Understanding disease suppressive soils: molecular and chemical identification of microorganisms and mechanisms involved in soil suppressiveness to Fusarium culmorum of wheat

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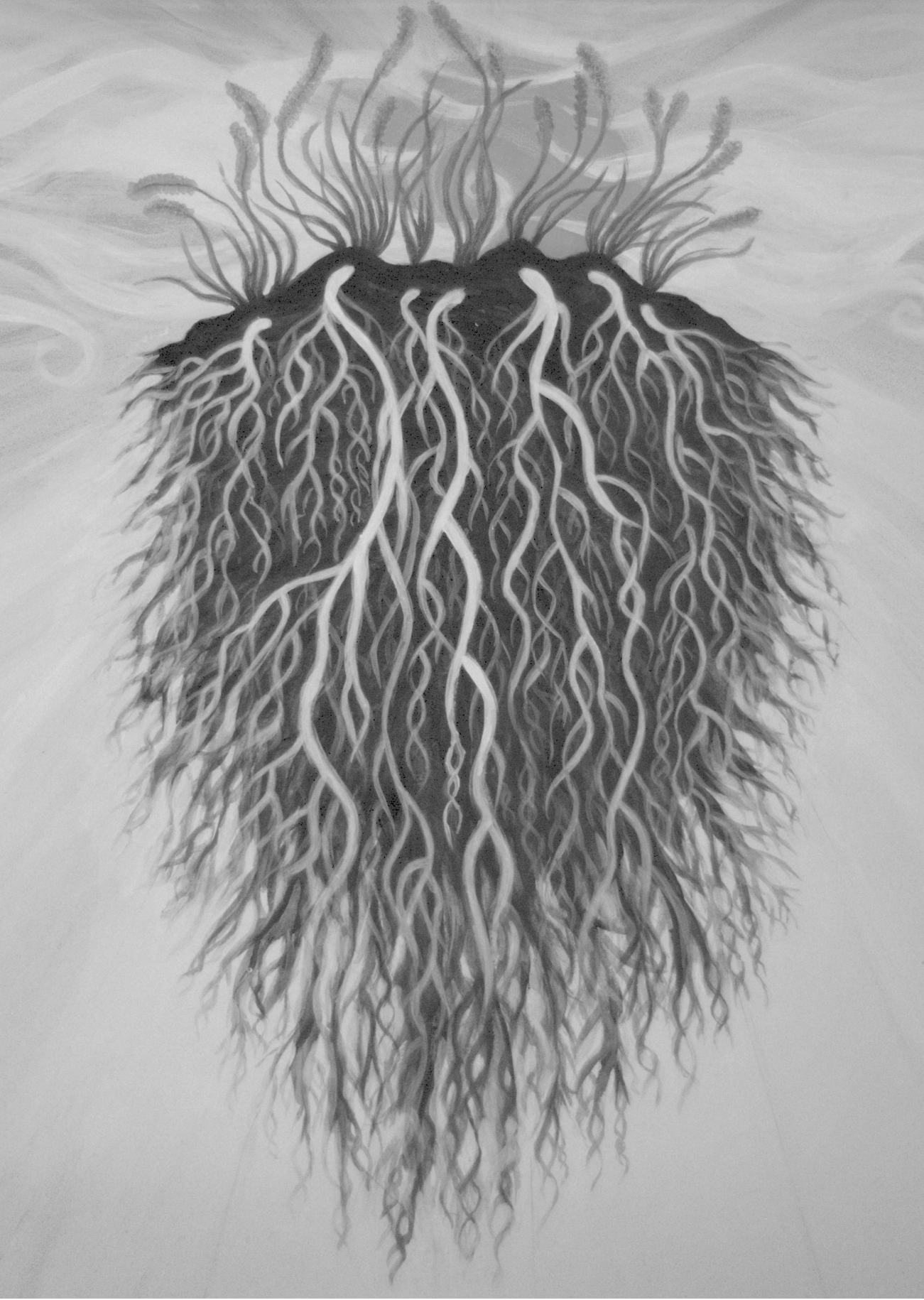

