CABI Agriculture and Bioscience

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Mind the blind spot: lessons from fungal community sequencing in a plant–soil feedback experiment

Mengshuai Liu[1,2], Jose G. Maciá-Vicente[2], Jasper van Ruijven[2], Wopke van der Werf[3], Zhenling Cui[1], Fusuo Zhang[1], Chunxu Song[1*] and Liesje Mommer[2]

Abstract

Background Plant–soil feedback (PSF) has gained increasing interest in agricultural systems. An important question is whether PSF differs between different cropping systems. Few attempts have yet been made to identify the pathogen species involved in negative PSF. Here, we hypothesize that the strength of negative PSF experienced by a crop species is determined by the relative abundance of host-specific soil-borne pathogenic fungi, that is in turn driven by the crop's relative abundance (in time).

Methods We performed a PSF experiment, with different soils originating from three cropping systems in the North China Plain and three crop species (wheat, maize, soybean) in a full factorial design. Soil fungal community composition and relative abundance of fungal (pathogen) species in each treatment was identified by metabarcoding using ITS (Internal Transcribed Spacer) sequencing.

Results PSF ranged from negative for wheat, neutral to negative for soybean and neutral to positive for maize, but the former density of a crop in a particular cropping system did not affect the strength of PSF experienced by each of the three. No relationships between fungal pathogen abundance and PSF were found, but we did find a surprisingly large enrichment across steps of the experiment of *Chaetomium* spp., a known cellulose-degrading fungus. This may be explained by addition of filter paper on the bottom of the pots.

Conclusions Our results suggest that the strength of PSF in these crops is not related to the relative abundance of specific fungal pathogens. However, we cannot rule out that our results were affected by the high abundance of one particular cellulose-degrading fungus. This highlights both the need to stop the practice of using filter paper in pot experiments, as well as the relevance of assessing the identity, relative abundance and potential functions of fungal taxa in PSF experiments.

Keywords Plant–soil feedback, Cropping system, Fungal community, Soil-borne pathogen

*Correspondence:
Chunxu Song
chunxu.song@cau.edu.cn
Full list of author information is available at the end of the article

Background

Plant–soil feedback (PSF) is the effect plants have on neighboring, newly established plants by altering the soil (a)biotic properties in their surroundings (Bever et al. 2012; van der Putten et al. 2013; Lekberg et al. 2018; Thakur et al. 2021). A large body of PSF research has shown that plant species influence the growth of microbes in their rhizosphere, including both pathogens (Raaijmakers et al. 2009; Philippot et al. 2013; Semchenko et al. 2022) and mutualists (Revillini et al. 2016). These microbes can in turn lead to positive or negative effects on plant growth (Lekberg et al. 2018; Reinhart et al. 2021; Semchenko et al. 2022).

Most work on PSF has been done in ecosystems with natural plant species (Kulmatiski et al. 2008; Mangan et al. 2010), but recent studies have also applied the PSF concept to agricultural systems (Mariotte et al. 2018) where negative feedbacks may be major constraints of crop production (Wei et al. 2018; Luo et al. 2019; Yang et al. 2019; Wang et al. 2021). For example, wheat, faba bean and maize have shown impaired productivity when grown on soils that previously hosted the same crops, as compared to soils conditioned with other species (Wang et al. 2017; Wang et al. 2019). Negative PSF is often considered the reason behind crop rotation in agriculture, as increasing crop diversity in time can prevent the accumulation of pathogens specific for a given crop species (Kirkegaard et al. 2008; Larkin 2015; Smith et al. 2015; Gong et al. 2021). However, despite the importance of PSF, the identity and host-specificity of soil and root-associated fungi potentially driving it have rarely been assessed in PSF experiments, in both natural and agricultural systems (van Ruijven et al. 2020; Semchenko et al. 2022).

In the present study, we use different crop rotation systems in the North China Plain (NCP) as a case study to investigate the role of soil-borne fungi in PSF in an agricultural setting. NCP is one of the most important regions for cereal production in China, accounting for 73% and 32% of all wheat and maize production, respectively (National Bureau of Statistics of China 2021). There, maize is produced either intensively in a continuous cropping system, or in rotation with wheat (wheat–maize rotation) to yield one harvest of each crop per year (Xu et al., unpublished data). A third system common in NCP includes rotation of maize, wheat and soybean, yielding one harvest of each crop every 2 years (National Bureau of Statistics of China 2021). These intensive agricultural practices used in continuous cropping may have contributed to the accumulation of soil-borne pathogens in the area (Sun et al. 2014; Xu et al. 2018; Ye et al. 2020). Continuous availability of a host species has been shown to promote the long-term accumulation of host-specific soil-borne pathogens that reduce biomass growth (Maron et al. 2011; Larkin 2015; Vandermeer 2011). In contrast, crop rotation systems such as the wheat–maize and wheat–maize–soybean combinations are expected to reduce pathogen pressure on the standing crop species by reducing the availability of hosts for crop-specific pathogens, and by interrupting the pathogens' transmission across harvests (Boudreau 2013). Consequently, negative PSF would be strongest in the continuous cropping system and have gradually decreasing effects on the wheat–maize and wheat–maize–soybean rotations, respectively. Despite this assumption, no data are available about the effects these alternative cropping systems have on soil biota and the productivity of each crop (Mariotte et al. 2018).

