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Allan Gammelgaard 201370890
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Hard wood biochar application on *Solanum tuberosum* growth, peel quality and severity of *Rhizoctonia solani* infections, a pot experiment.

Abstract

A short-term pot experiment on the effect from hardwood derived biochar, here, from wooden rafters, in relation to potatoes. The economic feasibility, yield, and severity of Rhizoctonia solani infections were examined, as well as peel quality meaning prettiness/skinfinish and equality in size of the potatoes were tested. The pot experiment was conducted using soil from a potato field and enriched sphagnum, the setup was divided into two groups containing 30 individuals each. Group A only experienced background infections from *R. solani* inherent in the field-soil, while inoculum containing *R. solani* was added to group B. No apparent effect from biochar application (0.0, 0.1, 0.5, 1.0, 3.0%) on any tested parameter was observed in group A, while a significant moderate positive correlation between biochar application and infection severity was observed in group B. This result was puzzling due to no significant differences in infection severity from *R. solani* between group A and B. Potential drivers for the observed result were explored, knowledge gaps as well as potential future issues caused by applying biochar in potato production were identified. Most importantly no direct desired or adverse effect from hardwood biochar on potatoes was observed in this experiment.

Introduction

Biochar is one of the co-products when charring organic matter under pyrolysis, mostly straw, wood and woodchips are used, but manure and other biological waste products are also viable feedstock. Biochar is increasingly being examined and applied in agricultural uses, because of its potential as a soil conditioner (Lehmann & Joseph, 2009) First off biochar have shown great promise by increasing nutrient retention, with an increase of N utilization efficiency and a decrease in N leaching (Zheng, Wang, Deng, Herbert, & Xing, 2013). Only biochar produced at lower temperatures than 600 C significantly increases P availability in soils, where off the application of biochar on acid (pH < 6.5) and natural soils (pH 6.5-7.5) increasing P availability by up to a factor 5.1 and 2.4 respectively (Glaser & Lehr, 2019). Increases in water retention have also been proven with up to 45% more water availability in coarse-textured soil, as well as 21% and 14% in medium and fine textured soils respectively, review by (Razzaghi, Obour, & Arthur, 2020). Furthermore, biochar increases soil organic C, cation exchange capacity, decreasing soil bulk density and have a high surface area, due to its pore structures. This high surface area gives in return a high potential for binding wanted as well as unwanted soil compounds (Debode et al., 2020; Nzediegwu et al., 2019; Rogovska, Laird, Leandro, & Aller, 2017). This porous structure of biochar, can also act as a shelter for soil microorganisms (Esmaeelnejad, Shorafa, Gorji, & Hosseini, 2017; Gao & DeLuca, 2018; Lehmann et al., 2011; Mahmoud, El-Beshbeshy, El-Kader, El Shal, & Khalafallah, 2019) Another quality of biochar is its potential to accumulate carbon deeper in the soil, decreasing chances of re-release into the atmosphere. This could significantly decrease the high CO2 emissions caused by the farming industry (Woolf, Amonette, Street-Perrott, Lehmann, & Joseph, 2010)

Potatoes being a high value agricultural crop often grown in sandy soils exhibiting low