Chapter 5

Impact of plastic mulch film residues on soil microbiome, disease suppressiveness, and plant growth

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Modified version was submitted for publication

Abstract

Plastic mulch film residues have been accumulating in agricultural soils for decades, but so far, little is known about its consequences on soil microbial communities and functions. Here, we tested the effects of plastic residues of low-density polyethylene and biodegradable mulch films on soil suppressiveness and microbial community. Soil suppressiveness is a microbial-driven phenomenon important for sustainable agriculture. The level of soil suppressiveness, plant biomass and nutrient status and microbial communities in rhizosphere and plastisphere were investigated using a controlled pot experiment in soil suppressive to *Fusarium culmorum*. The addition of 1% plastic residues to the suppressive soil did not affect the level of suppressions and the disease symptoms index. However, we did find that plant biomasses decreased, and that plant nutrient status changed in the presence of plastic residues. We did not observe significant changes in bacterial and fungal rhizosphere communities. Nonetheless, bacterial and fungal communities closely attached to the plastisphere were very different from the rhizosphere communities. The plastisphere revealed a high abundance of specific bacterial phyla (Actinobacteria, Bacteroidetes, and Proteobacteria) and fungal genera (*Rhizoctonia* and *Arthrobotrys*). Our work revealed new insights and raises emerging questions for further studies on the impact of microplastics on the agroecosystems.

Introduction

Soil plays a central role in supporting life and possess the highest microbial diversity known to date. Microbial communities are essential for promoting plant growth and suppressing soil-borne diseases (Cha et al., 2016; Lugtenberg et al., 2017). Plants are exposed to various abiotic and biotic stresses throughout their lives, yet certain soil microbes can help plants to overcome different stresses and improve growth (Gouda et al., 2018; Ilangumaran and Smith, 2017; Jochum et al., 2019).

Along with the abiotic and biotic stresses that can occur sequentially or simultaneously, plants also are challenged by anthropogenic soil pollution. Environmental pollution in soil caused by agrochemicals or the disposal of waste coming from industrial or urban sources may interfere with plant-microbe interactions and communication. One critical type of pollution emerging in agriculture is the increasing load of microplastics (defined as plastic particles < 5 mm) (de Souza Machado et al., 2018). Although, the effect of microplastics on the aquatic ecosystems have been intensively studied, their environmental impacts on the terrestrial ecosystem remain largely unexplored. According to recent literature, agricultural land may store more microplastics than oceans (Nizzetto et al., 2016b, 2016a), likely because there are multiple ways for microplastics to get into the soil (Ng et al., 2018). Plastic mulching is one of the primary sources contributing to the accumulation of microplastics in agroecosystems (Huang et al., 2020).

Plastic mulch films were applied to farmland for several purposes: retaining soil moisture, warming the soil and preventing weeds (Steinmetz et al., 2016). Unfortunately, it is not technically feasible for farmers to remove or recycle most of the mulch films used in the fields because the films are usually very thin (0.01-0.05 mm) (Kasirajan and Ngouajio, 2012). The accumulation of residual plastic mulch films in agricultural soils has raised concerns because it decreases soil productivity by blocking water infiltration, impeding soil gas exchange, and constraining root growth (Hegan et al., 2015; Jiang et al., 2017; Qi et al., 2020a). Plastic pollution is considered to be an emerging threat to soil ecosystem health and function (Dan Zhang et al., 2020). Biodegradable plastics were developed as promising environmentally sustainable alternatives to conventional low-density polyethylene films (Kasirajan and Ngouajio, 2012; Sintim and Flury, 2017). Biodegradable plastics are tilled into the soil where they are expected to be degraded by microbes. However, their impact on i) soil and rhizosphere microbiome, ii) the interactions between beneficial microbes and soil-borne pathogens and iii) the level of soil disease suppressiveness are largely unknown.

