotic factors associated with soil suppressiveness to *Rhizoctonia solani*. *Scientia Agricola*, *63*(2), 153-160.

Godfray, H. C. J., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Nisbett, N., … Whiteley, R. (2010). The future of the global food system. *Philosophical Transactions: Biological Sciences*, *365*(1554), 2769-2777. https://doi.org/ 10.2307/20752977

González García, V., Portal Onco, M. A., & Rubio Susan, V. (2006). Review. Biology and systematics of the form genus *Rhizoctonia*. *Spanish Journal of Agricultural Research*, *4*(1), 55-79. https://doi.org/10.5424/sjar/2006041-178

Grosch, R., Scherwinski, K., Lottmann, J., & Berg, G. (2006). Fungal antagonists of the plant pathogen *Rhizoctonia solani*: selection, control efficacy and influence on the indigenous microbial community. *Mycological Research*, *110*(12), 1464-1474. https://doi.org/10.1016/j.mycres.2006.09.014

Gupta, R., Awasthi, R. P., & Kolte, S. J. (2004). Effect of nitrogen and sulphur on incidence of *Sclerotinia* rot of mustard. *Indian Phytopathology*, *57*(2), 193-194.

Hojsgaard, S., & Halekoh, U. (2014). d*oBy: Groupwise statisticas, LSmeans, linear contrasts, utilities. R package version 4.5-13*. Retrieved on August 28, 2015. from http://cran.r-project.org/package=doBy

Höper, H., & Alabouvette, C. (1996). Importance of physical and chemical soil properties in the suppressiveness of soils to plant diseases. *European Journal of Soil Biology*, *32*(1), 41-58.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50(3)*, 346-363. https://doi.org/10.1002/bimj.200810425

Innocenti, G., Roberti, R., & Piattoni, F. (2015). Biocontrol ability of *Trichoderma harzianum* strain T22 against *Fusarium* wilt disease on water-stressed lettuce plants. *BioControl*, *60*(4), 573-581. https://doi.org/10.1007/s10526-015-9662-7

Jin, X., Harman, G. E., & Taylor, A. G. (1991). Conidial biomass and desiccation tolerance of *Trichoderma harzianum* produced at different medium water potentials. *Biological Control*, *1*(3), 237-243. https://doi.org/10.1016/1049-9644(91)90072-8

Kim, J. H., Kang, M. R., Kim, H. K., Lee, S. H., Lee, T., & Yun, S. H. (2012). Population structure of the *Gibberella fujikuroi* species complex associated with rice and corn in Korea. *Plant Pathology Journal*, *28*(4), 357-363. https://doi.org/10.5423/PPJ.OA.09.2012.0134

Kim, T. G., & Knudsen, G. R. (2013). Relationship between the biocontrol fungus *Trichoderma harzianum* and the phytopathogenic fungus *Fusarium solani* f.sp. *pisi*. *Applied Soil Ecology*, *68*(1), 57-60. https://doi.org/10.1016/j.apsoil.2013.03.009

Kinsbursky, R. S., & Weinhold, A. R. (1988). Influence of soil on inoculum density-disease incidence relationships of *Rhizoctonia solani*. *Phytopathology*, *78*(2), 127-130.

Kühn, J., Rippel, R., & Schmidhalter, U. (2009). Abiotic soil properties and the occurrence of *Rhizoctonia* crown and root rot in sugar beet. *Journal of Plant Nutrition and Soil Science*, *172*(5), 661-668. https://doi.org/10.1002/jpln.200700186

Lamichhane, J. R., Dachbrodt-Saaydeh, S., Kudsk, P., & Messéan, A. (2016). Toward a reduced reliance on conventional pesticides in European agriculture. *Plant Disease*, *100*(1), 10-24. https://doi.org/0.1094/PDIS-05-15-0574-FE

Lamichhane, J. R., Dürr, C., Schwanck, A. A., Robin, M.-H., Sarthou, J.-P., Cellier, V., … Aubertot, J.-N. (2017). Integrated management of damping-off diseases. A review. *Agronomy for Sustainable Development*, *37*(2), 1-25. https://doi.org/10.1007/s13593-017-0417-y

Lenz, G., Costa, I. F. D., Arrué, A., Karkow, D., Zemolin, C. R., Silva, T. M. B., … Marques, L. N. (2009). Suscetibilidade de cultivares de arroz, sob diferentes doses de nitrogênio, à *Rhizoctonia solani*. *Arquivos do Instituto Biológico*, *76*(4), 707-710.