water-retention as well as high leaching, thus promoting biochar as a promising soil conditioner. Although most meta-analysis on the positive and adverse effects of biochar in agricultural practices, show mostly promising results, thus meaning increased yields and productivity, still many independent studies display incohesive results and the underlying mechanisms are still not fully understood (Biederman & Stanley Harpole, 2013; Farhangi-Abriz et al., 2021; X. Liu et al., 2013) Most studies on potatoes grown in a biochar enriched soil have found an increase in plant growth, tuber yield and tuber quality in relation to chemical composition (Debode et al., 2020; Kalika P. Upadhyay, George, Swift, & Galea, 2014; Kalika Prasad Upadhyay, Dhami, Sharma, Neupane, & Shrestha, 2020; Youssef, Al-Easily, & A.S. Nawar, 2017). This, in relation to biochar also lowering production expenses, can significantly increase the net return of growing potatoes, as well as decrease pollution from those fields, a win-win condition for farmers and nature alike. (Farooque et al., 2020; Keske, Godfrey, Hoag, & Abedin, 2020; Metwaly & Mark, 2020; Youssef et al., 2017; Zheng et al., 2013). Because most Danish potato production is centralised in the western part of Jutland due to sandier soils making heavy potato farming equipment variable. This in combination with a low land to sea distance and high degrees of artificial irrigation methods, results in a land area high in nutrient leaching and thereby high risk of eutrophication (Andersen, 2003). The above stated properties of biochar are therefore of high interest to danish farmers as well as nature conservationist.

In contrast, some studies have found a negative effect from biochar on potato growth (C. Liu et al., 2017) or no significant direct effect from short term studies (Yang, Ravnskov, & Neumann Andersen, 2020).

This incoherence between studies on biochar in general are most likely due to the fact that feedstock, pyrolysis time, and temperature all affect the physical and chemical properties of the char, which in return affect the soil biota direct e.g toxicity from PAH's, metabolization of char, hideaway in porous structure or indirect e.g by changing cation exchange rate or water retention (Lehmann et al., 2011; Y. Liu et al., 2018; Mahmoud et al., 2019; Prodana et al., 2019). But soil biotic fauna is also dependent on soil type, geographic, and historic use of area, plant's reaction to biochar can also be directly related to the given biochar's properties or indirect through a change in plant-soil biotic interaction (Debode et al., 2020; Lehmann et al., 2011). Any variation in these factors stated above can drastically change an experimental result, thereby making comparison between studies and a one-model-fits-all impossible (Bonanomi, Ippolito, & Scala, 2015), to mitigate this, references as well as compared results from this experiment will primarily be related to studies conducted on biochar also derived from hardwood like the biochar used in this experiment.

The change in soil biota, have also spiked an interest in using biochar as a tool for combating different plant pathogens. A supressing effect have been shown against Fusarium spp., Phytophthora spp., Pythium spp., Sclerotinia spp., Sclerotium spp., Verticillium dahlia, and Rhizoctonia solani (review by (Bonanomi et al., 2015)). The model pathogen in this study is *Rhizoctonia. solani* (basal rot), *R. solani* is a plant pathogenic fungus which mostly infect younger plants by cellular penetration by hyphae (Bienkowski et al., 2010; Tsror, 2010). Suppression from biochar amendment have been observed in cucumber (Jaiswal, Elad, Graber, & Frenkel, 2014), common bean (Jaiswal, Frenkel, Elad, Lew, & Graber, 2015) as well as potatoes (Debode et al., 2020) . Often these response curves exhibits a hormesis effect, which is seen as a U-shaped curve, where low doses supressing the disease and high doses promoting the disease (Graber, Frenkel, Jaiswal, & Elad, 2014; Jaiswal et al., 2015), this influence on pathogens may

be direct e.g. supressing the pathogen directly, or indirect e.g. improve plant fitness, improving beneficial microbes or by organic material from the biochar inducing a systemic resistance (Graber et al., 2014; Rogovska et al., 2017)

The mechanism involved in supressing *R. solani* have yet to be found and no direct toxicity from biochar on *R. solani,* have been proven (Bonanomi et al., 2015) but biochar has been shown to up-regulate *R. solani* genes associated with carbohydrate metabolism, in a study conducted on soybeans (T. Copley, Bayen, & Jabaji, 2017). Increase in growth and hyphal extension caused by maple bark biochar, also suspected to be caused by metabolisms of organic compounds present in the biochar (T. R. Copley, Aliferis, & Jabaji, 2015). A shift in plant growth promoting rhizobacteria, in combination with a direct effect dependent on the type of biochar has been suggested as the driver of increased plant growth and suppressive effect on phytopathogenic fungi from biochar on soybeans and cotton (Egamberdieva, Wirth, Behrendt, & Allah, 2016; Pal, Tilak, Saxena, Dey, & Singh, 2000)

