



# Biochar, compost and arbuscular mycorrhizal fungi: a tripartite approach to combat *Sclerotinia sclerotiorum* in soybean

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## Abstract

*Sclerotinia* stem rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a major disease in soybean in many parts of the world. Sustainable control measures to combat this pathogen can be better achieved by combining different available tools. One element to control fungal diseases could be changing biological activities by adding organic matter inputs, such as biochar and compost, to the soil. Other players are arbuscular mycorrhizal fungi (AMF); bioprotective effects have already been documented for them. In the present study, we assessed the effect of organic matter inputs, such as compost alone at the application rate of 20% of the total substrate (v/v) and/or green waste biochar at the application rate of 3% (v/v) in combination with or without arbuscular mycorrhizal fungi, against *Sclerotinia sclerotiorum* and their effect on plant growth characteristics in soybean. Substrates including compost resulted in a lower disease severity in both, plants inoculated and non-inoculated with AMF. The AMF root colonization was highest in plants grown in the control treatment and green waste biochar substrate inoculated with *Sclerotinia sclerotiorum*; the lowest colonization was found in plants grown in substrates containing compost. Soil substrates, especially compost, affected shoot dry matter production in soybean plants inoculated with *Sclerotinia sclerotiorum* and in non-inoculated plants; compost alone was superior in treatments with and without AMF. Root morphological traits were more strongly influenced by AMF than by the substrate. Our findings suggest that compost has a positive effect in terms of soybean growth and diseases suppression, which is more pronounced than that of biochar and AMF.

**Keywords** *Sclerotinia* stem rot · Ascospore · Diseases suppression · Root morphology · Growth parameter

## Introduction

Soybean (*Glycine max* (L.) Merr.), one of the major crops providing protein and oil, can be affected by a number of plant diseases, which are causing serious yield losses worldwide. For example, in the USA annually 11% of the total soybean production get lost through soybean pathogens (Grau and Hartmann 2015). In Europe, *Sclerotinia* stem rot, caused by the ascomycetous fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is considered to be the most important soybean disease (Rüdelshiem and Smets 2012). The development of this pathogen is favored by the broad host range, the long-term viability of sclerotia in soils, even in the absence

of a host, and its ability to reproduce vegetatively or generatively, depending on the host plant and environmental conditions (Boland and Hall 1994). Sclerotia, the resting structures of the fungus, arise through aggregation of vegetative hyphae and are characterized by a multicellular, tuberoid structure enclosed by a melanized rind layer (Li et al. 2018). They function as nutrient source during myceliogenic or carpogenic germination (Li and Rollins 2009). During myceliogenic germination, hyphal strands break out of the sclerotia and subsequent basal infections of roots, crowns and other parts of plants that touch the ground occur. So far, this type of infection is mainly attributed to a stimulation by root exudates of specific host plants and to small sclerotia types, such as formed by *Sclerotinia minor*, the fungus that causes *Sclerotinia* blight (Purdy 1979). However, soybeans are primarily infected via carpogenic germination (Grau et al. 1982; Cline and Jacobsen 1983). In this event, sclerotia, preconditioned in a cool and moist environment and located at the upper 2–3 cm soil layer, are functional in

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forming apothecia and already a single mature apothecium is able to release millions of ascospores which are spread with the wind for several kilometers and, if landing on susceptible host plants, germinate and infect the plants (Abawi and Grogan 1975; Grau and Hartman 2015). Requirements for a successful ascospore germination and the infection of the host plant are temperatures in the range from 10–30 °C and the presence of free water (Abawi and Grogan 1975). Furthermore, exogenous nutrient sources, such as dead flower parts, pollen grains, wounds or senescent tissues, are needed to enable ascospores to penetrate the host plant (Abawi and Grogan 1975, 1979; Stelfox et al. 1978). Although plants exhibit a range of multifaceted defense mechanisms (recently reviewed by Wang et al. 2019), these are not sufficient to reduce the infection level reliably under the damage threshold.

The control of *Sclerotinia sclerotiorum* is challenging. Environment-friendly plant protection relies on combinations of various approaches, such as preventive, physical and biological measures, to fight against plant pathogens. One element to control fungal diseases could be changing biological activities by adding organic matter inputs, such as biochar and compost, to the soil. Compost gained increasing interest for nearly 100 years (Diaz and de Bertoldi 2007). Meanwhile, as comprehensively reviewed (Martínez-Blanco et al. 2013; Mehta et al. 2014), multiple positive effects were attributed to compost, such as improvement of nutrient supply, carbon sequestration, soil resilience to erosion, soil moisture household, soil aggregation, biodiversity, crop yield and not least pest and disease suppression. Only in the recent years, biochar, a carbon-rich pyrolyzed biomass, has risen much attention. Biochar, i.e., charcoal produced from plant matter, is also considered to improve physical, chemical and biological soil properties. For example, biochar can improve the available water capacity (Abel et al. 2013; Liang et al. 2014) and has a positive effect on the saturated hydraulic conductivity of the topsoil (Asai et al. 2009). The addition of biochar to the soil results in higher nutrient retention and nutrient availability due to increased surface area, higher exchange capacity and direct nutrient addition (Glaser et al. 2002). Nutrient availability can be improved by soluble nutrients contained in the biochar (Sohi et al. 2010; Major et al. 2010), but also by the mineralization of organically bound nutrients (Lehmann and Joseph 2009). The resulting effect of biochar on plant growth and yield ranges from positive (e.g., Glaser et al. 2002; Lehmann et al. 2006) to negative (e.g., Van Zwieten et al. 2010; Crane-Droesch et al. 2013; Borchard et al. 2014). Biochar could provide benefits in terms of plant health too. The addition of biochars added at up to 3% has been shown to reduce several foliar pathogens (Elad et al. 2010; Harel et al. 2012) and root pathogens (Matsubara et al. 2002; Bonanomi et al. 2007; Elmer and Pignatello 2011; Akhter et al. 2015

and 2016), with the mechanism of action likely due to an induced systemic response in the plant (Harel et al. 2012). The suppressive effect of compost against plant pathogens has been shown for a wide range of fungal diseases (Coventry et al. 2005; Noble and Coventry 2005; Termorshuizen et al. 2006; Morauf and Steinkellner 2015). Good efficiencies were already achieved with a 20% compost rate (Morauf and Steinkellner 2015). Beneficial effects were also evident when combining compost and biochar. In this regard, Schulz and Glaser (2012) reported on an improved plant growth and Akhter et al. (2015) on a reduction in *Fusarium oxysporum* f.sp. *lycopersici* disease severity in tomato.