In this study, we investigated how the three crop rotation practices in use at NCP, involving maize, wheat, and soybean, affect the buildup of crop-specific soil-borne fungal pathogens, and thereby determine the contribution of negative PSF to crop productivity. We used soils from agricultural fields at NCP representing the three cropping systems and evaluated their effects on growth of wheat, maize, and soybean after conditioning with either crop species. In addition, we monitored the build-up of crop-specific fungal pathogens by metabarcoding soil-borne fungal communities in every step of the experiment. We hypothesized that (1) the negative PSF experienced by each crop is related to its former abundance in a particular cropping system (e.g. maize growth would be lowest in soils that previously hosted maize in continuous cropping, and highest in soils from the wheat–maize–soybean rotation system); and that (2) the relative abundance of host-specific, soil-borne fungal pathogens is related to the strength of negative PSF (e.g. crop species exhibiting negative growth effects would host high abundances of crop-specific pathogens).

In contrast to our expectations, we only observed weak PSF effects in our system. Because we monitored the soil-borne fungal communities across all steps of our experiment via high-throughput sequencing, we could attribute our unexpected PSF results to methodological factors that prompted anomalous changes in fungal communities, unlikely to occur in real-case scenarios. Therefore, our study provides a case-study highlighting the importance of assessing the shifts in soil microbial communities alongside changes in plant biomass in PSF studies, to both identify the main microbial agents responsible for PSF and prevent potential 'blind spots' in the interpretation of the results.

Methods

Collection of soil samples

Samples from three agricultural soils were collected from a long-term field experiment at Quzhou experimental station (36.87°N, 115.02°E) in NCP (China) that was established in October 2007 on a clay loam soil (Gao et al. 2014; Meng et al. 2017). The soils originated from fields subject to three different cropping systems (Additional file 1: Fig. S1). A completely randomized design was employed with three treatments and four replicate fields. Each replicate field measured 1800 m^2 (30 × 60 m), and the distance between fields was 2 m. The three different cropping systems used in this experiment were as follows:

1. a maize monoculture with one harvest per year (M0). Maize was grown on the field from the late May to early October each year.
2. a wheat–maize double cropping system with two harvests per year (WM). Maize was grown from early June to early October, and wheat from mid-October to early June each year.
3. a maize–wheat–soybean–fallow system with three harvests in 2 years (WMS). Maize was grown from late May to early October, followed by wheat from early October to early June, and soybean from early June to early October each 2 years. After the soybean harvest, the field was not used (fallow) until the sowing of maize in the next growing season.

We calculated the temporal density of each crop in the 2 years previous to the sampling as the total duration of each crop (in days) divided by the total duration of the period (730 days) for each cropping system (Additional file 1: Table S1). Therefore, for each crop, their temporal densities at the three cropping systems were compared as follows: wheat, M0 < WMS < WM; maize, WMS < WM < M0; and soybean, M0 = WM < WMS.

Soil samples (0–15 cm depth) were collected using shovels (disinfected by 70% Ethanol between sites/fields) from five evenly distributed cores in each replicated field. From each field, 25 kg of soil were collected, pooled together per cropping system and homogenized by sieving the soil using a mesh (2 mm). Four 1 g subsamples from each pooled soil were taken and kept at − 80 °C for sequencing of fungal communities. Soil samples from M0 were collected in May 2020 before the sowing of maize, and the soil samples from WM and WMS were collected in June 2020, after the wheat harvest.

Plant material

The crop species used for the PSF experiment were wheat (*Triticum aestivum*) variety Liangxing 99, maize (*Zea mays* L.) variety Zhengdan 958, and soybean (*Glycine max*) variety Qihuang 34. These cultivars are commonly grown in the NCP, and are the same used in the fields at the Quzhou experimental station. Seeds of the three crop species, obtained from the previous season's inventory at the Quzhou experimental station, were surface sterilized by washing them in 10% (v/v) hydrogen peroxide for 30 min, followed by rinsing with demineralized water, after which they were germinated on moist filter paper at 25 °C for 24 h in the dark. Seeds were selected for uniformity prior to planting.

Plant–soil feedback: experimental procedures and statistics

Experimental set-up

The PSF experiment consisted of two phases, a conditioning phase and a feedback phase (Brinkman et al. 2010). In the conditioning phase, the three crop species were separately used to condition the soils originating from three different cropping systems, resulting in nine soil-crop combinations. In the feedback phase, each species was grown on each of the nine soil-crop combinations (Fig. 1). Each treatment in the conditioning and feedback phase was replicated eight times, although in some cases replication was lower due to missing data (data was not available). Each replicate pot (20 × 17 × 14 cm) contained 3 kg of soil and ten wheat, five maize, or five soybean individuals. The bottom of pots were lined with clean filter paper discs (125 mm diam., grade 1, Product No. NS1001-125; NEWSTAR Ind., China) to prevent soil run off. The pots were randomly placed in the greenhouse at Quzhou experimental station. During the experiment, crops were watered 3–5 times a week with deionized water. Seedlings of wheat, maize and soybean were thinned to eight, four, and three individuals, respectively within the first 15 days.

Conditioning phase

In this experimental phase, 72 pots were prepared, including three crop species × three soil origins × 8 replicates. Four weeks after planting, shoots were clipped at soil level and dried at 70 °C for at least 48 h before weighing. Rhizosphere soil was collected by brushing off the soil adhering to the roots from all plants in each pot, and a one-gram soil subsample was taken from each pot.