The aims of this study are to examine I: any potentially effects from biochar on the quality of the potato tubers as a food commodity hereby skin finish and equality in size. II: Plant growth and yield in response to biochar applications, and III: the effect of biochar on infection severity caused by *R. solani.*

Table 1:

Dry matter	63.00	%
Phosphorus (P)	960.00	mg/kg
Lead (Pb)	5.00	mg/kg
Cadmium (Cd)	1.40	mg/kg
Chrome (Cr)	59.00	mg/kg
Potassium (K)	6400.00	mg/kg
Mercury (Hg)	< 0.01	mg/kg
Nickel (Ni)	65.00	mg/kg
PAH's	> 470.00	mg/kg

Table 1: Analysis on biochar composition notable all polycyclic aromatic hydrocarbon (PAH) has been grouped together, and PAH amount exceed values acceptable for use in food production.

Materials and methods

Growing medium

The pots used in this experiment was 12 L buckets made of black 04-PE-LD plastic, in each pot was a mixture of 0.66 L gravel, 1.44 soil from a field containing potatoes in its crop rotation and 9.9 L of enriched sphagnum. The medium was mixed in a cement mixer for six pots at a time, which was done by applying 3.96 L gravel, 8.64 L soil and 59.4 L enriched sphagnum, a small dosage at a time and then letting the cement mixer run for a minimum of 15 minutes. One bag of enriched sphagnum could be distributed to 20 pots, each bag was enriched by1kg NPK 11-5-18 as well as micronutrients, thereby 50g in each pot which correspond to 5.5g N, 2.5g P and 9 K pr individual pot.

Biochar application

The biochar was created through pyrolysis of wooden rafters at a 500-600 ^{o}C, at Skive municipality and analysed by SEGES (see table: 1). Before any biochar was applied, the growing medium of the control groups six pots, where mixed, then dried and examined for moisture, when these six pots tested minimal moisture for 3 days consecutively, they were weighted and correlated for the weight of the bucket. To calculate a dry weight for a pot filled with growing medium to ensure a w/w percentage application of biochar, this resulted in a mean of 4.61 Kg medium in each pot. This weight was then used to calculate the desired biochar applications (0.1, 0.5, 1, and 3% w/w) which resulted in (4.61, 23.05, 46.10 and 138.3 g / pot) respectively. Before applying the biochar in the cement mixer, 4L of the mix was removed from the mixer and added to a bucket containing the required biochar and hand mixed, before added back to the cement mixer to ensure homogeneous distribution of biochar.

Experimental setup

The potato strain *Solist* was chosen, due to its early maturing, and high uses both commercial and privately in Denmark. All chosen tubers had a weight between 60-80 g and was planted at a 15 cm depth on the 12/04/21. Top was trimmed on the 25/06/21 to mature the tubers which were harvested 13 days after and examined on the 09/07/21 There were made two groups of 30 individuals, one of which not containing *R. solani* inoculum (Group A) and one including *R. solani* inoculum (Group B) these two groups were always kept at a medium of 3 meters apart to lower risk cross contamination from rain drop splashes. Each group contained 5 columns (0, 0.1, 0.5, 1.0, 3.0 %) biochar and 6 rows, each group was placed in a randomized block design, which were re-randomized once a week to mitigate potential border effects.

Pathogen application

The inocula containing *Rhizoctonia Solani,* were isolated from the potato stains Hermes and Bintje. Inocula were grown at Aarhus university's Flakkebjerg department, a total of 20 agar plates of each strain were cultivated. Like the biochar, a part of the growing medium mix was removed from the mixer and two agar plates of each *R, Solani* strain was hand mixed into the medium and added back to the cement mixer. All 30 pots which shouldn't be infected by *R. Solani* (Group A) were made and removed from the area before mixing the 30 infected pots.