Other players, which could alter biological activities in soil and subsequently in the plant, are arbuscular mycorrhizal fungi (AMF). The majority of crop plants form mutualistic symbioses with AMF, which enhance the growth and bioprotection of numerous plant species. Abundant data illustrate the bioprotective effect of AMF against soil-borne and foliar fungal plant pathogens (Singh et al. 2000; Azcon-Aguilar et al. 2002; Whipps 2004; Xavier and Boyetchko 2004; Fritz et al. 2006; St-Arnaud and Vujanovic 2007; Hage-Ahmed et al. 2013).

Compost, biochar and AMF as possible soil amendments function individually and together in terms of plant response. To our knowledge, the combined bioprotective effect of compost, biochar and AMF against *Sclerotinia sclerotiorum* has not been investigated yet. Compatible combinations of soil amendments might be efficient tools in order to alleviate plant stresses (Ohsowski et al. 2018) and to establish more environmentally friendly and sustainable plant diseases management strategies. We addressed the question of whether the addition of organic matter inputs, such as compost and biochar, in combination with AMF reduces ascospore infection in soybean and thus promotes plant health. The main objectives of this study were to assess the effect of biochar and compost in combination with or without AMF on *Sclerotinia sclerotiorum* disease suppression, growth of soybean and on changes in root morphological traits under a controlled greenhouse environment.

## Materials and methods

### *Sclerotinia sclerotiorum* inoculum production

*Sclerotinia sclerotiorum* was isolated from infected host plants (carrots, province Lower Austria, Austria) by surface sterilization of sclerotia with 50% household bleach ('Dan Klorix,' 2.8% NaOCl) for 4 min, followed by treatment with 70% ethanol for 4 min and subsequent washing with autoclaved distilled water three times for 2 min. The sclerotia were then bisected, placed on PDA (potato dextrose agar, Carl Roth GmbH + Co. KG, Karlsruhe, Germany)

and incubated in dark at 24 °C. Sclerotia derived from this culture were stored in a refrigerator until further use. Inoculum production was done according to a slightly modified method of Clarkson et al. (2003). For that purpose, 25 g of wheat grains and 50 g distilled water were put in 500-ml flasks and autoclaved for 20 min at 121 °C. Afterward, this medium was inoculated with two agar plugs (approx. 8 mm<sup>2</sup>) from the edge of a one-week-old *Sclerotinia sclerotiorum* colony and incubated at 24 °C under dark conditions. Sclerotia emerged after 3–4 weeks of incubation and were then used for the production of apothecia.

To induce carpogenic germination, the sclerotia were collected from the flasks and carefully packed in cheesecloth (approximately 50–100 g/bag). The cheesecloth bags were hung in a plastic container filled with tap water and stored in a cold room at 4 °C under dark conditions for 8 weeks. During this conditioning process, air circulation was provided by an aquarium pump, and the water was replaced every week. Afterward, the sclerotia were washed with distilled water, dried at room temperature under a fume hood and stored in a refrigerator at 4 °C until further use.

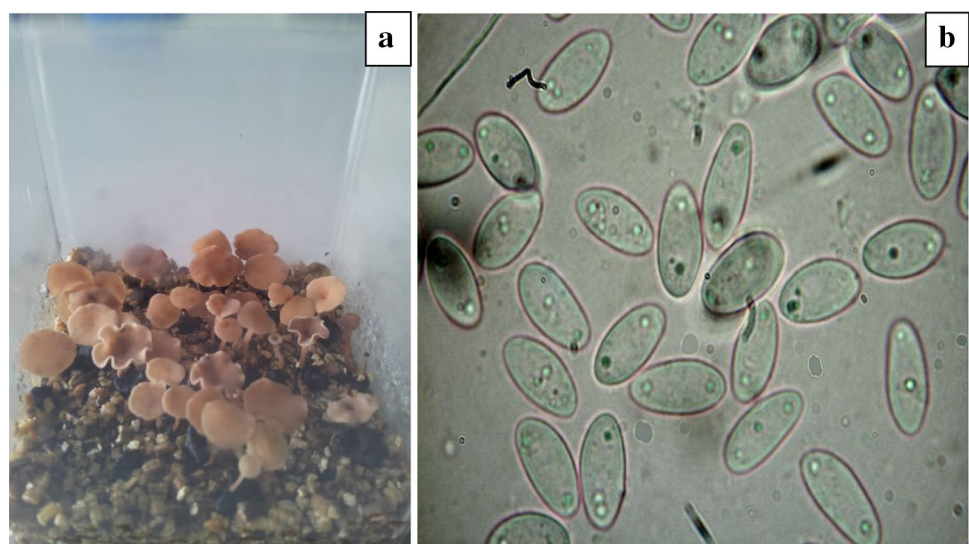
For germination of sclerotia, transparent tissue culture boxes (Magenta vessel GA-7, Sigma-Aldrich, Vienna, Austria) filled with vermiculite up to a quarter were autoclaved at 121 °C for 20 min and afterward, 30 conditioned sclerotia, which were about the same diameter (approx. 5–8 mm), were evenly placed close together on the top of the vermiculite surface. Distilled water was added up to saturation of the vermiculite, and the boxes were sealed with a lid to avoid water evaporation. The boxes were kept in a growth chamber with vertical light supply at a day/night photoperiod of 14/10 h at 15 °C until apothecia matured. After 6–8 weeks, the apothecia were matured (Fig. 1a) and ready for harvesting ascospores. For that purpose, matured apothecia were cut very carefully with

small cosmetic scissors under a fume hood. Five apothecia/boxes were sampled for three times (in total 15 apothecia/box within a period of six days). Five apothecia each were put in sterile 1.5-ml tubes filled with distilled water (1 ml per tube), closed, vortexed for 10 s and then stored at –80 °C. To avoid reducing ascospore germination ability and viability, this ascospore suspension (Fig. 1b) was used within 1 month after its preparation. For inoculation of the plants, the ascospore suspension was prepared by mixing the solution of tubes containing ascospores of three different harvests in a 50-ml falcon tube and adjusted to a final concentration of 10<sup>6</sup> ascospores ml<sup>-1</sup>.