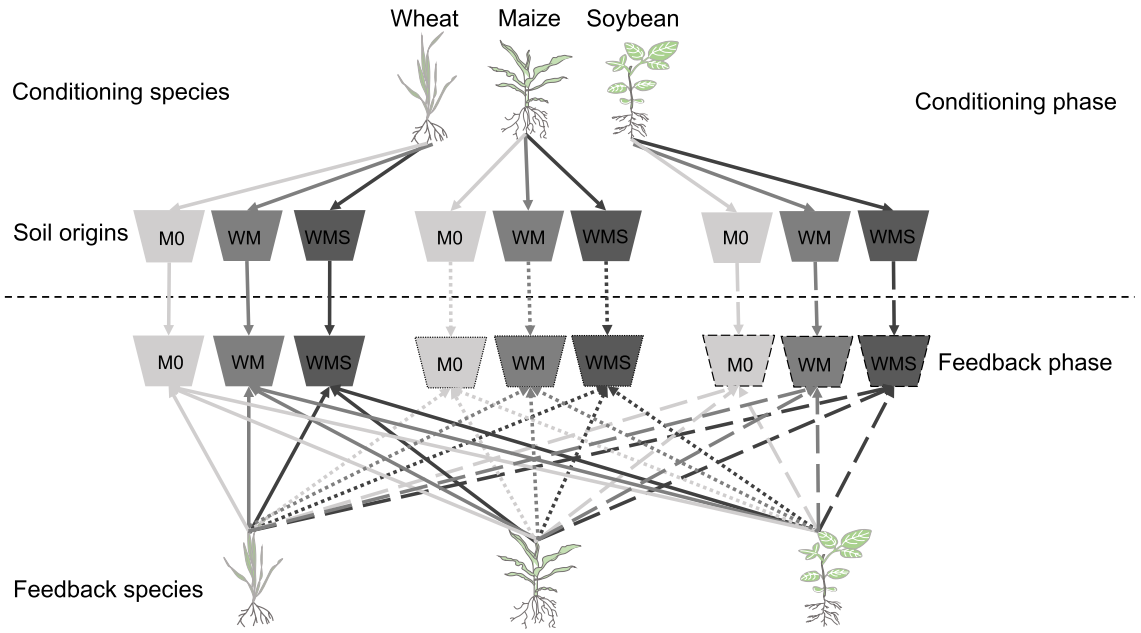

Fig. 1 Design of the PSF experiment. In the conditioning phase, three individual crops (wheat, maize, and soybean) were grown on soil originating from three rotation systems: M0 (only maize system), WM (wheat–maize rotation system), and WMS (wheat–maize–soybean rotation system). In the feedback phase, each species was grown on each of the nine soil-crop combination of the conditioning phase, N = 8

These samples were stored at − 80 °C for fungal community sequencing. The remaining soil from each pot was sieved using a 2 mm sieve, mixed well for each crop × soil treatment. The rest of the soil per pot was stored separately in a cold room (10 °C) for 1 week, until the start of the feedback phase.

Feedback phase

One week after the end of the conditioning phase, the soil of each pot from the conditioning phase was divided into three portions to plant wheat, maize, and soybean respectively. In this case, the soils were used as inoculum by mixing 15%(w/w) of conditioned soil with 85% soil from the corresponding cropping systems that had been sterilized by 25 kGγ at Hebei Nuclear Tongfang Irradiation Technology Co., Ltd., Baoding, China. After 4 weeks of growth, shoots for dry biomass measurements, rhizosphere and bulk soil samples for fungal community sequencing were collected as described above.

Calculations of PSF

PSF values for all crops in the different soil origins were calculated following Brinkman et al. (2010): PSF = ln (home/away). Here, home is the shoot dry biomass of wheat, maize or soybean grown on soil conditioned by conspecifics, and away is the average shoot dry biomass of the same crop species grown on soils conditioned by the other two crop species. Since each crop had two away soils (i.e., soils conditioned by two different other crop species), we calculated PSF effect in two ways. The first one was PSF using the average shoot biomass on the two away soils ($PSF_{average}$). In the second step, we calculated two PSF effects ($PSF_{specific}$): one for each of the two away soils.

Statistical analysis of shoot biomass and PSF

All statistical analyses were performed in R v4.1.2 (R Core Team 2021). Data of shoot dry biomass were natural log-transformed when necessary to meet assumptions of normality. For shoot dry biomass in the conditioning phase, an ANOVA with crop species, soil origin, and their interaction as fixed factors was used. For shoot dry biomass in the feedback phase, we used a three-way ANOVA to analyze the effects of conditioning species, soil origin, crop species and their interactions on shoot dry biomass. In follow-up analyses, we analyzed each crop species separately when interactions with species were significant. Similar models were used for PSF in the feedback phase. $PSF_{average}$ was analyzed using crop species, soil origin and their interaction as fixed factors in ANOVA analysis. To test whether PSF values depended on the identity of the away species used in the calculation, $PSF_{specific}$ was analyzed separately for each crop species using a two-way ANOVA model with soil origin, heterospecific conditioning species, and their interaction as fixed factors. Whenever the effect of a factor with more than two levels

was significant, a Tukey post-hoc test was performed to assess significant differences between levels. To determine the relationship between the PSF (i.e. $PSF_{average}$ and $PSF_{specific}$) experienced by one crop (i.e. wheat, maize and soybean) and their former abundance in a particular cropping systems, linear regression analyses were done for each crop species separately.

Amplicon sequencing of fungal communities

The DNA preparation and fungal amplicon sequencing was performed by Novogene (Tianjin, China). Total genomic DNA from 156 bulk [(3 original soils + 9 conditioning phase + 27 feedback phase) × 4 replicates] and 144 rhizosphere [(9 conditioning phase + 27 feedback phase) × 4 replicates] soil samples was extracted using a CTAB/SDS-based method as described by Healey et al. (2014). DNA concentration was determined on 1% agarose gels, and working aliquots were prepared at l μg μL^{-1} in sterile milliQ water. The ITS1 region of the fungal rDNA was amplified using the fungus-specific primers ITS1F and ITS2 (Gardes and Bruns 1993; White et al. 1990; respectively), modified to include tags for multiplexing in Illumina sequencing reactions. All PCR reactions were carried out in a volume of 15 μL including Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of each of ITS1F and ITS2 primers, and 10 ng of template DNA. Thermal cycling consisted of an initial denaturation at 98 °C for 1 min; followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s; and a final elongation step at 72 °C for 5 min. PCR products from all samples were pooled at equimolar concentrations, and the DNA pool was purified with the Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing was performed using the Illumina NovaSeq platform (Novogene Compay, Tianjin, China) to generate 250 bp paired-end reads.