Irrigation and Fertilization

The pots were placed outside and received natural watering from rain and 3 hours of sprinkler irrigation when any individual started to exhibit a lowering of water potential, this was done to stress individuals increasing chances of infection, as well as to increase potential positive effect from biochar's inherit water retention properties. No additional fertilizer was added doing growth.

Infection severity assessment

R. solani infection severity analysis was conducted in a lab at AKV langholt who's main work it to develop innovative and cost effecting potato cultivation strategies as well as industrial uses. Their disease severity assessment method is based on the SEGES created guidelines for assessment of national agricultural field studies. (SEGES, 2017). Every tuber was divided into 5 classes (0%, 2.5%, 7,5%, 15%, and 35%) coverage of *R. solani* sclerotia's on tuber, including pictures for comparison. The number of tubers in each class is then used to calculate an index value based on the following equation.

$$Index = \frac{(2.5 * CL.1) + (7.5 * CL.2) + (15 * CL.3) + (35 * CL.4) + 75\ CL.5}{Total\ Number\ of\ Tubers}$$

To ensure more data on potentially Phenotypic plasticity responses to an infection every class was also weighed under the assumption that an infected root might lose its connection to developing tubers due to root/stem cracks caused by *R, Solani* and thereby producing many small tubers. the number of tubers with deformities was also counted for each individual, this included all tubers exhibiting an irregular growth such as bumps and bends.

Quality criteria

The quality evaluation of the tubers was also conducted at AKV langholt, and was based on two parameters, skin finish and equalness in size, both parameters where personal assets a value between 1-10. Skin finish is the "cleanliness" of the skin where a value of 10 being equal to no marks of any kind on the tubers surface, while equalness was based of the approximately percentile number of tubers exhibiting the same size.

Statistical analysis

The statistical analysis of severity of infection caused by *Rhizoctonia* (IS), total top dry mass (TDM), Skinfinish (SF), tuber deformities (TD), tuber count (TC) tuber weight/yield (TW) and equalness size (ES) was conducted in R. Data

were tested for normality and homogeneity of variance, infection severity (IS) and deformities (TD) was highly positively skewed and where (log10 + 1) transformed, there after only highly non-significant results from shapiro test conducted with the function "shapiro.test" from the "stats" package in R. Residuals vs fitted plots showed homogeneity of variance for all tested parameters. Interactions was examined by a correlation analysis in R with the "cor" function from the *"stats"* package, assuming non monotonic relationship only worsen the correlations so pearsons's r was chosen as the desired cor test, this was supported with at principal component analysis (PCA) (see fig 1).
T-test was also conducted to examine differences between group A and B, by using the "t.test" function of the "stats" package in R

Results

Skin finish

No effect from biochar on skin finish was found, only a negative correlation from deformities (TD) in both groups and infection severity (IF) but only in group A (see table 2) which is also seen in the PCA biplot by the isolation of BC in group A (see fig 1), SF was also negatively correlated whit TDM, IS, TD and TC in group A

Growth and yield

Yield was generally low due to early harvest, cold may, insufficient fertilization, and high amount of phytopathogen infections. tuber yield (WT) and total top dry mass (TDM) was positively correlated in both groups. Biochar (BC) and infection severity (IS) had no effect on (WT) and (TDM) in group A but had a negatively correlation in group B at the same degree of correlation as between (IS) and (WT) (TDM) (see table 2)

