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BRIEF COMMUNICATION

Interactions Between High Load of a Bio-based and Biodegradable Plastic and Nitrogen Fertilizer Affect Plant Biomass and Health: A Case Study with *Fusarium solani* and Mung Bean (*Vigna radiata* L.)

Sarah-Maria Scheid[1,2] · Kantida Juncheed[3] · Benjawan Tanunchai[1,4] · Sara Fareed Mohamed Wahdan[1,5] · François Buscot[1,6] · Matthias Noll[4,7] · Witoon Purahong[1]

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Abstract

Bio-based and biodegradable plastics such as mulching films are widely used in agricultural field sites. However, there are limited studies of their impact on plant development and health even though an important soil-borne plant pathogen *F. solani* has been reported to associate with various types of bio-based and biodegradable plastics, especially polybutylene succinate-co-adipate (PBSA). To evaluate the influence of PBSA amendment in soils on plant development and health, *F. solani* and mung bean (*V. radiata*) were used as models in a modified petri-dish test using soil suspensions. Mung bean seeds were incubated in suspensions with two dilutions (high vs. low dilution with low vs. high PBSA amendment) of soils pre-incubated 1 year with PBSA under different treatments (combinations of N fertilizer (ammonium sulfate) and PBSA load) in the modified petri dish test. Plant development and disease incidence were recorded with both microscopic and molecular techniques (specific PCR and Illumina amplicon sequencing). Treatment with PBSA and N fertilizer in non-sterile soil suspensions strongly increased the disease caused by *F. solani* on *V. radiata* at both low and high soil dilution. At high soil dilution, the *F. solani* disease incident was 67.5% while at the low dilution the disease incidence reached 92.5%. In contrast, in treatments PBSA but without N fertilizer, non *F. solani* disease was observed. Apart from *F. solani* infection, other soil fungi can also infect the mung bean seedlings, especially at low soil dilution levels. Nevertheless, based on this short-term study, we found no evidence that PBSA alone can significantly increase the overall disease incidence.

Keywords PBSA · Plant health · Ammonium sulfate fertilization · Mulching film · Plastic accumulation

Introduction

Mulching film has been used globally across agro-ecosystems [1]. It is an integral part of modern agriculture due to the effect of climate change, which caused serious drought

Sarah-Maria Scheid, Kantida Juncheed, and Benjawan Tanunchai have contributed equally to this work.

Matthias Noll and Witoon Purahong are senior authors.

✉ Witoon Purahong
witoon.purahong@ufz.de

François Buscot
francois.buscot@ufz.de

Matthias Noll
Matthias.noll@hs-coburg.de

1 Department of Soil Ecology, UFZ-Helmholtz Centre for Environmental Research, Theodor-Lieser-Str. 4, 06120 Halle (Saale), Germany

2 Institute for Integrated Natural Sciences, University of Koblenz and Landau, Universitätsstraße 1, 56070 Koblenz, Germany

3 Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand

4 Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, 95440 Bayreuth, Germany

5 Botany Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt

6 German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Leipzig, Germany

7 Institute of Bioanalysis, Coburg University of Applied Sciences and Arts, 96450 Coburg, Germany

problems [2]. Mulching films preserve water from evaporation and maintain soil moisture [3]. Furthermore, mulching films can also improve the microclimate around the plants, by enhancing the water and dissolved nitrogen (N) ions (i.e. NH_{4+}, NO_{3-}) use efficiency and regulating increasing soil temperature, based on a "greenhouse-effect"[4–7]. Consequently, the growth rates (in both root and shoot), biomass and yield production of plants are promoted [8, 9]. Due to the wide advantages of mulching films, their usages in farmlands are increasing annually. Polyethylene (PE) is the material used for decades in the production of mulching films [10]. European agriculture uses about 570,000 tons of PE annually [11]. Unfortunately, PE mulching films are considered as the major source of macro-, micro- and nano-plastics contamination in agricultural soils [12]. Currently, bio-based and biodegradable plastics are becoming an important alternative material for mulching films used in agriculture. The major advantages of bio-based and biodegradable plastics compared with conventional petroleum-based plastics included the use of renewable resources and the reduction of petroleum and energy consumption [13]. Additionally, the bio-based production reduces emission of greenhouse gases [14]. There are many bio-based and biodegradable plastics used as mulching film such as starch-based polymers, polylactic acid (PLA), polybutylene adipate terephthalat (PBAT), polybutylene succinate (PBS), and polybutylene succinate-co-adipate (PBSA) [15–17]. Due to the biodegradability of bio-degradable plastics in soil environments, after cultivation period, farmers may leave residues of mulching films to decompose in their agricultural fields in order to save time and labor costs [18, 19]. In case of PBSA, it can be degraded approximately 30% per year in temperate regions [20]. Thus, the PBSA residues can be accumulated in the soil systems over time [10].