Processing and analysis of amplicon sequencing data

Sequence reads were processed using the DADA2 pipeline (Callahan et al. 2016) for quality filtering, dereplication, removing chimeric sequences, and grouping of reads into amplicon sequence variants (ASVs) (Callahan et al. 2017). After the dereplication step, paired forward and reverse reads were merged. The code used in this process was performed according to a previously described protocol (Maciá-Vicente et al. 2020). Fungal ASVs were taxonomically annotated by comparing against the UNITE database of fungal ITS sequences (Kõljalg et al. 2005) based on the Naive Bayesian Classifier (Wang et al. 2007) available in MOTHUR v1.39.5 (Schloss et al. 2009). Then, BLASTN v2.2.31 + was used to compare ASVs against NCBI GeneBank records in order to remove non-fungal sequences. ASVs potentially belonging to plant pathogens were identified by collating the ASV taxonomic annotations against the FungalTraits database (Põlme et al. 2020).

Subsequent analyses of sequencing data were done in R, with use of functions within the package vegan v 2.5-7 (Oksanen et al. 2020). First, ASVs represented globally by less than five reads were discarded, and we investigated completeness of sequencing depth per sample using ASV rarefaction curves. We performed in parallel the analyses of fungal community structure for total and pathogenic fungal communities, by calculating Bray–Curtis dissimilarities across samples and normalizing them with the Hellinger transformation (Legendre and Gallagher 2001). We visualized patterns in fungal community variation using a non-metric multidimensional scaling (NMDS) ordination, and used permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) to test the contribution of experimental factors (i.e. soil origin, phase) on fungal community variation. The variation in the relative abundances of the main fungal genera across phases were evaluated using Kruskal–Wallis tests with Bonferroni–Holm adjustment of p values.

Results

Effects of conditioning species and soil origin on shoot biomass

In the conditioning phase of the experiment, shoot dry biomass differed significantly between crop species (Additional file 1: Table S2). For each soil origin, shoot dry biomass of maize was biggest, wheat was lowest, and soybean was intermediate (Additional file 1: Fig. S2). No significant main effect of soil origin was found, but the interaction between crop species and soil origin was significant (Additional file 1: Table S2). When the crop species were analyzed separately, wheat and maize showed no significant differences in biomass across the three soils, while the effect of soil origin on biomass of soybean was marginally significant (Additional file 1: Table S3).

In the feedback phase of the experiment, both crop and the former conditioning species had significant effects on shoot dry biomass. No overall effect of soil origin was found, but a significant three-way interaction was observed (Additional file 1: Table S4). Separate analyses for each crop revealed different effects for each of the crop species (Table 1). Soil origin had a significant effect on shoot dry biomass of wheat (Table 1), which was highest in WMS, lowest in M0, and intermediate in WM soil (Fig. 2a). However, in each soil, wheat grew worse in soil conditioned by wheat than by soybean. Soil conditioned by maize showed intermediate values (Fig. 2a). In contrast, maize showed a significant interaction between soil

Table 1 ANOVA results of the effects of soil origin, conditioning species, and their interactions on shoot dry biomass for each crop in the feedback phase

Factor	*df*	Wheat		Maize		Soybean	
		F	*p*	F	*p*	F	*p*
Soil origin (S)	2	25.771	**< 0.001**	0.9932	0.376	2.851	0.0655
Conditioning species (D)	2	9.318	**< 0.001**	21.1012	**< 0.001**	15.383	**< 0.001**
S × D	4	1.331	0.269	8.0811	**< 0.001**	1.011	0.4087