Table 2. Pearson's correlation coefficient in both groups

Group A

	BC	TDM	SF	IS	TD	TC	TW	ES
BC	1.000	-0.017	0.001	0.004	0.015	0.008	0.105	-0.079
TDM	-0.017	1.000	-0.547	0.042	0.567	0.567	0.461	-0.192
SF	0.001	-0.547	1.000	-0.250	-0.655	-0.351	-0.054	0.409
IS	0.004	0.042	-0.250	1.000	0.090	0.069	-0.049	-0.169
TD	0.015	0.567	-0.655	0.090	1.000	0.406	-0.224	-0.411
TC	0.008	0.538	-0.351	0.069	0.406	1.000	0.155	-0.452
TW	0.105	0.461	-0.054	-0.049	-0.224	0.155	1.000	0.212
ES	-0.079	-0.192	0.409	-0.169	-0.411	-0.452	0.212	1.000

Group B

	BC	TDM	SF	IS	TD	TC	TW	ES
BC	1.000	-0.405	0.012	0.668	-0.123	-0.337	-0.541	0.091
TDM	-0.405	1.000	-0.096	-0.357	0.437	0.357	0.539	-0.165
SF	0.012	-0.096	1.000	-0.107	-0.139	-0.099	0.246	0.486
IS	0.668	-0.357	-0.107	1.000	-0.036	-0.382	-0.515	0.035
TD	-0.123	0.437	-0.139	-0.036	1.000	0.572	-0.101	-0.592
TC	-0.337	0.357	-0.099	-0.382	0.572	1.000	0.070	-0.316
TW	-0.541	0.539	0.246	-0.515	-0.101	0.070	1.000	0.330
ES	0.091	-0.165	0.486	0.035	-0.592	-0.316	0.330	1.000

Pearson correlation coefficients between parameters, group A: non purposely infected with *Rhizoctonia Solani* only background infection from field soil, group B: purposely infected with inoculum containing *R. Solani.* Values between 0.3-0,5 indicates a weak/low correlation, 0.5-0.7 moderate correlation, 0.7> strong correlation. Biochar (**BC**), Top Dry Mass (**TDM**), SkinFinish (**SF**), Infection Severity (**IS**), Tuber Deformities (**TD**), Tuber Count (**TC**), Tuber Weight (**TW**), and Equalness of Size (**ES**)

Infection severity

In group A, 25 individuals where infected with *Rhizoctonia Solani* while all were infected in group B, there was no significant difference between group A and B (p-value = 0.91) in relation to (IS) (see fig 2), highest variation and highest infection index values were found in group A. in group A (IS) did not correlate with any other variable, except a low non-significant with (SF) while it had a significant moderate positive correlation with biochar in group B as well as a negative correlation with TDM and WT (see table 2 and fig 1). notable did the diseases potato late blight as well as common scab also afflict most individuals in both groups.

Discussion

The wood biochar used in this short-term experiment showed no effect in group A on any parameters of growth, yield, skin finish and equalness of size which all are of high importance for the food industry both in production and sales. Group B did only see a correlation with a lowering in yield but this lowering in group is most likely caused indirectly by biochar through an increase of *R. solani* infection severity.

Infection severity between the two groups was not significantly different (p =0.96), thereby each group should have the same degree of infection caused by *R. solani* overall, the only difference between the two groups