Li, H., Yan, J., Yue, X., & Wang, M. (2008). Significance of soil temperature and moisture for soil respiration in a Chinese mountain area. *Agricultural and Forest Meteorology*, *148*(3), 490-503. https://doi.org/10.1016/j.agrformet.2007.10.009

Liu, S., & Baker, R. (1980). Mechanism of biological control in soil surressive to *Rhizoctonia solani*. *Phytopathology*, *70*(5), 404-412.

Lorito, M., Woo, S. L., Harman, G. E., & Monte, E. (2010). Translational Research on Trichoderma : From 'Omics to the Field. *Annual Review of Phytopathology*, *48*(1), 395-417. https://doi.org/10.1146/annurev-phyto-073009-114314

Matson, P. A. (1997). Agricultural intensification and ecosystem properties. *Science*, *277*(5325), 504-509. https://doi.org/10.1126/science.277. 5325.504

Moraes, A., Carvalho, P. C. F., Anghinoni, I., Lustosa, S. B. C., Costa, S. E. V. G. A., & Kunrath, T. R. (2014). Integrated crop-livestock systems in the Brazilian subtropics. *European Journal of Agronomy*, *57*(1), 4-9. https://doi.org/10.1016/j.eja.2013.10.004

Nash, S. M., & Snyder, W. C. (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology*, *52*(6), 567-572.

Pérez-Brandán, C., Huidobro, J., Grümberg, B., Scandiani, M. M., Luque, A. G., Meriles, J. M., & Vargas-Gil, S. (2014). Soybean fungal soil-borne diseases: a parameter for measuring the effect of agricultural intensification on soil health. *Canadian Journal of Microbioogy*, *60*(2), 73-84. https://doi.org/10.1139/cjm-2013-0792

Pontes, L. S., Carpinelli, S., Stafin, G., Porfírio-da-Silva, V., & Santos, B. R. C. (2017). Relationship between sward height and herbage mass for integrated crop-livestock systems with trees. *Grassland Science*, *63*(1), 29-35. https://doi.org/10.1111/grs.12147

R Core Team. (2015). *R*: A language and environment for statistical computing. Viena. Retrieved on August 28, 2015. from http://www.r-project.org/

Rajput, A. Q., Khanzada, M. A., & Shahzad, S. (2014). Effect of different substrates and carbon and nitrogen sources on growth and shelf life of *Trichoderma pseudokoningii*. *International Journal of Agriculture & Biology*, *16*(5), 893-898.

Rodrigues, F. A., Carvalho, E. M., & Vale, F. X. R. (2002). Severidade da podridão-radicular de *Rhizoctonia* do feijoeiro influenciada pela calagem, e pelas fontes e doses de nitrogênio. *Pesquisa Agropecuária Brasileira*, *37*(9), 1247-1252.

Samavat, S., Heydari, A., Zamanizadeh, H. R., Rezaee, S., & Aliabadi, A. A. (2014). Application of new bioformulations of *Pseudomonas aureofaciens* for biocontrol of cotton seedling damping-off. *Journal of Plant Protection Research*, *54*(4), 335-339. https://doi.org/10.2478/jppr-2014-0050

Sant, D., Casanova, E., Segarra, G., Avilés, M., Reis, M., & Trillas, M. I. (2010). Effect of *Trichoderma asperellum* strain T34 on *Fusarium* wilt and water usage in carnation grown on compost-based growth medium. *Biological Control*, *53*(3), 291-296. https://doi.org/10.1016/j.biocontrol.2010. 01.012