Significant effects ($p < 0.05$) showed in bold

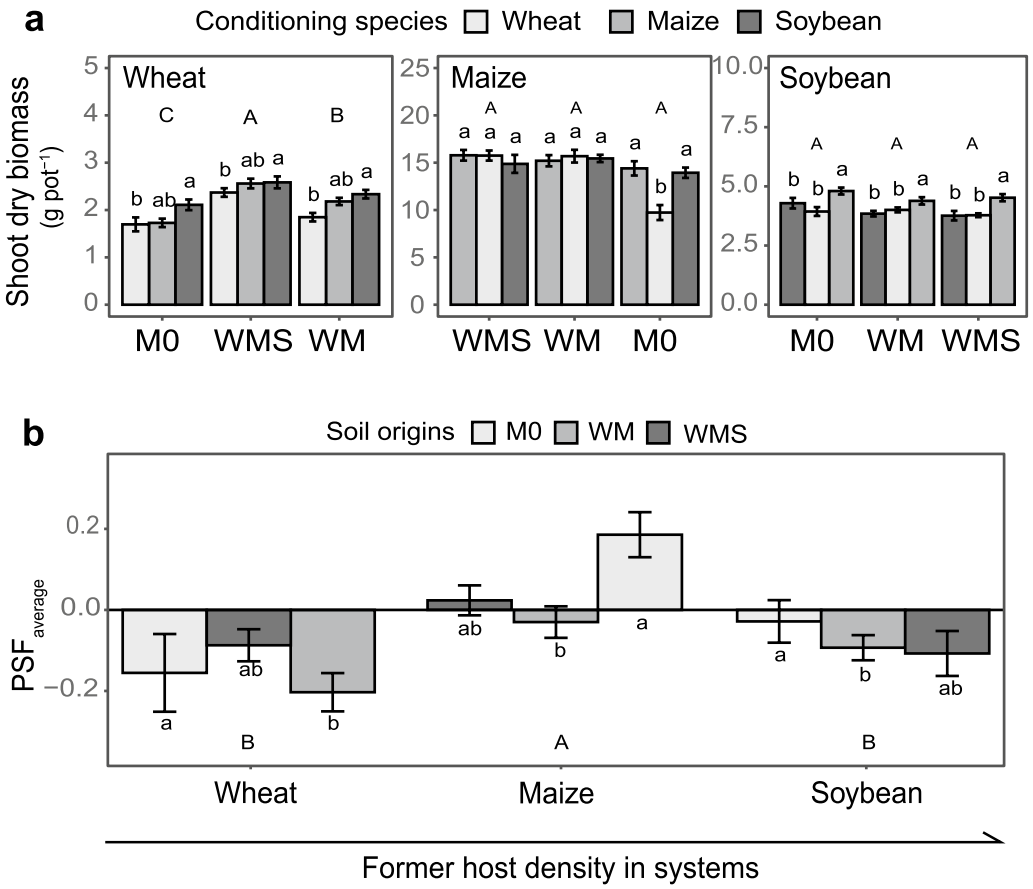

Fig. 2 Shoot dry biomass in the feedback phase (**a**) and (**b**) plant–soil feedback ($PSF_{average}$) for each crop on each soil origin. In both, soil origins from left to right are ranked by increasing former host density on each soil. In **a**, different capital letters indicate significant differences between soil origins, and different small letters indicate significant differences between conditioning species. In **b**, different capital letters indicate significant differences between crop species and different small letters indicate significant differences between soil origins. Bars show means ± SE, N = 6–8 (see "Methods" section)

origin and conditioning species (Table 1). Maize grew worse in soil conditioned by wheat, but only in M0 soil (Fig. 2a, Additional file 1: Table S5). Finally, soybean was only affected by the conditioning species (Table 1). It grew better in soils conditioned by maize than in soils conditioned by the other two species, irrespective of soil origin (Fig. 2a).

Table 2 ANOVA results of the effects of crop species, soil origin, and their interaction on $PSF_{average}$ based on shoot dry biomass

Factor	*df*	F	*p*
Crop species (C)	2	12.341	**< 0.001**
Soil origin (S)	2	3.810	**0.028**
C × S	4	1.627	0.179

Significant effects ($p < 0.05$) showed in bold

Strength and direction of PSF across soil origin

Plant–soil feedback based on the average of the two 'away' soils ($PSF_{average}$) was significantly affected by crop species and soil origin, but not by their interaction (Table 2). Wheat and soybean showed significantly stronger negative feedback than maize in each soil (Fig. 2b). Across crop species, $PSF_{average}$ was most negative in WM soils and least negative in M0 soils. WMS soils showed intermediate values (Fig. 2b).

For wheat, PSF was independent of the identity of the heterospecific conditioning species: $PSF_{specific}$ was not affected by conditioning species or soil origin (Additional file 1: Table S6). However, for maize, a significant interaction between soil origin and conditioning species on PSF was observed (Additional file 1: Table S6). The positive $PSF_{average}$ of maize in M0 soil (see Fig. 2b) was particularly strong when soil was conditioned by wheat, but close to zero for soybean-conditioned soil (Additional file 1: Fig. S3). For the remaining two soils, no effect of conditioning species on the PSF experienced by maize was found. For soybean, the effect of conditioning species was significant (Additional file 1: Table S6). $PSF_{specific}$ was neutral in wheat-conditioned soils, but negative in maize-conditioned soils (Additional file 1: Fig. S3).

No significant effect of the former density in a particular cropping system was apparent for $PSF_{average}$ experienced by wheat, maize and soybean (Fig. 2b, Additional file 1: Table S7). Moreover, when we analyzed $PSF_{specific}$ experienced by each crop as a function of their former density in a particular cropping system, we did not find any significant effects for wheat, maize and soybean (Additional file 1: Fig. S3, Additional file 1: Table S7).

Soil-borne fungi across the phases in the PSF experiment

Illumina sequencing of 300 bulk soil and rhizosphere samples taken from the PSF experiment yielded 26,793,444 sequence reads, representing 4003 fungal ASVs after quality filtering. Visualization of changes in fungal community structure across samples using an NMDS ordination showed that bulk soil fungal