Figure 1: Principal component analysis (PCA) biplot

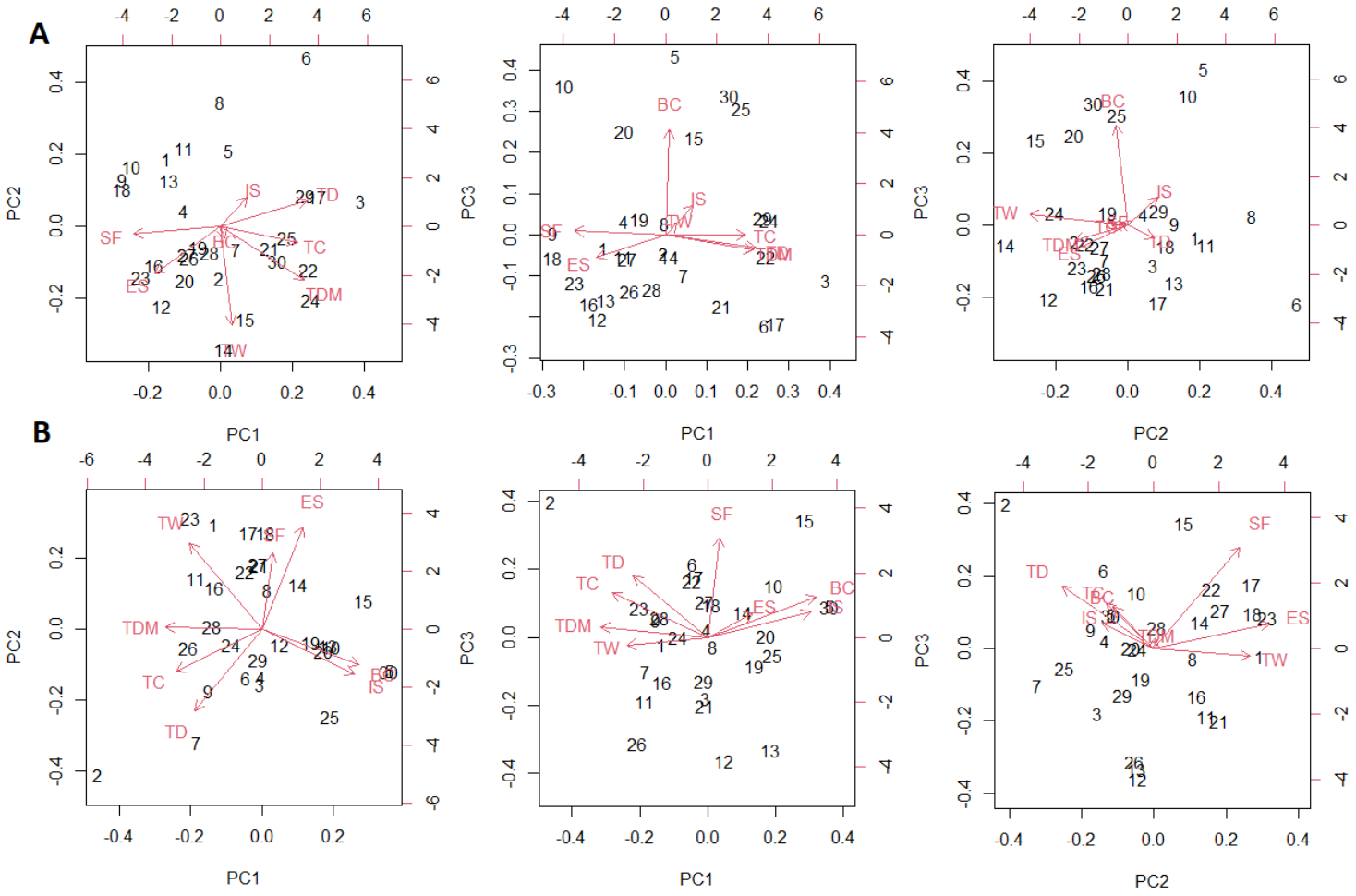

Figure 1: The Principal component analysis (PCA) biplot shows the PC scores of individual plants in each group while the eigenvector show the loading of the individual variables, meaning vector length show the amount of influence while the angel between vectors illustrate the relation, positive correlated (< 90°), no relation (~ 90°) and negative correlated (> 90°), Biochar (**BC**), Top Dry Mass (**TDM**), Skinfinish (**SF**), Infection Severity (**IS**), Tuber Deformities (**TD**), Tuber Count (**TC**), Tuber Weight (**TW**), and Equalness of Size (**ES**)

should only be the application of pathogenic infected inoculum. The result of non-significant correlation to biochar from any parameters in group, A have also been seen in other experiment on potatoes yields conducted with hard-wood biochar (Debode et al., 2020; Jay, Fitzgerald, Hipps, & Atkinson, 2015; Nzediegwu et al., 2019; Yang et al., 2020)