### Arbuscular mycorrhizal fungi inoculum production

For the AMF plant inoculation, a commercially available inoculum of *Funneliformis mosseae* (BEG 12, Biorize/Agrauxine, Quimper, France) multiplied in the host plant *Sorghum sudanense* cv. Piper was used. The start inoculum had been stored at 4 °C. Pots with a diameter of 14 cm were filled with an autoclaved substrate consisting of sand (Quarzsand 0.5–2.0 mm, Quarzwerke Österreich GmbH, Melk, Austria) and expanded clay (Liapor fit 1–4 mm, Lias Österreich GmbH, Fehring, Austria) (1:1 ratio v/v). Thereafter, the start inoculum (15 ml) was added and 5–7 sorghum seeds were sown and then covered with the substrate. The pots were cultivated in the greenhouse and watered with tap water according to plant demands. For preparing the inoculum, the plants were stopped watering and the sorghum shoots were cut off. Finally, the AMF inoculum was prepared by cutting the fresh roots of mycorrhizal *Sorghum sudanense* in small pieces of approx. 2–4 mm and mixing the root pieces with the colonized substrate (spores, hyphae, sand and expanded clay).

**Fig. 1** Apothecia formation on vermiculite (a) and ascospores obtained from apothecia (b)



## Organic soil amendments

The compost (Comp) was obtained from the municipal compost works in Klosterneuburg (Lower Austria). The compost was categorized as quality A + according to the Austrian compost regulation (BGBl. II Nr. 929/2001). The green waste biochar (GWB) was made from garden waste residues at pyrolysis temperature of 500 °C. The physicochemical parameters of compost and biochar are summarized in Table 1.

## Greenhouse experiment

The experimental setup included four different substrates, those were control, green waste biochar (GWB), compost (Comp) and GWB + Comp, inoculated with *Sclerotinia sclerotiorum* (+Scl) or without (–Scl) in the presence or absence of arbuscular mycorrhizal fungi (AMF). For that purpose, soybean seeds (*Glycine max* L. cv. Gallec, kindly provided by Raiffeisen Ware Austria AG) were surface-sterilized by soaking in 50% household bleach (2.8% NaOCl) for 5 min and subsequently washed three times with autoclaved distilled water for 2 min. Three soybean seeds were sown in each of a 1 L pot filled with four different substrates: I) an autoclaved (20 min at 121 °C) mixture of sand (Quarzsand 0.5–2.0 mm, Quarzwerke Österreich GmbH, Melk, Austria), soil (Aussaaterde, Gramoflor GmbH & Co. KG, Vechta, Germany; pH 5.2–6.2; 50–300 mg/l N; 80–300 mg/l P<sub>2</sub>O<sub>5</sub>; 80–400 mg/l K<sub>2</sub>O) and expanded clay (Liapor fit 1–4 mm, Lias Österreich GmbH, Fehring, Austria) (1:1:1, v/v/v) without additional amendments ('Control'), the same mixture II) in combination with compost at 20% (v/v) ('Comp'), III) in combination with green waste biochar at 3% (v/v) ('GWB')

**Table 1** Physicochemical characteristics of compost and green waste biochar

Parameters	Compost	Green waste biochar
pH	7.10 <sup>a</sup>	9.03 <sup>b</sup>
Carbon (%)	27.00	79.78
Nitrogen (%)	2.20	0.65
Ash contents (%)	n.a	19.30
Zinc (mg/kg)	321.00	95.00
Cadmium (mg/kg)	0.14	<2.00
Copper (mg/kg)	86.00	21.00
Conductivity (mS/cm)	1.40	1.67
Density (kg/l)	0.77	0.34
CEC (mmol 100/ml)	n.a	12.85

<sup>a</sup>pH in CaCl<sub>2</sub> and <sup>b</sup>pH were measured in de-ionized water. n.a. Parameters were not analyzed

and IV) in combination with compost at 20% (v/v) and green waste biochar at 3% (v/v) ('GWB + Comp').

For AMF inoculation, the above-mentioned inoculum was mixed with sand (1:1 ratio v/v) and 30 ml of the mixture was added subsequently to the planting hole during the potting procedure. One week after sowing, the soybean plants were thinned from 3 to 1 even uniform plant per pot. After 3 weeks, the axillary bud tissue at the third node of the soybean plants was slightly scratched by a scalpel blade and inoculated by pipetting 10 µl of ascospore suspension (+Scl) or water (–Scl) in the center of the scratched tissue. Each plant was carefully covered with a transparent plastic bag for 48 h to prevent a spread of ascospores to other plants and to maintain the moisture needed to germinate them. The plants were grown for 8 weeks in a greenhouse with a photoperiod of 14 h. The temperature ranged from 18 °C at night and 24 °C during the day, and the relative humidity was approximately 60%. Additional light was provided when outside photosynthetically active radiation (PAR) was below 367.43 µmol m<sup>-2</sup> s<sup>-1</sup> during the day. The plants were irrigated regularly according to their moisture requirement with a nutrient solution (Steinkellner et al. 2005). The experimental design was a completely randomized design, and each treatment consisted of six pots. The entire experiment was repeated three times (in total 18 pots/treatment).