Unfortunately, there is little knowledge about the degradation process of bio-based and biodegradable plastics and their effects on soil microbial activity, especially those obtained by high resolution molecular approaches such as Next Generation Sequencing (NGS) [21]. Furthermore, the impact of bio-based and biodegradable plastics on plant health is still unclear. Thus, we need more experiments that determine whether bio-plastic substances affect soil functions and plant health. Recently, Sforzini et al. (2016) introduced a biotest method to determine the soil ecotoxicity after exposure to high concentration of biodegradable plastics on autotrophic organisms, bacteria, protozoa and invertebrates such as *Daphnia magna* (crustacea) and earthworm *Eisenia andrei* as model organisms [22]. Choices of model organisms were made on the basis of the different trophic levels of which they are representative in food chains of terrestrial and aquatic ecosystems. Corn starch-based plastics and biodegradable polyesters were shown to have no significant effect on plant development and soil (micro-) organisms [22]. The quality of soils after input at high concentration of starch-based plastic (1%) has been assessed with a large array of biotests based on the same model organisms, and these plastics were classified as not harmful to agricultural utilizations [22]. In contrast, Qi et al. (2018, 2020) showed different negative effects of low-density PE and starch-based biodegradable plastics on wheat growth [23, 24]. In comparison to petroleum-based plastics, they found that bio-based and biodegradable mulch films have potentially a higher negative impact on growth of wheat. Indeed, when macro-plastics (particle size > 5 mm) degrade in water or soil, it breaks up into smaller particles, called micro-plastics (particle size < 1 mm). Micro-plastics revealed stronger negative effects on wheat growth than macro-plastics [23].

Moreover, some studies reported that various plant pathogenic fungi can colonize bio-degradable plastics [20, 21, 25, 26]. Thus, leaving the degraded mulching film in soils, can trigger some problems related to plant health and productivity as many plant fungal pathogens can colonize, grow and reproduce on residue of bio-based and biodegradable plastics. This situation can lead to the promotion of the diseases of various plants, such as cereal crops, vegetables, trees, and ornamental plants. Recently, PBSA has been reported to be colonized by *F. solani* [17, 20]. Furthermore, our earlier study demonstrated that *F. solani* strongly enriched in soil that was highly amended with PBSA and N fertilizer (e.g. ammonium sulfate) [21]. *F. solani* is an important soil-borne plant pathogen, which causes diseases in many agricultural crop species, including e.g. *Cucurbitaceae* and *Fabaceae* [27, 28]. This fungus infects plant roots, seedlings and can cause the soybean sudden death syndrome [29]. It is especially harmful for bean species. Nevertheless, we do not know, if such enrichment of *F. solani* caused by the interactions between PBSA and ammonium sulfate can significantly affect plant biomass and health.

Hence, in the study we aimed to (i) evaluate the effects of high load of PBSA amendment in soils and its interaction with N fertilizer (in the form of ammonium sulfate) to plant biomass (including shoot and root biomass) and health, (ii) to demonstrate that mismanagement of PBSA mulching film (for instance as agricultural waste) can cause negative effects on plant health. We use *F. solani* and mung bean (*V. radiata* L.) as a model for this evaluation. We used suspensions of soils pre-incubated 1 year with PBSA under different treatments encompassing combinations of N fertilizer (ammonium sulfate) and PBSA load to realise a petri dish test on the effect of different experimental treatments on growth and infection of seedling by *F. solani*. We hypothesize that high load of *F. solani* in soil as feedback to addition of PBSA and N fertilizer can significantly increase the *F. solani* disease incident in mung bean. In treatment without addition of N fertilizer, we expect that the *F. solani* disease incident is similar to the one in control soils without PBSA addition. Two

dilution levels of PBSA in soil (1:10 and 1:5) were used, which accounts for 0.6% and 1.2% initial PBSA (wt/wt) in soil suspensions, respectively, as is in line with previously reported plastic contamination in agricultural soils (approx. 1% PBSA in soil) [30, 31]. Nevertheless, in our opinion there is high possibility that the concentrations of bio-based and biodegradable plastics in soil can be even higher than 1%, especially if plastic mulch films are intentionally left in the field after usage. This model experiment enables us to anticipate a potential risk in plant health stemming from the mismanagement of bio-based and biodegradable plastic PBSA in agricultural field.

Materials and Methods

Experimental Design

To evaluate the effects of PBSA amendment in water/soils on the mung bean biomass and development, we did the following 9 treatments: (i) sterile water (SW); (ii) sterile soil (SS); (iii) sterile soil and PBSA (SSP); (iv) sterile soil and N fertilizer (SSN); (v) sterile soil, PBSA and N fertilizer (SSPN); (vi) non-sterilized soil = soil (S); (vii) soil and N fertilizer (SN); (viii) soil and PBSA (SP); and (ix) soil, PBSA and N fertilizer (SPN). The soil used for the 8 treatments was a Haplic Chernozem, C:N ratio ~ 10, pH = 7.16 ± 0.02 (mean ± SE) from central Germany on which a 1-year PBSA decomposition experiment with 6% (wt/wt, PBSA (BioPBS FD92, percent bio-based carbon = 35%, PTT MCC Biochem Company Limited, Thailand) had been run at 22 °C in darkness by maintaining 17.5% water content) as previously described by Tanunchai et al. [21]. The PBSA film was a double-layer thin film (21 cm × 30 cm) with 50 µm thickness used in the 1-year pre-incubation study. High load of ammonium sulfate $(NH_4)_2SO_4$ (equivalent to 280 kg N per hectare) was used as N fertilizer in SSN, SSPN, SN, and SPN treatments. After 1 year of PBSA decomposition, soil pH was significantly lowered in pre-treatments with N fertilizer regardless of PBSA addition (~ 2 level of pH). We homogenized the soil/PBSA carefully. In PSN, all PBSA were decomposed after 1 year whereas in PS treatment there were > 50% of PBSA remaining. Thus, soil from PSN, SN and control treatments were homogenized the same way by well mixing with sterile spatula and separated into 4 subsamples. For PS treatment, we separated PBSA and soil, mixed each compartment and pooled them based on weight into 4 subsamples. Two subsamples of each treatment were autoclaved for 3 times and used as sterile treatments whereas other two subsamples were used as non-sterile treatment. The soil or soil-PBSA subsamples from each treatment were diluted with sterile distilled water to a 1:10 (high dilution, low concentration, accounting for 0.6% initial PBSA) and 1:5 (low dilution, high concentration, accounting for 1.2% initial PBSA). Initial PBSA content in high and low dilutions were accounting for 0.6 and 1.2%. We previously analyzed fungal communities in all soil treatments using Illumina amplicon sequencing as explained earlier [21]. In this current study, *F. solani* was found enriched in treatment SPN (~ 33%) as compared with other treatments (~ 2% or less). PBSA addition or N fertilization alone did not increase the relative abundances of *F. solani* [21].