Disease suppressive soils protect plants from root pathogens despite the presence of favourable conditions for disease development (Deacon, 1984). Enhancing soil suppressiveness is of great agronomic interest to achieve sustainable management for plant disease control (Ghorbani et al., 2009; Singh and Vyas, 2009). Many previous studies investigated the link between soil disease suppressiveness and the soil microbiome and such findings have been summarized in several review articles (Kinkel et al., 2011; Schlatter et al., 2017b; Weller et al., 2002b). For instance, the presence of some microbial taxa in soil were associated to the development of soil suppressiveness (Gomes Exposito et al., 2017) and the disturbance in microbiome composition lead to losing the ability of microbiome to protect plants (Carrión et al., 2019; Cha et al., 2016).

Recently, we revealed that microplastics could have strong effects on plant growth, the blend of volatiles emitted in the rhizosphere, and the assembly of the rhizosphere communities (Qi et al., 2020b).

The aim of the present study was to understand the impact of microplastic pollution on the level of soil disease suppressiveness, plant growth and nutrient status and on microbial community. Recently, Ossowicki et al. (2020) screened soils from 28 different sites in the Netherlands and Germany for their level of suppressiveness to *Fusarium culmorum*. The microbiological basis of the suppressiveness were characterised in four different field-soils displaying clear and reproducible disease suppressiveness (Ossowicki et al., 2020). In this work, we therefore, tested the effect of plastic residues on i) the level of soil disease suppressiveness (using previously characterized suppressive soil), ii) rhizosphere microbial communities composition and iii) plant growth. Besides, we analysed the microbiome of the so-called plastisphere, which may host a distinct microbial colony on the plastic debris.

Materials and Methods

Growth conditions and materials

A pot experiment was conducted in a growth cabinet (MC 1750 VHO-EVD, Snijders Labs) with photoperiod of 12 h day/12 h night at 20°C and 60% relative humidity. Plants were watered every two days and supplemented weekly with a 0.5 Hoagland solution (1 ml per 80 cc of the soil, 0.5 M $Ca(NO_3)_2 \cdot 4H_2O$, 1 M KNO_3, 1M KH_2PO_4, 0.5 M $MgSO_4 \cdot 7H_2O$ and 98.6 mM ferric EDTA).

The disease-suppressive soil (S11) used in this study was found to be highly suppressive against *F. culmorum* in wheat (Ossowicki et al., 2020). Soil was collected in agricultural field, air-dried at room temperature, homogenized, sieved through a 4 mm sieve, and stored at 4°C. The soil was sandy with an organic matter content of 3.48 ± 0.47% and a pH of 7.28 ± 0.19. We used a gamma-sterilized sand collected near Bergharen, the Netherlands as a standard substrate. More information about the suppressive soil and Bergharen sand is provided in Table S1. Wheat seeds (*Triticum aestivum,* JB Asano variety) were obtained from Agrifirm (the Netherlands). Seeds were surface sterilized and pregerminated on sterile moist filter paper in order to use in the experiment.

Two types of plastic mulch films (PMF) were used in this study: a low-density polyethylene (LDPE) and a starch-based biodegradable plastic (Bio). Two sizes of plastic residues (macro and micro) were prepared as described in a previous study (Qi et al., 2018). Macro-sized plastic pieces were made by cutting PMF into 5 mm × 5 mm squares by hand. The micro-sized powders were obtained through cryogenic grinding, then sieved to obtain a powder size ranging from 50 µm to 1 mm. All plastic materials were sprayed with 70% ethanol and air-dried in a fume cupboard to minimize microbial contamination.

The fungal pathogen *F. culmorum* PV was propagated on 1/4 potato dextrose agar (PDA) and incubated at 20 ºC for two weeks. Plugs with a diameter of 6 mm were cut from the border zone of *F. culmorum* hyphae. One plug was mixed with 10 cc of soil for treatments and, in controls without the pathogen, sterile 1/4 PDA plugs were used instead.