## Disease rating

The analysis of disease severity was performed in weeks 1–4 after inoculation. *Sclerotinia* disease severity was determined visually according to the following scale: (0) no symptoms, (1) first signs of symptoms through changing the color on the buds, (2) lesions on lateral branches only, (3) lesion on the main stem but little or only through changing the color, (4) lesions on main stem resulting in plant death, (5) dead plants. The formula given by Grau and Radke (1984) was used to calculate the disease rating as follows:

Diseases severity index (DSI) =  $\Sigma$  (severity class x number of plants per class) × 100 / (total number of plants x total number of classes with symptoms).

## Agronomic and physiological parameters

Eight weeks after sowing, the plants were removed from the substrate, gently rinsed under running tap water, taking care to preserve the root system, and dried between paper towels. The roots and shoots were separated, and their fresh weight, number of pods, shoot length and number of leaves were recorded. Thereafter, the roots were divided into two similar parts of 4 cm from taproots and maintained in 50-ml falcon tubes filled with 30% ethanol in 4 °C until further use. One part was used to determine the degree of mycorrhization. For that purpose, the roots were kept overnight



and then clarified by boiling for 8 min in 10% KOH, rinsed three times with tap water and stained by boiling for 4 min in a 5% ink (Sheaffer black)–vinegar solution according to the method of Vierheilig et al. (1998). Then, the AMF root colonization was determined microscopically according to the counting procedure of Giovannetti and Mosse (1980). After that, both root parts were digitized by a modified flat-bed scanner (Epson Perfection V700 Photo) and root morphological traits [root length (cm), root volume (cm<sup>3</sup>), root surface area (cm<sup>2</sup>) and average diameter (mm) of roots] were measured by means of the software WinRHIZO® Regular (Regent Instruments Inc. Ltd., Quebec, Canada). After imaging, root and shoot dry weight was calculated after drying the samples at 105 °C for 24 h.

### Statistical analysis

The data analysis was performed using IBM SPSS Statistics V21.0.0 software. Analyses of variance (ANOVA) were carried out after a variance check by the Levene's test. The data of plant growth and root morphological parameters were analyzed by two-way ANOVA with the main factors 'Substrate' (Control (I), GWB (II), Comp (III) and GWB + Comp (IV)) and 'Scl' (–Scl and + Scl). In addition, in the presence of significant interaction effects, a simple effect analysis test was performed. Moreover, AMF root colonization rate and disease severity index assessment data were analyzed by one-way ANOVA. Mean values were compared using Tukey's post hoc test at the significance level of  $p \leq 5\%$ .

## Results

### Disease severity index

The assessment of Scl disease severity on soybean plants was performed in week 1–4 after inoculation (Table 2). In

contrast to plants grown in the control and GWB substrate, plants grown in substrates including compost did not show any diseases symptoms 7 days after inoculation (DAI). The diseases severity rose up clearly between 7 and 14 DAI. ANOVA on mean disease severity (%) indicated a significant difference at 14 DAI ( $F_{(7, 16)} = 34.85, p < 0.001$ ). The disease severity was highest for plants grown in the control substrate and lowest in substrates including compost. The picture was similar for both –AMF- and + AMF-treated variants. This trend continued in the following weeks [21 DAI ( $F_{(7, 16)} = 22.43, p < 0.001$ ) and 28 DAI ( $F_{(7, 16)} = 37.88, p < 0.001$ )]. 28 DAI, the disease severity in –AMF plants grown in the control substrate was 1.5 times higher than in plants grown in compost and 2.5 times higher than in plants grown in compost and GWB. Compost alone showed a tendency to lower diseases severity in + AMF plants compared to –AMF plants.

### AMF root colonization rate

AMF root colonization for soybean plants grown in different soil substrates ( $F_{(7, 136)} = 47.72, p < 0.001$ ) is shown in Fig. 2. The mycorrhization was highest in plants grown in the control and GWB substrate inoculated with *Sclerotinia sclerotiorum* followed by non-inoculated plants grown on these substrates. The lowest colonization was found in plants grown in compost (22.61% in 'Comp–Scl' and 21.39% in 'Comp + Scl'). There was only a tendency toward a higher AMF root colonization by combining compost and GWB.

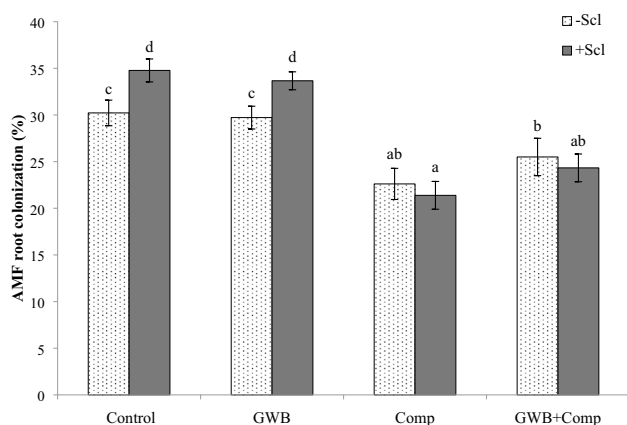
### Plant growth parameters

The soil substrates affected plant growth and biomass production in soybean plants (Tables 3 and 4). For all factors, except factor Scl in + AMF, and their interactions, a significant difference in shoot dry mass according to Tukey's post hoc test ( $p < 0.05$ ) has been observed. In the absence

**Table 2** Disease severity index (%) for *Sclerotinia sclerotiorum* in soybean plants grown in different soil substrates, with (+ AMF) or without AMF (–AMF) at different days after inoculation (DAI)