Modified Petri-dish Assay to Study *Fusarium* Disease Incidence in Mung Bean

Petri-dish assay to study *Fusarium*–based disease incidence of *V. radiata* seeds was modified from Purahong et al. [32]. *V. radiata* seeds (VIG 1631) were ordered from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany. To avoid contamination, the seeds were surface sterilized with a 2% (vol./vol.) NaClO solution for 8 min under a lamina air flow. Later, the seeds were rinsed 6 times with distilled water to remove any chemical residues [32]. After that, the seeds were dried on sterile filter papers for 1 h and kept inside the sterile lamina air flow.

For seed incubation, 8 seeds were placed in an autoclaved glass petri-dish (9 cm in a diameter) on tissue paper supplied with 10 mL sterile water. Incubation of the seeds were carried out under an illuminated air flow to avoid contamination. Additionally, the seeds were disposed in two rows with the seedling embryo turned upwards. We added 1 mL of soil solution (low or high concentration of soil) to each petri dish (125 µL soil solution per seed). The experiment was run with five independent replicates for each treatment and for each soil dilution concentration. To keep the moisture inside the petri-dishes, all five plates from the same treatment were packed in a clean PE bag with sterilized wet tissue paper and were securely closed with a wire. All plates were incubated in an incubator at 22 °C for six days in the darkness. After two, four and six days of germination, the biomass, the plant development and disease incidence of each seedling were examined, and the numbers of germinated and infected seedlings were counted. The disease incident was calculated in percentage. Within the petri dish, four seedlings were randomly collected from each plate for DNA extraction. Initial mung bean seeds and 1-year soil from SPN treatment were also taken for DNA extraction. The other 4 seedlings for each plate were separated with a scalpel into root and shoot and taken for measurement of biomass. Shoots and roots of seedling were dried in an oven at 105 °C for 24 h. Thereafter dry weight was measured using a five-digit balance (Mewes Wägetechnik, Haldensleben, Germany). Each seedling was optically observed and divided into healthy (seedling have the similar appearance than negative control without any additions) and diseased seedlings (seedling have the similar

appearance than same seedling with addition of *F. solani* (DSMZ 1164) as positive control) from each treatment were separated. The modified petri-dish assay was repeated with three replicates and the results are presented in (Supplementary Material Tables S1, S2, S3).

DNA Extractions and Detection Of *F. solani*

All seedlings were taken from each treatment separately into 50 mL tubes and washed with 50 mL of 0.1% (vol./vol.) sterile tween solution for three times to remove any soil particles. Thereafter the seedlings were washed again four times with double-distilled water to remove tween solution residues. At the end, each tube was filled with 15 mL nuclease and proteinase-free water (AppliChem, Darmstadt, Germany) and incubated for 1 h at 4 °C. After incubation the water solution was discarded. Each washed seedling was homogenized separately by adding liquid N_2 and crushing with a sterile nail. DNA was extracted using QIAGEN DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. For extraction 100 mg of homogenized seedling was used. The quality of DNA was checked using the nanodrop. The detection of *F. solani* were carried out from healthy and infected seedlings with specific PCR by employing the primer pair 1- TEF-Fs4f [5'-ATC GGCCACGTCGACTCT-3'] and TEF-Fs4r [5'-GGCGTC TGTTGATTGTTAGC-3'] [33]. DNA extract of *F. solani* (DSMZ 1164) was used as positive control. PCR assays were performed in 20 µL reaction mixtures containing 4 µL FIREPol® master mix ready to load (Solis BioDyne, Tartu, Estonia), 1 µL of each forward and reverse primer, 1 µL genomic DNA template and 13 µL nuclease- and proteinase-free water. The PCR products were checked in a 1.5% agarose-gel. The presence of *F. solani* was attested by the presence of the expected band of 658 bp. Fungal community composition in the used 1-year soil with PBSA pre-incubation and seedlings from SPN treatments were analyzed using Illumina amplicon sequencing as described in our previous study [21]. Briefly, DNA extracts from all seedlings of SPN treatment were pooled to get a composite sample. DNA from 1-year soil from SPN treatment was extracted using QIAGEN DNeasy Power Soil Kit (Qiagen, Hilden, Germany) according to manufacturer`s instructions. For construction of the fungal amplicon libraries, the fungal ITS2 gene was amplified using the fungal primer pair fITS7 [5'-GTGART CATCGAATCTTTG-3'] [34] and ITS4 primer [5'-TCC TCCGCTTATTGATATGC-3'] [35] with Illumina adapter sequences. Amplifications were performed using 20 µL reaction volumes with 5 × HOT FIRE Pol Blend Master Mix (Solis BioDyne). The amplicon products from three technical replicates were then pooled in equimolar concentrations. Paired-end sequencing (2 × 300 bp) was performed on the pooled amplicon products using a MiSeq Reagent kit v3 on an Illumina MiSeq system (Illumina Inc., San Diego, CA, United States) at the Department of Soil Ecology, Helmholtz Centre for Environmental Research, Germany. The bioinformatics analyses were carried out using pipeline Dadasnake as described by earlier [36]. Assembled reads fulfilling the following criteria were retained for further analyses: a minimum length of 70 nucleotides, quality scores at least equal to 9 nucleotides with maximum expected error score of 5 nucleotides for forward and reverse sequences and no ambiguous nucleotides. Merging was conducted with 2 nucleotide mismatches allowed and a minimum overlap of 20 nucleotides. Fungal ASVs were classified against the UNITE v7.2 database [37] using the Bayesian classifier as implemented in the mothur classify.seqs command, with a cut-off of 60 nucleotides. The ASV method is used to infer the biological sequences in the sample, as described previously [38]. Rare ASVs (singletons) and chimeras, which potentially represent artificial sequences, were removed. The fungal ecological function of each ASV was determined using FUNGuild as described in our previous study [21].