While the result of an increase in *R. solani* infection as a response to higher (1%-5%) hardwood-biochar doses have also been observed in soybeans (T. Copley et al., 2017) and lettuce (Debode et al., 2020). The above stated studies used a similar pathogen application approach by firmly mixing inoculum into the growing medium. This higher virulence might be supported by wood biochar that has been shown to up-regulate *R. solani* genes associated with carbohydrate metabolism in a study conducted on soybeans (T. Copley et al., 2017), and general increased growth rate of *R. solani* (T. R. Copley et al., 2015). in contrast, if biochar positively and directly influences *R. solani,* some degree of correlation should have been seen in group A, however, no correlation was found, and in Fig 2, infection severity seems stochastic in group A as well as a clear isolation of (BC) in comparison to the (BC)-(IS) clustering seen in the PCA bi plot (see fig 1).

The positive growth and disease suppression effect derived from biochar is suspected to be driven by an altering of soil chemistry in favour of Plant growth promoting bacteria (PGPB) (Bertola, Mattarozzi, Sanangelantoni, Careri, & Visioli, 2019; Wang et al., 2020), some of which also produce a wide array of anti-fungal substances. Some PGPB have proven too readily grow on different types of biochar so that biochar is suggested as a suitable carrier for these bacterial inoculants (Egamberdieva et al., 2018; Hale, Luth, Kenney, & Crowley, 2014; S. Liu et al., 2017). Using biochar as a bacterial inoculant carrier have been done with *Bacillus mucilaginosus* (S. Liu et al., 2017), *Bradyrhizobium sp*

Figure 2

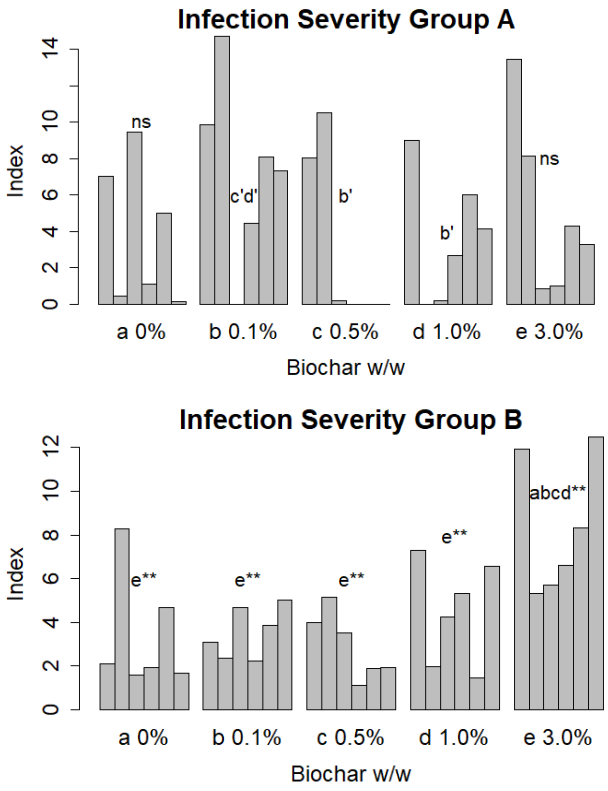

Figure 2: Bar plot visualizing index values for Rhizoctonia solani infection severity (**IF**) in relation to biochar application (**BC**), significance difference between groups denoted by (ns) non-significant, (‘) p = 0.1-0.05, (*) p 0.05-0.01, (**) p = < 0.01

(Araujo, Díaz-Alcántara, Urbano, & González-Andrés, 2020), and *Enterobacter cloacae* (Hale et al., 2014), all PGPB’s.