AMF	Substrate	Diseases severity index (%)						
		7 DAI	14 DAI	21 DAI	28 DAI			
– AMF	Control	10 ± 3.33	44.44 ± 6.94	d	57.77 ± 8.38	d	65.55 ± 1.92	e
	GWB	7.77 ± 1.93	27.78 ± 3.85	c	44.44 ± 1.92	c	53.33 ± 3.33	cd
	Comp	0	13.33 ± 3.33	ab	37.77 ± 1.93	bc	44.44 ± 5.09	bc
	GWB + Comp	0	4.44 ± 5.09	a	23.33 ± 3.33	a	26.66 ± 3.33	a
+ AMF	Control	10 ± 3.33	27.77 ± 1.93	c	47.77 ± 1.93	cd	61.11 ± 5.09	de
	GWB	5.55 ± 1.92	22.22 ± 5.09	bc	42.22 ± 8.39	c	56.66 ± 3.33	de
	Comp	0	7.77 ± 1.93	a	27.77 ± 1.93	ab	35.55 ± 5.09	ab
	GWB + Comp	0	4.44 ± 1.92	a	22.22 ± 1.92	a	26.66 ± 5.77	a

Means and standard deviations were calculated for total number of plants in each treatment ( $n = 18$ ) and tested for significant differences by one-way ANOVA followed by Tukey's test. For each DAI set, means not sharing same letters in subscripts are significantly different (Tukey's test,  $p \leq 5\%$ )



**Fig. 2** AMF root colonization (%) of soybean plants in different soil substrates un-inoculated (–Scl) and inoculated (+Scl) with *Sclerotinia sclerotiorum*. Each bar represents mean  $\pm$  standard deviation ( $n=18$ ). Means means not sharing same letters are significantly different (Tukey’s test,  $p \leq 5\%$ )

of *Sclerotinia sclerotiorum* (–Scl), the treatments including compost significantly increased the shoot dry mass compared to the control or GWB, where compost alone performed better than GWB + compost. In the presence of the pathogen (+Scl), all treatments performed better than the control. Compost alone was superior both in treatments with and without AMF. The results of root dry mass showed significant differences between variants with or without Scl and between the different substrates in both + AMF and –AMF variants (no significant interactions). In the absence as well as in the presence of AMF, the amendment of compost with or without GWB increased the root dry mass compared to the control and GWB treatment. The effect of the different Scl inoculations and substrates on the number of leaves was not that pronounced. However, the sole compost treatment showed an increased number of leaves. Compost with and without GWB as soil amendment significantly improved the pod set in –AMF soybean plants. The picture looked different in the treatments + AMF; there were significant interactions between the substrates and pathogen.

**Table 3** Effect of AMF and *Sclerotinia sclerotiorum* on shoot and root dry mass, number of leaves and pods of soybean plants (*Glycine max* L. cv. Gallec) 8 weeks after planting grown soil substrates of different compositions

AMF			Shoot dry mass (g/ plant)		Root dry mass (g/plant)		Number of leaves		Number of pods				
– AMF	– Scl	Control	5.97 $\pm$ 0.34	$\alpha^a$	0.93 $\pm$ 0.14	$A^b$	30.61 $\pm$ 4.59	A	5.11 $\pm$ 1.13	A			
		GWB	5.97 $\pm$ 0.62	$\alpha$	0.93 $\pm$ 0.16		32.33 $\pm$ 3.66		5.06 $\pm$ 1.69				
		Comp	7.70 $\pm$ 0.69	$\gamma$	1.17 $\pm$ 0.17		34.89 $\pm$ 4.39		9.17 $\pm$ 1.29				
		GWB + Comp	7.34 $\pm$ 0.59	$\beta$	1.09 $\pm$ 0.17	Ia <sup>c</sup>	33.06 $\pm$ 4.10	Ia	8.72 $\pm$ 1.27	Ia			
	+ Scl	Control	4.49 $\pm$ 0.39	$\alpha$	0.84 $\pm$ 0.14	B	IVb	29.83 $\pm$ 2.68	B	IVa	3.61 $\pm$ 1.53	B	IVb
		GWB	4.95 $\pm$ 0.39	$\beta$	0.90 $\pm$ 0.12			29.67 $\pm$ 3.64			4.44 $\pm$ 1.42		
		Comp	7.65 $\pm$ 0.42	$\gamma$	1.08 $\pm$ 0.17			34.83 $\pm$ 4.17			8.50 $\pm$ 1.50		
		GWB + Comp	7.68 $\pm$ 0.45	$\gamma$	1.09 $\pm$ 0.15			31.67 $\pm$ 2.89			9.00 $\pm$ 1.23		
+ AMF	– Scl	Control	5.72 $\pm$ 0.48	$\alpha$	0.74 $\pm$ 0.10	A		33.22 $\pm$ 4.68	A		6.39 $\pm$ 1.61	$\beta$	
		GWB	5.92 $\pm$ 0.37	$\alpha$	0.76 $\pm$ 0.10			33.83 $\pm$ 4.38			5.22 $\pm$ 1.39	$\alpha$	
		Comp	7.57 $\pm$ 0.48	$\gamma$	1.08 $\pm$ 0.16			31.83 $\pm$ 4.98			8.00 $\pm$ 1.08	$\gamma$	
		GWB + Comp	7.03 $\pm$ 0.44	$\beta$	1.01 $\pm$ 0.12		Ia	33.67 $\pm$ 4.74	Ia	9.06 $\pm$ 1.58	$\delta$		
	+ Scl	Control	5.68 $\pm$ 0.51	$\alpha$	0.79 $\pm$ 0.16	A	IVb	31.17 $\pm$ 3.22	A	IVa	5.22 $\pm$ 1.59	$\alpha$	
		GWB	6.29 $\pm$ 0.51	$\beta$	0.80 $\pm$ 0.11			31.33 $\pm$ 2.63			6.00 $\pm$ 1.64	$\alpha$	
		Comp	7.27 $\pm$ 0.57	$\gamma$	1.00 $\pm$ 0.11			32.83 $\pm$ 4.21			7.67 $\pm$ 1.37	$\beta$	
		GWB + Comp	7.09 $\pm$ 0.54	$\gamma$	0.94 $\pm$ 0.13			32.67 $\pm$ 3.34			8.44 $\pm$ 1.50	$\beta$	