Statistical Analysis

The data analysis was performed using PAST (version 2.17c) [39]. The disease incidence and plant development and biomass (shoot and root dry weight) were analyzed with a Jarque–Bera test and Shapiro-Wilk W test to determine the distribution of data. A one-way analyses of variance (ANOVA) incorporating the Tukey´s pairwise comparisons test was performed. The data were log-transformed when necessary. When Levene's test for homogeneity of variance based on medians was significant either based on non-transformed or log-transformed datasets, the data was analyzed with a Kruskal–Wallis test and followed by a Mann–Whitney pairwise comparison test.

Results

Plant Growth Parameters

At low PBSA concentration, all treatments did not significantly affect plant biomass parameters including both shoot dry weight and root dry weight (Fig. 1). On the other hand, at high PBSA concentrations we detected significant differences in the plant biomass parameters for some treatments. Specifically, shoot dry weight was highest in the SSPN treatment (0.080 ± 0.005 g, mean ± SE) and lowest in the SN treatment (0.044 ± 0.004 g, mean ± SE) (Fig. 1b). Root dry weight was also lowest in the SN (0.008 ± 0.002 g, mean ± SE) but highest in the SPN treatments (0.034 ± 0.011 g, mean ± SE) (Fig. 1d). In the sterile soil and water control treatments, the effects of PBSA