Silva, A. S., Colozzi Filho, A., Nakatani, A. S., Alves, S. J., Andrade, D. S., & Guimarães, M. F. (2015). Microbial characteristics of soils under an integrated crop-livestock system. *Revista Brasileira de Ciência do Solo*, *39*(1), 40-48. https://doi.org/10.1590/01000683rbcs20150185

Stockdale, E. A., & Brookes, P. C. (2006). Detection and quantification of the soil microbial biomass – impacts on the management of agricultural soils. *Journal of Agricultural Science*, *144*(4), 285-302. https://doi.org/10.1017/ S0021859606006228

Sun, R., Zhang, X.-X., Guo, X., Wang, D., & Chu, H. (2015). Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biology and Biochemistry*, *88*(1), 9-18. Retrieved on August 28, from https://doi.org/10.1016/j.soilbio.2015.05.007

Tripathi, P., & Dubey, N. K. (2004). Exploitation of natural products as an alternative strategy to control

postharvest fungal rotting of fruit and vegetables. *Postharvest Biology and Technology*, *32*(3), 235-245.

Tsror, L. (2010). Biology, epidemiology and management of *Rhizoctonia solani* on potato. *Journal of Phytopathology*, *158*(10), 649-658.

Van Bruggen, A. H. C., & Semenov, A. M. (1999). A new approach to the search for indicators of root disease suppression. *Australasian Plant Pathology*, *28*(1), 4-10.

Van Bruggen, A. H. C., Sharma, K., Kaku, E., Karfopoulos, S., Zelenev, V. V., & Blok, W. J. (2015). Soil health indicators and *Fusarium* wilt suppression in organically and conventionally managed greenhouse soils. *Applied Soil Ecology*, *86*(1), 192-201. doi.org/10.1016/j.apsoil.2014.10.014

Van der Heijden, M. G. A., & Wagg, C. (2013). Soil microbial diversity and agro-ecosystem functioning. *Plant and Soil*, *363*(1-2), 1-5. https://doi.org/10.1007/s11104-012-1545-4

Wall, P. C., Neate, S. M., Graham, R. D., Reuter, D. J., & Rovira, A. D. (1994). The effect of rhizoctonia root disease and applied nitrogen on growth, nitrogen uptake and nutrient concentrations in spring wheat. *Plant and Soil*, *163*(1), 111-120.

Weller, D. M., Raaijmakers, J. M., Gardener, B. B. M., & Thomashow, L. S. (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology*, *40*(1), 309-348. https://doi.org/10.1146/annurev.phyto.40.030402.110010

Westphal, A., Li, C., Xing, L., McKay, A., & Malvick, D. (2014). Contributions of *Fusarium virguliforme* and *Heterodera glycines* to the disease complex of sudden death syndrome of soybean. *PLoS ONE*, *9*(6), 1-13. https://doi.org/10.1371/journal.pone.0099529

Wiseman, B. M., Neate, S. M., Keller, K. O., & Smith, S. E. (1996). Suppression of *Rhizoctonia solani* anastomosis group 8 in Australia and its biological nature. *Soil Biology and Biochemistry*, *28*(6), 727-732. https://doi.org/10.1016/0038-0717(95)00178-6

Wrather, A., Shannon, G., Balardin, R., Carregal, L., Escobar, R., Gupta, G. K., … Tenuta, A. (2010). Effect of diseases on soybean yield in the top eight producing countries in 2006. *Plant Health Progress*. https://doi.org/10.1094/PHP-2010-0125-01-RS

Wrather, J. A., & Koenning, S. R. (2006). Estimates of disease effects on soybean yields in the United States 2003 to 2005. *Journal of Nematology*, *38*(2), 173-80.

Yangui, T., Rhouma, A., Triki, M. A., Gargouri, K., & Bouzid, J. (2008). Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. *Crop Protection*, *27*(2), 189-197. https://doi.org/10.1016/j.cropro.2007.05.005

Zhang, H., van der Lee, T., Waalwijk, C., Chen, W., Xu, J., Xu, J., … Feng, J. (2012). Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. *PLoS ONE*, 7(2), 1-14. https://doi.org/10.1371/journal.pone.0031722

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