The potentially microbial benefactors from the biochar, both growth promoting and pathogenetic species, would have had an equal playing field in the soil when the biochar was applied in group A. while in group B, the high abundance of *R. Solani* could have given the pathogen a lead in dominating the soil biota, in the course of time this could naturalize and enter some sort of biota equilibrium (Bertola et al., 2019).
A similar result as seen within group B but a more favourable results have been seen in (Yang et al., 2020) where biochar alone had no effect on potato yield but the application of biochar together with arbuscular mycorrhiza fungi (AMF) inoculums increased yield more than AMF application alone, same

results have been shown on maize (Mau & Utami, 2014; Warnock et al., 2010) also driven by highest increasement of P-uptake in AMF+biochar.

Another take on the observed results in group B could be due to *R. Solani* already being in its active phase after being kept at room temperature before application, while the inherent field-soil *R. solani* were only waking up (Orozco-Avitia et al., 2013). Thereby the mycelium in group B had less competition when colonising the pot enhanced by a metabolic rate increment induced from the biochar (T. Copley et al., 2017), thereby an increase in potential attack pathways before any Induced systemic resistance by the host plant. But this would apparently not be the case under more natural field circumstances in group A

Implications that can be derived from this include a risk of higher *R. solani* longevity in soils in and around the porous biochar, which could facilitate the need for a longer potato crop rotation. No studies on increased longevity have been found, but an increasement in soil biotic diversity and function is often seen (Lehmann et al., 2011; Nguyen et al., 2018; Prodana et al., 2019; Thies, Rillig, & Graber, 2019), like most other studies conducted on biochar some studies indicates no change or a decrease in soil biota diversity (Lehmann et al., 2011; McCormack et al., 2019). Another implication is to use biochar as a booster when applying PGPB or AMF or as the carrier agent, either by direct inoculation of the desired microorganisms onto the biochar or as a seed-coating agent which have been tested for *Pseudomonas libanensis* and readily available phosphorus on to maize seeds, decreasing sprouting time and increasing plant growth (Martyna, Husk, & Schwinghamer, 2016). Furthermore the antifungal characteristics of *Pseudomonas* strains have been tested and suggested as a means of biological control of *R. Solani* (Crowe & Olsson, 2001; Pal et al., 2000) which also have been tested in a study using *P, Fluorescens* in micro bio-capsules, as a treatment against *R, Solani* on potatoes successfully suppressing 90% the disease (Fathi, Saberi-riseh, & Khodaygan, 2021) Furthermore other PGPBs from Arthrobacter, Pseudomonas, Microbacterium, Bosea, and Variovorax genres, have shown to increases in abundance over time in older long-term biochar-amended soil, of which PGPBs were able to colonise the pore structure of biochar, already present (Bertola et al., 2019). Using biochar to guide or purposely shift the soil biota in a desired direction might be a better way to approach the use of biochar, this is supported by pine-bark biochar Inoculation with *Bradyrhizobium* strains have shown a shelf time up to a year which is an increase relation to traditional inoculum mediums (Araujo et al., 2020)

Conclusion

Beside no direct adverse short-term effect from hard wood biochar on commodity important parameters, like skinfinish and equalness of size was found, no increasement in yield nor suppression of *R. solani* was found either. Even thou all null hypothesis of no effect was accepted, the carbon, water and nutrient retention properties of biochar is still favourable in comparison to contemporary potato farming practice. Implication that studies conducted on biochar as an amendment to increase resistance to soil born pathogenic attack, might need a closer look in relation to inoculum application method, to identify potential type II errors. Further studies should also be conducted on the possibility on increased longevity of soil borne pathogen due to biochar. Standardization of biochar type should be considered, to avoid PAH accumulation in soils as well as stronger comparison between studies. Even thou soil-biota composition analysis wasn't possible in this study, mainly due to economical restraints. Biota analysis should be included in future studies conducted on the potential uses of biochar in agricultural practice. The implications in using biochar as a bacterial and

AMF carrier are also in highly need of closer examinations. Biochar as carrier might also be where the highest economic gain as well as pathogenic prevention can be derived from soil amendment, through biochar.

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