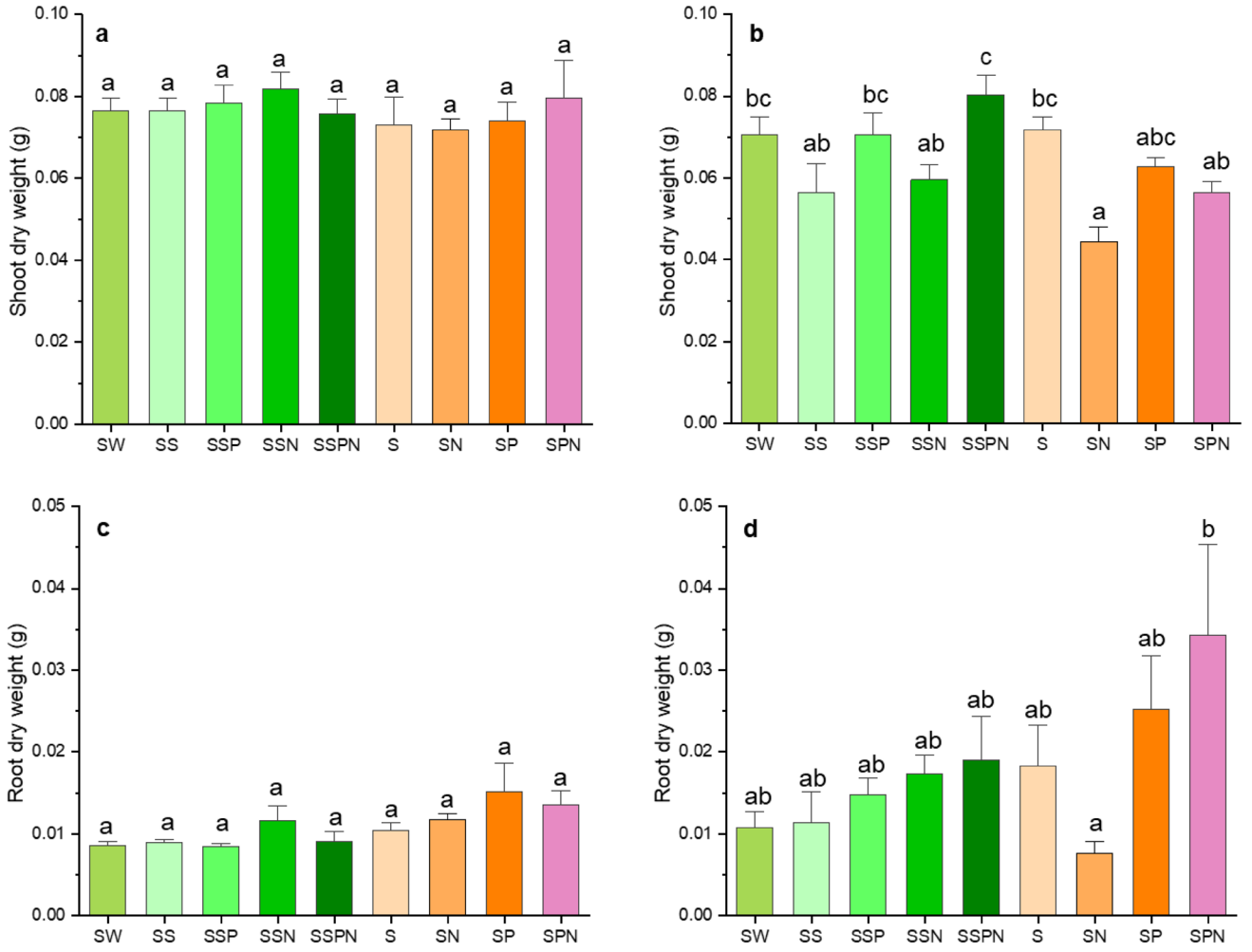

Fig. 1 Shoot and root biomass weight after nine treatments (mean ± SE, $n = 5$) with high (**a**, **c**) or low (**b**, **d**) soil dilution concentration. Different letters above the bars indicate significant differences ($P < 0.05$) according to one-way analysis of variance (ANOVA) or Kruskal–Wallis test. Abbreviations are SW, sterile water; SS, sterile soil; SSP, sterile soil and PBSA amendment; SSN, sterile soil and N fertilizer; SSPN, sterile soil with PBSA amendment and N fertilizer; S, soil; SN, soil and N fertilizer; SP, soil and PBSA amendment; SPN, soil with PBSA amendment and N fertilizer

addition, N fertilization as well as their combined effect on both shoot and root dry weights were almost negligible (Fig. 1). The exception was found for the combined effect of PBSA and N fertilization for the sterile soil (SSPN) treatment (0.080 ± 0.005 g, mean ± SE) which showed higher shoot dry weight as compared with SSN (0.060 ± 0.004 g, mean ± SE)and SS (0.056 ± 0.007 g, mean ± SE) treatments. In contrast, we detected a significant negative effect of N fertilization on shoot and root dry weight in the non-sterile soil treatment (SN).

F. solani Disease Incidence

Either amendment of PBSA (SSP and SP) or N fertilizer (SSN and SN) alone did not increase *F. solani* disease incidence in both experiments using high or low soil dilution concentrations (Table 1). In contrast, treatment with PBSA amendment and N fertilizer in non-sterile soil (SPN) strongly increased the *F. solani* disease incident. At the high soil dilution concentration, the *F. solani* disease incident was 67.5% while at the low dilution concentration disease incident reached 92.5% (Table 1; Fig. 2, S1). These results were confirmed by specific *F. solani* PCR (Fig. S1).

The independent replication of the experiments showed similar *F. solani* disease incident of 83.3% and 87.5% from low or high dilutions in the soil suspension, respectively (Supplementary Material Table S2). Therefore, a higher concentration of PBSA combined with N fertilizer strongly negatively affected plant development. Nevertheless, also other fungal pathogens infected the mung bean seedlings of the non-sterile treatments (S, SN, SP, and SPN; Table 2

Table 1 *F. solani* disease incident in percent after nine treatments (mean ± SE, $n = 5$) with low or high soil dilution concentration

Soil dilution concentration	SW	SS	SSP	SSN	SSPN	S	SN	SP	SPN	*P* value
High	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	67.5 ± 6.4b	$P < 0.001$
Low	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	92.5 ± 3.1b	$P < 0.001$

Different letters indicate significant differences ($P < 0.05$) according to Kruskal–Wallis test. Abbreviations can be found in legend of Fig. 1