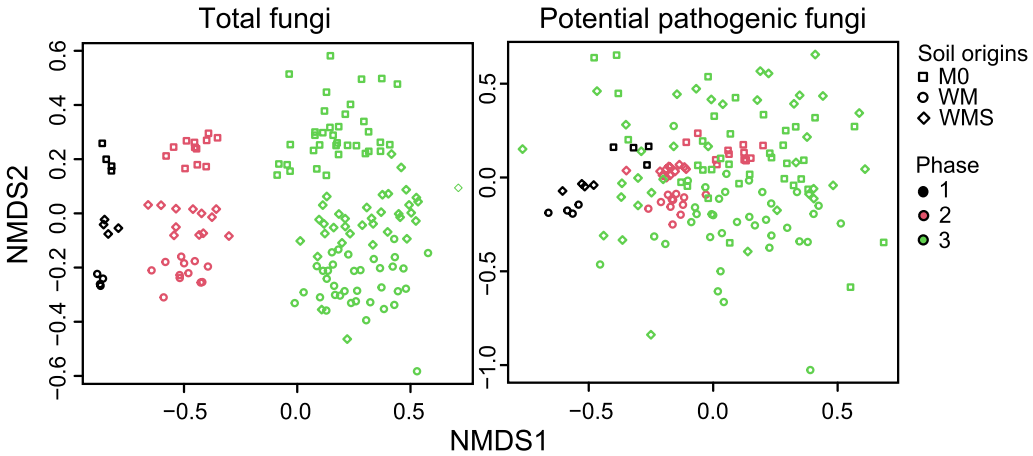

Fig. 3 Total fungal and potential pathogenic fungal community structure in the PSF experiment. Nonmetric multidimensional scaling (NMDS) ordinations show the association of the total fungal (stress = 0.12) and potential pathogenic fungal (stress = 0.24) assemblages of bulk soil samples in three soil origins across the phases in the PSF experiment. Samples from different soil origins are represented by the following symbols: squares, M0; circles, WM; diamonds, WMS. The black, red, and green symbols represent samples from phase 1, 2, and 3, respectively. Abbreviations: M0, only maize system; WM, wheat–maize rotation system; WMS, wheat–maize–soybean rotation system; 1, phase 1 (original soils); 2, phase 2 (conditioning phase); 3, phase 3 (feedback phase)

Table 3 PERMANOVA results of the effects of soil origin, phase, and their interactions on total and potential pathogenic fungal community variation from the bulk soils

Factor	*df*	Total fungi			Potential pathogenic fungi		
		F	R^2	*p*	F	R^2	*p*
Soil origin (S)	1	13.154	0.05587	**< 0.001**	10.2922	0.05680	**< 0.001**
Phase (P)	1	66.040	0.28052	**< 0.001**	17.7599	0.09801	**< 0.001**
S × P	1	4.230	0.01797	**< 0.001**	1.1521	0.00636	0.304

Significant effects ($p < 0.05$) showed in bold

assemblages varied similarly with the phases of the PSF experiment (Fig. 3). The PERMANOVA analysis showed that the experimental phase was indeed the strongest predictor of fungal community variation, explaining 28.1% of total variation (Table 3). In contrast, soil origin explained only 5.6% in this community (Table 3).

When we focused on the changes of the potential pathogenic fungal community in bulk soil, the largest variation (9.8%) was found across experimental phases (Table 3), but the separation of assemblages between conditioning (phase 2) and feedback phase (phase 3) was not clear (Fig. 3). Similarly, the changes in total and potential pathogenic fungal communities in the rhizosphere were consistent with those found in bulk soil (Additional file 1: Table S8, Additional file 1: Fig. S4). PERMANOVA analysis showed that the experimental phases again explained the most variation in the rhizosphere total and potential pathogenic fungal communities (Additional file 1: Table S8).

In terms of community composition, most fungal ASVs belonged to the Ascomycota (63.8%), followed by the Basidiomycota (11.9%) and the Glomeromycota (9.3%) (Additional file 2: Table S9). Of these, 703 ASVs were potentially assigned a plant pathogenic lifestyle based on comparisons with the FungalTraits database (Additional file 2: Table S10). However, when we attempted to monitor the changes in relative abundances of potential plant pathogens across treatments, we found that these were strongly reduced along experimental phases as a result of a gradual and steep increase of ASVs classified in genus *Chaetomium* (Fig. 4). In all cases, including different soil origins and crop species, fungal communities in bulk soil showed a progressive shift from high evenness (i.e. multiple taxa with similar relative abundances) in the original soils, to a strong dominance by *Chaetomium* ASVs in the feedback phase (Fig. 4), ranging from 55.5 to 94.3% of all reads (Additional file 2: Tables S11, S12, S13). The increase in the relative abundance of *Chaetomium* ASVs across phases was significant (Additional file 2: Tables S11, S12, S13, Fig. 4 and Additional file 1: Fig. S5), and contrasted with a significant decrease of pathogenic fungi in the three crop species, such as *Bipolaris*, *Fusarium*, *Macrophomina*, and *Gaeumannomyces* ASVs (Additional file 2: Tables S11, S12, S13, Additional file 1: Figs. S6, S7, S8, S9). The patterns in the rhizosphere fungal communities (phases 2 and 3 only) mirrored those in bulk soil, with a significant enrichment of genus *Chaetomium* in the feedback phase (Additional file 2: Tables S14, S15, S16, Additional file 1: Figs. S5 and S10) accompanied by a decrease in the relative abundances of ASVs identified as *Bipolaris*, *Fusarium*, *Macrophomina*, and *Gaeumannomyces* ASVs (Additional file 2: Tables S14, S15, S16, Additional file 1: Figs. S6, S7, S8, S9).

Discussion

We found weak negative PSF effects among the three crop species tested. Moreover, contrary to our hypothesis, the strength of PSF effects on each crop did not relate to the previous density of the same crop in the cropping systems. Our approach to characterize by amplicon