Means and standard deviations were calculated for total number of plants in each treatment ( $n=18$ ) and tested for significant differences by two-way ANOVA followed by Tukey’s test. Means not sharing same letters in subscripts are significantly different (Tukey’s test,  $p \leq 5\%$ ) or simple effect analysis

<sup>a</sup>In the case of interaction effects, a simple effects analysis was performed on all substrate levels for ‘–Scl’ and ‘+Scl,’ respectively. Results from the simple effect analyses are indicated by Greek letters

<sup>b</sup>Uppercase letters indicate differences in the main factor ‘Scl’ according to the Tukey’s test at the 5% level of significance

<sup>c</sup>Roman numbers (Control (I), GWB (II), Comp (III) and GWB + Comp (IV)) in combination with lowercase letters indicate differences in the main factor ‘Substrate’ according to the Tukey’s test at the 5% level of significance

**Table 4** Two-way ANOVA analysis growth parameters of soybean plants grown in different soil substrate compositions with or without AMF (–/+ AMF) according to the main factors (Substrate and Scl) and their interaction (Substrate × Scl)

Factors	df	Shoot dry mass (g/plant)		Root dry mass (g/plant)		Number of leaves		Number of pods	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>–AMF</i>									
Substrate	3	244.71	< <b>0.001</b>	23.57	< <b>0.001</b>	10.20	< <b>0.001</b>	113.45	< <b>0.001</b>
Scl	1	43.98	< <b>0.001</b>	3.82	0.053	3.68	0.057	7.19	<b>0.008</b>
Substrate × Scl	3	25.77	< <b>0.001</b>	0.80	0.494	0.75	0.522	2.43	0.068
Error df	<b>136</b>								
<i>+AMF</i>									
Substrate	3	95.77	< <b>0.001</b>	41.96	< <b>0.001</b>	0.39	0.757	38.71	< <b>0.001</b>
Scl	1	0.08	0.783	0.79	0.376	2.78	0.098	1.81	0.180
Substrate × Scl	3	2.82	<b>0.041</b>	2.54	0.059	1.30	0.278	2.73	<b>0.046</b>
Error df	<b>136</b>								

Data are presented as degrees of freedom (df), *F* values and level of significance ( $P < 0.05$ ). Significant values are highlighted in bold

## Root morphological traits

The two-way ANOVA revealed a significant effect of the substrate on root length and root surface area in the absence of AMF. Here, compost alone lowered these root traits compared to the control. Furthermore, a significant effect of Scl and significant interactions between the main factors in root average diameter in the absence of AMF were found. In treatments + AMF neither the pathogen nor the substrate affected the root morphological traits significantly (Tables 5 and 6).

## Discussion

In this study, we analyzed for the first time the simultaneous use of compost, biochar and AMF on *Sclerotinia* disease caused by foliar infection of soybeans. Our data show that the amendment with both, compost and biochar, can cause a delay in symptom development and is able to reduce the susceptibility to *Sclerotinia sclerotiorum* in soybean. This beneficial impact was even more pronounced, when the plants were grown in compost, and the combination of both even showed a tendency to improve the performance of compost alone. The microbial communities present in compost are the most frequently reported drivers in diseases suppression (Mehta et al. 2014) which has been documented for numerous soil-borne pathogens (Termorshuizen et al. 2006). Of course, the amendment with compost and biochar significantly altered the environment for microbes in our soil substrate, in particular in consideration of the autoclaved control substrate. However, as we inoculated above ground plant parts with the pathogen, a direct impact on *Sclerotinia sclerotiorum* based on the main modes of action of beneficial microbes, such as competition, antibiosis, hyperparasitism

or ineffective pathogen proliferation (Termorshuizen et al. 2006; Mehta et al. 2014), is unlikely. A recent study (Aggeli et al. 2020) found clear evidence for an early triggering of salicylic acid (SA), jasmonate- or ethylene-dependent plant defenses for *Sclerotinia sclerotiorum* in lettuce subjected to plant biocontrol strains isolated from a diseases suppressive compost. Therefore, although we did not address the microbial communities in detail, this mechanism might to some extent explain our results.

In fact, the addition of compost to the soil substrate has led to a higher nutrient input compared to biochar and thus might have improved more the plant's nutritional status. It is generally thought that a high nutrient availability in the soil, achieved by nitrogen-rich manures and fertilizers, alters the plant growth and accelerates the canopy closure and thereby benefits the pathogen's development (Wallace et al. 1990; Schmidt et al. 2001). However, in our experimental setup diseases promoting environmental conditions due to canopy closure play a minor role. Furthermore, specific data regarding host nutrition in *Sclerotinia* symptom development are lacking or conflicting. Couper (2001) reported on a reduction of *Sclerotinia* in carrot upon low nitrogen supply, while the opposite trend was shown for oil seed rape (Söchting and Verreet 2004). Recent data pointed out the importance of Zn (Helferstein et al. 2015). In their study, a significant reduction in *Sclerotinia sclerotiorum* has been reported for soybeans grown with a normal or increased Zn-fertilization compared to plants grown without or reduced Zn-status. In our study, the compost used was characterized by a high Zn-content. This might have contributed to the lower disease development in compost-treated plots, although we did not directly focus on specific nutrients.