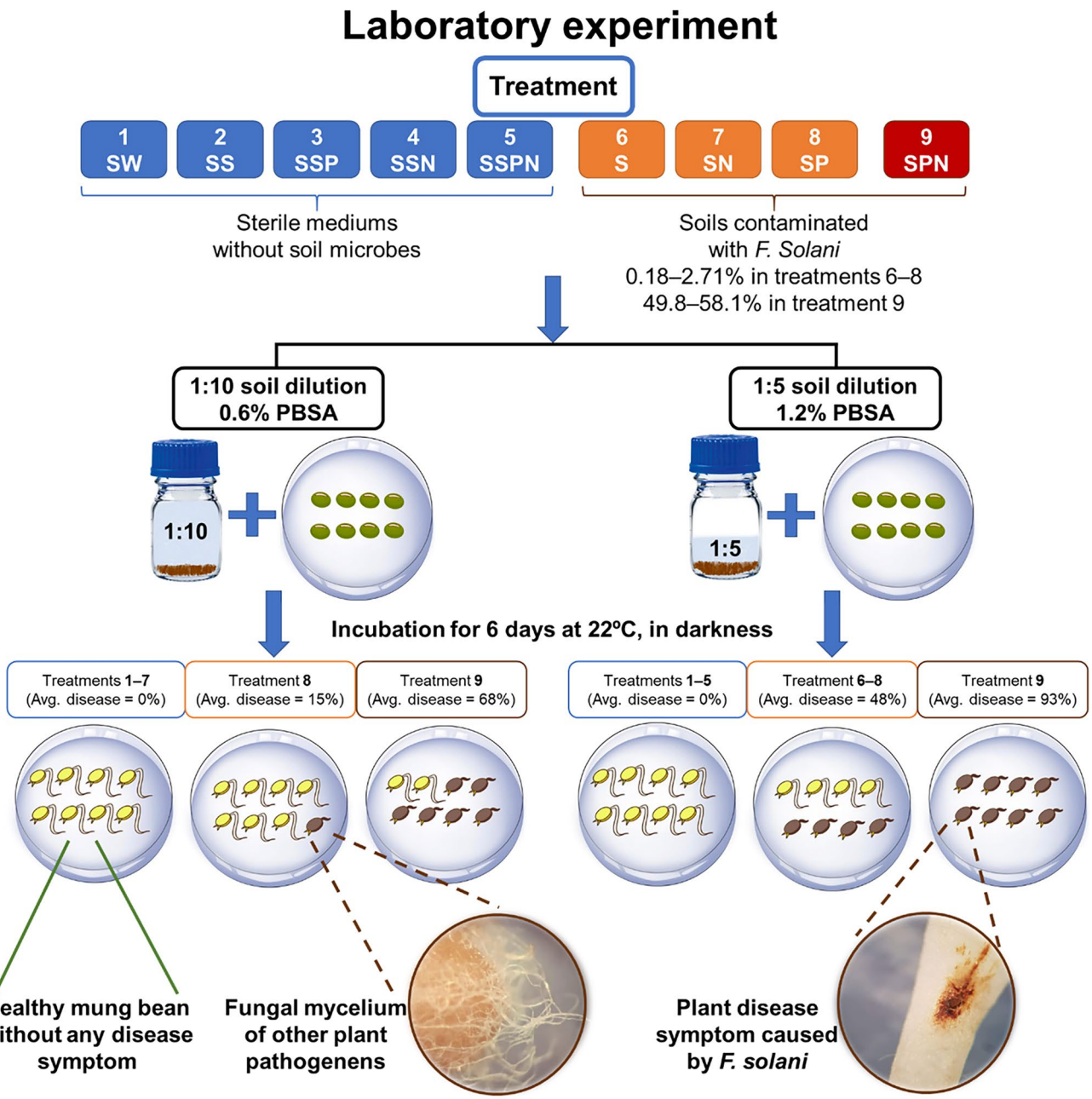

Fig. 2 Microscopic and PCR results of *F. solani* disease incidence after nine treatments and its respective *F. solani* frequency of each seedling and treatment. Abbreviations are *SW* sterile water; *SS* sterile soil; *SSP* sterile soil and PBSA amendment; *SSN* sterile soil and N fertilizer; *SSPN* sterile soil with PBSA amendment and N fertilizer; *S* soil; *SN* soil and N fertilizer; *SP* soil and PBSA amendment; *SPN* soil with PBSA amendment and N fertilizer. Two soil dilution levels were used: 1:10 (high dilution, low soil or soil/PBSA concentration) and 1:5 (low dilution, high soil or soil/PBSA concentration) ($n = 5$)

Table 2 Overall disease incident in percent after nine treatments (mean ± SE, $n = 5$) with high or low soil dilution concentrations

Soil dilution concentration	SW	SS	SSP	SSN	SSPN	S	SN	SP	SPN	*P* value
High	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	15 ± 7.3a	67.5 ± 6.4b	$P < 0.001$
Low	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	45 ± 22.6bc	80 ± 20bc	17.5 ± 9.4ab	92.5 ± 3.1c	$P < 0.001$

Different letters indicate significant differences ($P < 0.05$) according to Kruskal–Wallis test. Abbreviations can be found in legend of Fig. 1

and Supplementary Material Table S3). Especially in SN treatment, we found very high disease incident rate of other fungi (80%), which caused strong reductions of both shoot and root weight (Table 2; Fig. 2). Among the non-sterile soil treatments, PBSA amendment treatment (SP) resulted in the lowest overall disease incidents, which was not significantly different from control non-sterile soil (S) as well as all the sterile water and soil treatments (SW, SS). Both SW and SS treatments were completely without any disease incidents of *V. radiata* seedlings. In addition, *F. solani* was not detected in any seedling from these treatments. In contrast, majority of infections in non-sterile soil treatments with PBSA and N fertilizer (SPN) were caused by *F. solani* (Tables 1 and 2; Fig. 2). We summarized the experimental setup, microscopic results of healthy and infected plants (*F. solani vs*. other fungi) and the overall results in Fig. 2.