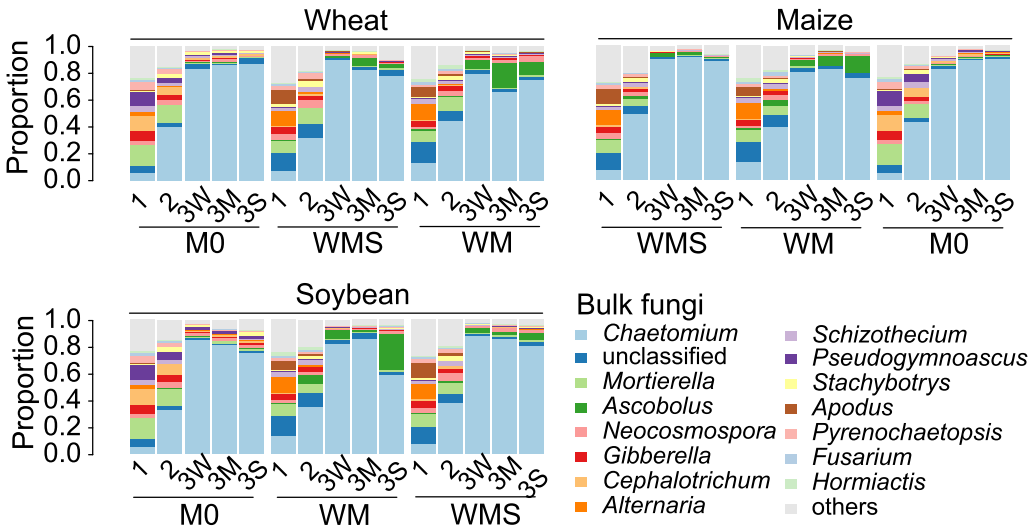

Fig. 4 Taxonomic structure of bulk soil fungal communities across the phases in the PSF experiment. The bar plots show the relative proportion of abundances numbers for the top 15 fungal genera in three soil origins across the phases of the PSF experiment. The soil origins from left to right are ranked by increased host density of the former system. Abbreviations: M0, only maize system; WM, wheat–maize rotation system; WMS, wheat–maize–soybean rotation system; 1, phase 1 (original soils); 2, phase 2 (conditioning phase); 3W, wheat-conditioned in the phase 3 (feedback phase); 3 M, maize-conditioned in the phase 3 (feedback phase); 3S, soybean-conditioned in the phase 3 (feedback phase)

sequencing the bulk soil and rhizosphere-associated fungal communities throughout the experiment suggests that the weak PSF effects resulted from an artifactual buildup of specific saprotrophic fungal taxa in the soil pots, rather than from specific pathogenic effects of the crop species. Our results thus highlight the importance of monitoring microbial community changes in PSF experiments to interpret the results.

Only a few experimental studies have tested PSF effects on the growth of the three crop species used in our study (Hol et al. 2013; Wang et al. 2017; Wang et al. 2019; Kuerban et al. 2022). Each of these showed strong PSF effects, which contrasts with our results. For instance, Kuerban et al. (2022) observed significantly positive PSF effects on wheat and negative PSF on maize and soybean, while Wang et al. (2019) also reported negative PSF effects on the growth of maize. In line with our second hypothesis, Wang et al. (2021) found negative PSF effects in faba bean to be associated with a build-up in the population of putative pathogens within the genus *Fusarium*. Whereas the discrepancies between the results of these studies and ours could be attributed to differences in the biological materials employed (e.g. nature/origins of the soils or different plant genotypes) or the experimental conditions, we argue that the weak PSF effects we observed do not reflect real-case scenarios. Instead, they may have resulted from the use of filter paper lining the bottom of the pots in our experiment, which we speculate would have caused an artifactual stimulation in soil of fungal populations with strong cellulolytic activity. This may have interfered with the normal dynamics in soil-borne fungal communities to be expected in the context of PSF under conditions closer to natural.

In particular, we observed a gradual but steep increase in the relative dominance of fungal communities by ASVs within the genus *Chaetomium* along the phases of the experiment, which was consistent across treatments comprising different soils or conditioning plant species. These ASVs accounted for 80.3% of the total relative abundance in fungal communities in the feedback phase in each treatment, a level of dominance that is exceptionally high in comparison with those commonly reported in soil-borne fungal communities, either in PSF experiments (Miller et al. 2019; Pineda et al. 2020) or in the field (Maciá-Vicente et al. 2020; Maciá-Vicente and Popa 2022). Species within the fungal genus *Chaetomium* have well known cellulolytic abilities (Zhang et al. 2006; Katrolia et al. 2012; Koechli et al. 2019), and have been shown to become dominant in soil fungal communities in response to amendments with paper pulp or straw (Banerjee et al. 2016; Clocchiatti et al. 2020), reaching levels of relative abundance similar to the ones we report. Therefore, we speculate that the enrichment of *Chaetomium* ASVs in our experiment may be caused by our addition of filter paper at the bottom of soil pots. Filter paper is commonly used in plant growth experiments to prevent soil leakage through the drainage holes at the bottom of potting containers upon watering, and is frequently reported in PSF studies (Xue et al. 2018a; Xue et al. 2018b; De Long et al. 2021; Oschrin and Reynolds 2020). Based on these results, we advise against such use of filter paper in future experiments, which can be easily replaced by alternative drainage layering materials, such as clay pellets.

Because PSF effects on plant growth are hypothesized to be mainly driven by microbes (Mariotte et al. 2018; van Ruijven et al. 2020; Semchenko et al. 2022), it seems logical that PSF studies include the monitoring of shifts in microbial communities along changes in plant biomass. However, most PSF studies have traditionally focused on plant biomass while neglecting the soil microbial communities that presumably drive differences in plant growth. In a review of the recent literature (Method S1), we found that only 21% of the PSF studies published in the last 5 years included some characterization of the microbial communities associated with soil or plants (Additional file 2: Table S17). Our results highlight the importance of monitoring the changes in microbial communities in PSF studies, both to identify the mechanisms underlying PSF effects and to rule out potentially misleading outcomes (as was our case), and thus we advocate for these assessments to become standard practice in future PSF research.

Conclusion

PSFs of the different crops in our study were not related to their former density in a particular cropping system. Instead, the results were most likely affected by the enrichment of one particular cellulose-degrading fungus in response to the use of filter paper at the bottom of the pots, which is a common practice in experiments of this sort. Our study warns against using filter paper in such studies, as this may cause a strong shift in the soil microbial community. Here we call this effect 'the blind spot', because if the microbial community is hidden in PSF research, conclusions might go in different directions than anticipated. Deeper knowledge about soil microbial communities is essential to understand the mechanisms underlying plant–soil feedback, and how it can contribute to more sustainable agrosystems.