In a study on soybean charcoal root rot, caused by *Macrophomina phaseolina* (Spagnoletti et al. 2020), it has been concluded that AMF might limit soybean diseases, even

**Table 5** Effect of AMF and *Sclerotinia sclerotiorum* on roots morphological parameters of soybean plants (*Glycine max* L. cv. Gallec) 8 weeks after planting grown in different soil substrate compositions

AMF	Scl	Substrate	Root length (cm)	Root surface area(cm <sup>2</sup> )	Root volume(cm <sup>3</sup> )	Root average diameter(mm)			
- AMF	- Scl	Control	1754.99 ± 381.87	165.84 ± 36.74	1.25 ± 0.28	0.301 ± 0.003	α <sup>a</sup>		
		GWB	1283.89 ± 404.89	124.35 ± 35.65	0.96 ± 0.25	0.311 ± 0.015	αβ		
		Comp	1268.03 ± 143.23	127.85 ± 18.45	1.03 ± 0.22	0.322 ± 0.018	β		
		GWB+Comp	1375.12 ± 145.56	135.76 ± 13.34	1.07 ± 0.12	0.315 ± 0.014	αβ		
	+ Scl	Control					Ia		
							IIa		
							IIIa		
							IVa		
		GWB						IIa	
								IIIa	
								IVa	
								IVa	
Comp						Ia			
						IIa			
						IIIa			
						IVa			
GWB+Comp						Ia			
						IIa			
						IIIa			
						IVa			
+ AMF	- Scl	Control	1780.52 ± 392.13	173.26 ± 36.16	1.34 ± 0.27	0.311 ± 0.007	A		
		GWB	1596.57 ± 334.64	154.49 ± 32.01	1.19 ± 0.25	0.309 ± 0.014			
		Comp	1798.05 ± 227.30	179.00 ± 25.92	1.42 ± 0.24	0.316 ± 0.013			
		GWB+Comp	1733.62 ± 317.17	174.64 ± 25.75	1.40 ± 0.16	0.322 ± 0.012	Ia		
	+ Scl	Control					Ia		
							IIa		
							IIIa		
							IVa		
		GWB						Ia	
								IIa	
								IIIa	
								IVa	
Comp						Ia			
						IIa			
						IIIa			
						IVa			
GWB+Comp						Ia			
						IIa			
						IIIa			
						IVa			

Means and standard deviations were calculated ( $n = 6$ ) and tested for significant differences by two-way ANOVA followed by Tukey's test. Means not sharing same letters in subscripts are significantly different (Tukey's test,  $p \leq 5\%$ ) or simple effect analysis

<sup>a</sup>In the case of interaction effects, a simple effects analysis was performed on all substrate levels for '-Scl' and '+Scl', respectively. Results from the simple effect analyses are indicated by Greek letters

<sup>b</sup>Uppercase letters indicate differences in the main factor 'Scl' according to the Tukey's test at the 5% level of significance

<sup>c</sup>Roman numbers (Control (I), GWB (II), Comp (III) and GWB + Comp (IV)) in combination with lowercase letters indicate differences in the main factor 'Substrate' according to the Tukey's test at the 5% level of significance



**Table 6** Two-way ANOVA analysis roots morphological parameters of soybean plants grown in different soil substrate compositions in the presence or absence of AMF (–/+AMF) according to the main factors (Substrate and Scl) and their interaction (Substrate x Scl)

Factors	df	Root length (cm)		Root surface area (cm <sup>2</sup> )		Root volume (cm <sup>3</sup> )		Root average diameter (mm)	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>–AMF</i>									
Substrate	3	3.91	<b>0.015</b>	3.36	<b>0.028</b>	2.63	0.063	2.48	0.075
Scl	1	0.29	0.590	1.02	0.318	2.01	0.164	5.52	<b>0.024</b>
Substrate × Scl	3	1.93	0.140	1.44	0.246	1.08	0.369	3.59	<b>0.022</b>
Error df	<b>40</b>								
<i>+AMF</i>									
Substrate	3	0.99	0.406	1.61	0.203	2.35	0.087	2.38	0.084
Scl	1	0.15	0.703	0.01	0.933	0.06	0.805	3.18	0.082
Substrate × Scl	3	0.08	0.970	0.06	0.979	0.15	0.930	1.52	0.225
Error df	<b>40</b>								

Data are presented as degrees of freedom (df), *F* values and level of significance ( $P < 0.05$ ). Significant values are highlighted in bold

when the disease development is promoted by an increased nitrogen fertilization. This is in contrast to our data, as we could neither find an increased risk of diseases upon an improved nutrient status nor a sustainable bioprotective AMF effect, since the significant disease reduction in the control treatment by +AMF two weeks after inoculation was already compensated four weeks later. For all other substrates, we found no or only a tendency for a lower diseases severity index in +AMF plants compared to –AMF plants.

Compared to the pathogen effect, our data showed a reverse soil substrate effect regarding AMF root colonization. Here, compost decreased mycorrhization, while GWB kept it stable on the level of the control substrate. This is contrasting to other studies on compost or biochar and shows the complexity of plant–microbe interactions. Most frequently, adding biochar to the soil substrate is attributed to an enhanced AMF activity (Ishii and Kadoy 1994; Solaiman et al. 2010; Rillig et al. 2010). However, other studies found this effect only under specific conditions. These are, for instance, the use of grounded biochar as shown in marigold (Ezawa et al. 2002), specific growth stages and fertilization rates in soybean (Saito 1990) or a certain threshold of biochar application (Warnock et al. 2010). Nevertheless, our study revealed that the 3% addition of biochar to the soil substrates could not affect the mycorrhization rate. This underlines the observations that biochar can show its AMF-stimulating potential mainly at higher rates (Solaiman et al. 2010; Rillig et al. 2010). Furthermore, there is evidence that the AMF-promoting effect of biochar can only be utilized at high N levels in the soil substrate (Le Croy et al. 2013). This is in turn contrary to our data, as we found a lower AMF root colonization in the GWB + comp treatment compared to GWB alone.