Illumina amplicon sequencing of 1 year-soil and mung bean plants in SPN treatment supported these findings as *F. solani* was the most dominant plant pathogen (Table 3 and Supplementary Material Table S4). Top-20 fungal ASVs that had the highest average relative abundances in 1 year-soil and *V. radiata* in SPN treatment are presented in Table 3. The overall relative abundance of *F. solani* was approximately 33% in the soil, while it was highly enriched

Table 3 Top-20 fungal ASVs that had the highest average relative abundances in 1 year-soil and mung bean plants (*V. radiata)* in SPN (soil, PBSA and N fertilizer) treatment, which contributed for 94.9 and 99.7% of the total detected sequences from SPN derived from soil and seedling, respectively

ASV	SPN soil	SPN seedling	Class	Family	Species	Functional group
ASV_000005	6.95	78.41	Sordariomycetes	Nectriaceae	*Fusarium solani*	Plant Pathogen
ASV_000001	25.31	5.46	Sordariomycetes	Nectriaceae	*Fusarium solani*	Plant Pathogen
ASV_000002	16.23	0.00	Eurotiomycetes	Herpotrichiellaceae	*Exophiala equina*	Animal Pathogen-Saprotroph
ASV_000012	14.76	0.84	Leotiomycetes	Pseudeurotiaceae	–	Saprotroph
ASV_000058	6.81	0.03	Leotiomycetes	Myxotrichaceae	*Oidiodendron echinulatum*	Ericoid Mycorrhizal
ASV_000027	0.00	11.31	Sordariomycetes	Nectriaceae	*Fusarium solani*	Plant Pathogen
ASV_000065	5.87	0.00	Leotiomycetes	Myxotrichaceae	*Oidiodendron echinulatum*	Ericoid Mycorrhizal
ASV_000024	3.73	0.00	Sordariomycetes	Nectriaceae	*Fusicolla aquaeductuum*	Mycoparasite
ASV_000068	2.53	0.00	Leotiomycetes	Pseudeurotiaceae	–	Saprotroph
ASV_000032	0.29	3.59	Sordariomycetes	Nectriaceae	*Fusarium* sp.	Plant Pathogen
ASV_000083	2.28	0.01	Leotiomycetes	Pseudeurotiaceae	–	Saprotroph
ASV_000132	1.94	0.04	Sordariomycetes	Ophiocordycipitaceae	*Purpureocillium lilacinum*	Animal parasite
ASV_000029	1.94	0.00	Eurotiomycetes	Herpotrichiellaceae	*Exophiala* sp.	Animal Pathogen-Saprotroph
ASV_000098	1.50	0.00	Leotiomycetes	Pseudeurotiaceae	–	Saprotroph
ASV_000166	0.96	0.00	Leotiomycetes	Myxotrichaceae	*Oidiodendron truncatum*	Ericoid Mycorrhizal
ASV_000009	0.90	0.00	Sordariomycetes	Chaetomiaceae	*Chaetomium* sp.	Saprotroph-Endophyte-Plant pathogen
ASV_000007	0.87	0.00	Sordariomycetes	Nectriaceae	*Ilyonectria macrodidyma*	Plant Pathogen
ASV_000195	0.75	0.00	Leotiomycetes	–	–	–
ASV_000037	0.71	0.00	Sordariomycetes	Microascaceae	*Pseudallescheria boydii*	Saprotroph
ASV_000214	0.55	0.00	Leotiomycetes	Pseudeurotiaceae	–	Saprotroph

Taxonomic information of all detected fungi can be found Supplementary Table S4

(95% of total sequences) in the seedlings growing in the SPN treatment (Table 3 and Supplementary Material Table S4). Apart *F. solani*, another plant pathogen (*Fusarium oxysporum*) also colonized seedling with low relative abundances (Supplementary Material Table S4).

Discussion

PBSA Alone Does Not Significantly Negatively Impact on Plant Growth and Disease Incidence

Based on a limited number of studies, low concentration of PBSA based plastics have no adverse effects on soil microorganisms (bacteria and fungi) and soil ecosystem functions (including nitrification potential and soil esterase activity) [15, 40]. These results are consistent with those from studies on other bio-based and biodegradable plastics (e.g., PBS and PBAT), where the incorporation of such plastics into soil is generally not harmful to biological soil health, microbial diversity, and soil ecosystem functions, such as nitrification potential, nutrient cycling, and fertility [41, 42]. Nevertheless, a recent study based on high resolution molecular techniques showed that high load of PBSA (6%) caused a significantly decline of archeal, bacterial and fungal richness [21]. The bacterial and fungal community composition also significantly changed after the addition of such high load of PBSA. In addition, another recent study based on high resolution molecular techniques revealed that rhizospheric soil bacterial community composition and their volatile profiles associated with wheat plants were significantly altered by microplastics of starch-based biodegradable plastic mulching film [24].

In this study, we showed that PBSA alone does not significantly negatively impact on plant biomass and *F. solani* disease incidence, even at high PBSA concentrations. Our results on plant biomass are in line with other studies, which focused on similar biotest model ecotoxicity of 1% concentration bio-based and biodegradable plastics degraded under controlled conditions for 6 months on growth of plants (the monocotyledon *Sorghum saccharatum* and the dicotyledon *Lepidium sativum*) [22]. However, these findings were in contradiction to our findings as amendment of 1% low-density PE and a starch-based biodegradable plastic mulching film in soil caused negative effects on wheat growth [23]. Therefore, negative effects of bio-based and biodegradable plastics on plant development may be related to many factors such as types and concentrations of bio-based and biodegradable plastics, plant species and other environmental conditions such as the application of N fertilizer [21, 27]. It is important to examine the impact of bio-based and biodegradable plastics on plant development from seedlings to maturation stages as well as on soil microbial communities. Bandopadhyay et al. (2020) showed that amendment of biodegradable and bio-based plastics had no significant effect on the soil microbial community composition [43]. Moreover, such microbial compositions were rather mostly affected by microclimate, seasonal and local parameters, which may govern the microbial community composition and hide other effects [43]. Soil health and shifts in microbial community composition may influence plant performance including plant growth, plant mass and plant health [44, 45]. Still, we know very little on bio-based and biodegradable plastic microbiome [20] and their relationships between the degradation processes and its impacts on plant development.