Abbreviations

PSF	Plant–soil feedback
NCP	North China Plain
ITS	Internal transcribed spacer
M0	Continuous cropping of maize
WM	Wheat–maize rotation with two harvests per year
WMS	Wheat–maize crop rotation including soybean
NMDS	Non-metric multidimensional scaling
PERMANOVA	Permutational multivariate analysis of variance

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43170-023-00147-5.

Additional file 1. Fig. S1. The soil origins. **Fig. S2.** The effects of soil origins on crop shoot dry biomass in the conditioning phase. **Fig. S3.** $PSF_{specific}$ based on crop shoot dry biomass in three soil origins and two different conditioning species. **Fig. S4.** Total fungal and potential pathogenic fungal community structure in the PSF experiment. **Fig. S5.** Relative abundance of *Chaetomium* spp. reads as a proportion of all fungal reads in the bulk and rhizosphere soils and the phases in the PSF experiment. **Fig. S6.** Relative abundance of *Bipolaris* spp. reads as a proportion of all fungal reads in the bulk and rhizosphere soils and the phases in the PSF experiment. **Fig. S7.** Relative abundance of *Fusarium* spp. reads as a proportion of all fungal reads in the bulk and rhizosphere soils and the phases in the PSF experiment. **Fig. S8.** Relative abundance of *Macrophomina* spp. reads as a proportion of all fungal reads in the bulk and rhizosphere soils and the phases in the PSF experiment. **Fig. S9.** Relative abundance of *Gaeumannomyces* spp. reads as a proportion of all fungal reads in the bulk and rhizosphere soils and the phases in the PSF experiment. **Fig. S10.** Taxonomic structure of fungal communities for rhizosphere soils across the phases (phase 2 and 3) in the PSF experiment. **Table S1.** The host density in the former cropping systems. **Table S2.** ANOVA results of the effects of crop species, soil origin, and their interaction on shoot dry biomass in the conditioning phase. **Table S3.** ANOVA results of the effects of soil origin on shoot dry biomass for each crop in the conditioning phase. **Table S4.** ANOVA results of the effects of crop species, soil origin, conditioning species, and their interactions on shoot dry biomass in the feedback phase. **Table S5.** ANOVA results of the effects of conditioning species on shoot dry biomass of maize for M0, WM, and WMS soil origin in the feedback phase. **Table S6.** ANOVA results of the effects of soil origin, heterospecific conditioning species, and their interaction on $PSF_{specific}$ based on shoot dry biomass of wheat, maize, and soybean. **Table S7.** The results from linear regression models for the relationship between the PSF (i.e. $PSF_{average}$ and $PSF_{specific}$) experienced by a crop (i.e. wheat, maize and soybean) and their former abundance in a particular cropping system. **Table S8.** PERMANOVA results of the effects of soil origin, phase, and their interactions on total and potential pathogenic fungal community variation from the rhizosphere soils. **Method S1.** Method to retrieve publications about PSF experiments from Web of Science.

Additional file 2. Table S9. Taxonomic classification of fungal ASVs. **Table S10.** Taxonomic classification of potential pathogenic fungal ASVs. **Table S11.** Variation across phases in the abundance of fungal genera in bulk soils (phase1, 2 and 3) of wheat. **Table S12.** Variation across phases in the abundance of fungal genera in bulk soils (phase 1, 2 and 3) of maize. **Table S13.** Variation across phases in the abundance of fungal genera in bulk soils (phase 1, 2 and 3) of soybean. **Table S14.** Variation across phases in the abundance of fungal genera in rhizosphere soils (phase 2 and 3) of wheat. **Table S15.** Variation across phases in the abundance of fungal genera in rhizosphere soils (phase 2 and 3) of maize. **Table S16.** Variation across phases in the abundance of fungal genera in rhizosphere soils (phase 2 and 3) of soybean. **Table S17.** List of publications about the plant–soil feedback retrieved from Web of Science.

Acknowledgements

We would like to thank the students and staffs at Quzhou Experimental Station for their great assistance with the experiment, especially for the help to the development of ideas from Feng Zhu, Zhengyuan Liang and Shuaimin Chen, and suggestion on the retrieving method from Zhan Xu. ML thank the financial support from China Scholarship Council (No. 201913043) and Hainan University. The authors are also grateful to anonymous reviewers and the editor for their constructive comments that greatly improved the manuscript.

Author contributions

LM, CS, JVR, and WVW designed the project. ML performed the experiments. JVR and ML analyzed statistics of PSF data. JMV and ML analyzed the sequencing data. ZC and FZ managed the field regarding the soil data. ML, JMV, JVR, CS and LM wrote the first version of the manuscript. All authors read and approved the final manuscript.

Funding

This work has received funding from The National Key Research and Development Program of China (2021YFD1900200), China Scholarship Council (No. 201913043), the Program of Advanced Discipline Construction in Beijing (Agriculture Green Development), and the 2115 Talent Development Program of China Agricultural University to CS.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

Jose G. Maciá-Vicente is an associate editor of CABI Agriculture and Bioscience.

Author details

[1]College of Resources and Environmental Sciences, National Academy of Agriculture Green Development, Key Laboratory of Plant-Soil Interactions, Ministry of Education, National Observation and Research Station of Agriculture Green Development (Quzhou, Hebei), China Agricultural University, Beijing 100193, China. [2]Plant Ecology and Nature Conservation, Wageningen University & Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands. [3]Centre for Crop Systems Analysis, Wageningen University & Research, P.O. Box 430, 6700 AK Wageningen, The Netherlands.

Received: 7 September 2022 Accepted: 9 March 2023
Published online: 27 March 2023

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