On the other hand, we found a reduction of mycorrhization in the treatments containing compost. Our data on

AMF root colonization are contrary to a recent study on soybean (Yang et al. 2018) which reported no effect of low compost levels but increasing mycorrhization with increasing compost levels. Therefore, our findings are in line with a comparatively small number of studies, as according to Cavagnaro (2015) only 8% of studies report on such detrimental effects of compost on AMF. On the one hand, this has been attributed to the larger supply of *P* and *N* through compost (Baon et al. 1992). On the other hand, under low *P* availability an increase in AMF root colonization is well described (Smith et al. 2011; Ryan et al. 2016; Wen et al. 2020). Unfortunately, the specific *P* content in our compost was not available to us. However, in general, *P* contents in commercial composts from biogenous waste are in the range of 0.09–0.22% in dry matter (Baumgarten et al. 2010), and thus, a distinct phosphorus effect in our study seems to be unlikely, even more so as all plants were watered regularly with the same low dosage of nutrient solution. With 2.2%, the *N* content in the compost we used was in the upper range for composts (0.6–2.3% in dry matter, according to Baumgarten et al. 2010).

Another interesting point in our experiment was the increasing AMF colonization after shoot inoculation with *Sclerotinia sclerotiorum* only in the control and GWB treatment. Both treatments were also characterized by a higher diseases severity compared to the compost treatments. This indicates an alteration in the host plant physiology upon pathogen infection at lower nutrient supply. Here, we can only speculate on possible changes in nutrient availability and/or the release of diverse inorganic and organic compounds that the plants exude via the roots into the rhizosphere in response to biotic and abiotic stresses (Bais et al. 2006; Toljander et al. 2007) which in turn promote root colonization by AMF in our study.

In our study, the shoot growth of soybean was clearly improved in soil substrates amended with compost. In fact, the quality and characteristics of organic amendments such as biochar and compost clearly depend on feedstock and production conditions used. For example, Al-Wabel et al. (2013) reported that biochar produced at higher pyrolysis temperature has a higher carbon and nutrient content than biochar produced at lower pyrolysis temperatures. Furthermore, the feedstock can modify the soil organic matter and nutrient status (Mukherjee and Zimmerman 2013; Kloss et al. 2014). In our study, the plants were subjected to a regular, low-dose fertilization. The amendment of compost is known to enhance plant nutrient availability (Epstein 1997; Kawasaki et al. 2008) and thus exhibited a growth-promoting effect. A short-term immobilization of *N* in substrates, as known for higher C/N ratios (Scherer et al. 1996), is unlikely in our study. The compost we used was characterized by a relatively low C/N ratio and thus makes the sufficient *N*-availability plausible. Our data suggest that under a lower nutrient availability, *Sclerotinia sclerotiorum* boosts the mycorrhization of soybean roots which in turn compensates the negative impact on plant growth, whereas in general, the plant growth effects were more pronounced in the shoot compared to the root.

In a study on sorghum, Le Croy et al. (2013) found that biochar and AMF did not alter the shoot and root biomass under low *N* levels. Furthermore, the application of biochar at a rate of 2.6% and/or AMF did not affect the plant growth, but at higher *N* rates the combination of biochar and AMF lowered the growth-promoting *N*-effect. This is similar to our data on shoot and root growth of soybean under lower nutrient supply (control and GWB). However, it could not be confirmed under higher nutrient supply as given by the use of compost in our study. Here, we found only a slight trend toward a growth reduction due to AMF and biochar. Another factor, which might have contributed to our outcome, is the soil *P* availability. The addition of biochar might have led to a decline in *P* availability and thus the plant growth parameters (Clark and Zeto 1996; Warnock et al. 2010), although, contrary to Warnock et al. (2010) in our study, the AMF root colonization was not reduced in GWB compared to the control treatment.

Similarly, to the shoot dry weight, the number of pods/plant was altered by the addition of compost to the soil substrate in –AMF treatments. Besides of nutrient effects, this could also be attributed to the microbial community in compost. As mentioned above, the beneficial microbes in compost might be a trigger for a plant hormone-regulated responses (Aggeli et al. 2020), which may not only affect plant defense but also may be involved in the development of plant parts. This has at least been reported for *Arabidopsis* NahG transgenic lines and *sid2* mutants (Abreu and Munné-Bosch 2009), where decreasing SA concentrations improved

the number of seeds and pods. However, our study was characterized by significant substrate x *Scl* effects in + AMF treatments. This indicates that AMF intervenes differently here, but the exact role of the AMF remains unclear.

Observations in tomato showed that the substrate and a pathogen infection can cause a decrease in root morphological traits (Morauf and Steinkellner 2015). Our data suggest that AMF can compensate the negative effects of the pathogen. In our study, root length and root surface area were lowered in –AMF treatments, but in + AMF treatments morphological traits were not affected neither by the soil substrate nor by the pathogen. However, this is in contrast to Wang et al. (2011) who reported that AMF significantly decreased these variables in two soybean genotypes under low *P* and *N*-levels, but even at higher nutrient levels they found only a neutral response in terms of these root traits. Similar data are also available for other plants, e.g., for *Plantago lanceolata* (Forbes et al. 1996) or tomato (Trota et al. 1996). Generally, such modifications in root traits can allow for an improved *P* acquisition (Lambers et al. 2006; Shen et al. 2011), but as shown by Wen et al. (2020) this response is highly dependent on the plant genotype. In particular, thick rooted genotypes with large amounts of rhizosheath carboxylates are considered to respond less markedly to the *P* availability in soil. In our study, under conditions providing a low-dose fertilization, AMF were able to increase root length, root surface area and root volume, in particular in substrates amended with compost and/or biochar. Our data are in agreement with some other AMF–plant interactions. For instance, enhanced lateral root development upon AMF inoculation has been shown for mycorrhizal rice plants (*Oryza sativa*) (Gutjahr et al. 2009), peanut (*Arachis hypogaea*) or pigeon pea (*Cajanus cajan*) (Yano et al. 1996). The latter assumed that nutrient-poor conditions might foster mycorrhizal roots and subsequently trigger local modifications in root traits. However, our data reveal a trend toward a decrease in the mentioned root traits in soil substrates including compost and/or biochar. There is therefore much to suggest that AMF can compensate a potential impact of possibly growth-inhibiting microbes introduced via these amendments. However, our findings suggest that compost has a positive effect in terms of soybean growth and diseases suppression, which is more pronounced than that of biochar and AMF.

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## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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