Combination of Amendement of High PBSA Concentration and N Fertilizer Causes Significantly Negative Effect on Plant Health

Some studies have found that bio-based and biodegradable plastics enhanced soil enzyme activity and microbial biomass [41, 46]. High soil quality is linked to higher microbial biomass and enzyme activities compared to those with lower ones [47, 48]. However, increase of microbial biomass and enzyme activity may not necessarily result into a benefit for soil systems or plant development. For example, if the increased microbial biomass and enzyme acticvities are associated with fungal plant pathogens. Therefore, an effect of plastic derivatives on microbial functional traits requires careful interpretation considering its consequences for soil ecosystem services and plant development.

In our previous study we showed that after amendment of high concentration of PBSA in combination with N fertilizer, the fungal plant pathogen *F. solani* was enriched in soil [21], here we show that such enrichment can affect the health of seedlings. Although the shoot and root biomass of seedlings was not significantly impacted by the SPN treatment, the seedlings were heavily infected with *F. solani*. Such tremendous infections during the developmental stage make the seedlings less competitive and their survival less likely [49, 50]. In view of the dramatic effects observed after high PBSA load and N fertilisation in our petri dish test system with soil water suspension, one can hypothesize that if the experiment would be conducted in the pot experiment or in the field for longer time, the biomass of *V. radiata* in the SPN treatments would be greatly reduced or totally loss.

It has been reported that *F. solani* can colonize various types of biodegradable plastics, especially PBS and its derivatives (PBSA) [26, 51]. Due to their degradability in field soil conditions, PBS and PBSA have been used as mulching films. Meanwhile, farmers may leave such mulching films in the field to reduce the time and labor cost, thus these plastic can

highly accumulate in soil [18, 19, 21]. The PBSA films are described to fragment into smaller pieces (macro plastics), particles (micro-and nano-plastics) or molecules (metabolites and CO_2) [52]. In combination with high load of N fertilizer in the field, *F. solani* can be enriched in both soil and PBSA, which subsequently can increase the disease incident of plant roots and shoots. The usage of high doses of N fertilizer can enrich soil N, which will be taken up by both plant and microbes. It has been shown in our previous study that the same kind of N fertilizer supported *F. solani* to outcompete other fungal PBSA colonizers and become the most dominant fungi in PBSA-soil system [21]. This implies that in conventional agriculture, which involves PBSA based mulching films and high load N fertilizer applications may lead to significant enrichment of *F. solani* in the soil. To prevent such scenario, we suggest ideally to remove the biodegradable mulching films from the field for recycling [21].

Although we successfully demonstrate the clear negative effect of the combination of PBSA load and N fertilizer on plant heath, in this treatment we co-manipulated two factors, the plastic addition and the *F. solani* propagule concentration. Thus, what we measured, is the effect of these two factors, and we cannot distinguish whether the effect was due to one factor or the other, or even to the sum of their effects. When only one factor was applied (N fertilization or PBSA addition), we did not detect the negative effect. Clearly, there is a confounding factor (and/or their interactions) and we cannot conclude whether the effect were due to the PBSA load or the *F. solani* propagule quantity. In this regard, our control with sterilized soil helps a little, as it suppressed the propagules, however to fully distinguish the effects of the plastic from the one of the inoculated propagules, we should add an another treatment inoculating a known propagule quantity after the sterilization.

Future Study

To avoid potentially negative effects of different types of bio-based and biodegradable plastics on plant development, caused by fungal plant pathogens, it is urgently needed to examine shifts in the soil microbiome during degradation process of bio-based and biodegradable plastics using high-resolution molecular approaches. In addition, the ecotoxicity effects of different types of bio-based and biodegradable plastics on plant developments deserve also more attention by testing against different model plants as well as other economic plants. The effect of different concentrations and types of fertilizers should be tested in presence of PBSA to evaluate its impact on seedling development. Moreover, future studies should also focus on evaluating the effect of bio-based and biodegradable plastics on the long-term effects on soil microbiome, plant development and fitness under various field and weather conditions.

Conclusions

In this work, we raised the concern of the interaction between high load of bio-based and biodegradable plastics (PBSA) and N fertilizer to plant development. Our study suggests that PBSA alone in the soil environment do not significantly negatively impact disease incidence and plant development. However, when N fertilizer and PBSA are simultaneously present in soil, plant pathogen *F. solani* is enriched and thereby plant development was weakened. Thus, it is essential to plan and manage the use of bio-based and biodegradable plastics as mulching films after the agricultural uses.

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Author Contributions SS, KJ, BT, MN and WP conceived and designed the study. SS, KJ, BT and WP led the laboratory experimental set-up. WP, FB and MN contributed reagents and laboratory equipment. BT, SS, KJ and WP led the DNA analysis. SW led bioinformatics. WP and SS led the microbial taxonomy and data analyses. SS, KJ, BT and WP wrote the manuscript. MN and WP supervised SS, KJ, BT. MN, SW and FB reviewed and gave comments and suggestions for manuscript. All of the authors gave final approval for manuscript submission.

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Declarations

Conflict of interest The authors declare that they have no competing interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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