Experimental setup

Prior to the experiment, the soil was “activated” to induce microbial activity by growing wheat for two weeks. Afterwards, plants along with the whole root system were removed and the soil was mixed and prepared as follows. The suppressive soil was mixed 2:1:1 in volume with sterile Bergharen sand and sterile vermiculite (Agra-vermiculite, the Netherlands). Sterile Bergharen sand was mixed with vermiculite 3:1 in volume for negative controls. For each pot, 140 g of the soil mixture, 1.4 g of the plastic residues (except for the controls) and plugs with or without the pathogen were added to pots and manually mixed. One pre-germinated wheat seed was transferred into each pot and grew for three weeks. After this time, disease symptoms were assessed, and rhizosphere samples collected.

Treatments and replicates

Four types of plastic residues were mixed separately with suppressive soil at 1% (w/w). This concentration is environmentally relevant and consistent with our previous studies (Qi *et al.*, 2018). Two positive controls with only suppressive soil and without the addition of plastic residues were used to control for disease suppressiveness (S11_FC and S11_NF). Two negative controls with sterilized Bergharen sand were used to control for the pathogenicity of *F. culmorum* (BS_FC and BS_NF). Eight treatments were tested with 10 replicates in fully randomized design (Table 1).

Table 1 The pot experiment treatments.

Treatment	Plastic residues	*F. culmorum*	Soil
LDPE_Ma	LDPE macro	✓	S11 (Suppressive soil)
LDPE_Mi	LDPE micro	✓	S11 (Suppressive soil)
Bio_Ma	Bio macro	✓	S11 (Suppressive soil)
Bio_Mi	Bio micro	✓	S11 (Suppressive soil)
S11_FC	/	✓	S11 (Suppressive soil)
S11_NF	/	/	S11 (Suppressive soil)
BS_FC	/	✓	Bergharen sand
BS_NF	/	/	Bergharen sand

Disease symptoms assessment and analysis of plant biomass and plant nutrient status

To assess the disease symptoms, the wheat plants were carefully removed, the excess of soil was shaken off, and the roots were cleaned with water. The root system was visually inspected for brown/black lesions or rotting and the stem base/coleoptile was inspected for rotting and the presence of pink-white fungal hyphae. Plants were scored from 0-5 for disease symptoms (Ossowicki et al., 2020). Statistical differences in disease symptoms between treatments and controls were assessed using the chi-square test, with an alpha cutoff of $p < 0.05$.

After the screening, plants were separated into shoots and roots. Dry biomass was recorded after drying at 70 ºC for 48 h. Dried shoot and root tissues were then digested using 65% HNO_3 at 120 ºC. The mineralized samples were transferred into polypropylene test tubes. Samples were diluted 1:40 in MILLI-Q water and the concentration of metal elements was measured by Inductively Coupled Plasma-Mass Spectrometry ICP-MS (BRUKER Aurora- M90 ICP-MS) as previously described (Vigani *et al.*, 2017, Martín-Sánchez *et al.*, 2020). Differences among the mean values of inter-groups were analysed by one-way ANOVA and the post-hoc tests considered were: Tukey HSD (in the case of Levene's test $p > 0.05$) and Tamhane (in the case of Levene's test $p < 0.05$).

DNA extraction for rhizosphere soil and plastisphere

For six randomly selected replicates in each treatment, rhizosphere soil samples were collected. After the plants were taken out of the pots, the excess soil was removed and the root system with adhering soil was placed in a sterile paper bag. Soil particles (rhizosphere) were detached from the roots by rigorously shaking. Rhizosphere soil samples were stored at -4 ºC and the DNA was isolated using a DNeasy PowerSoil Kit (QIAGEN, the Netherlands) within one week.

Six to ten pieces of macroplastics were collected from each pot of treatments LDPE_Ma and Bio_Ma. The plastic pieces were stored in Eppendorf tubes in glycerol stock at -80 ºC before DNA extraction. DNeasy PowerSoil Kit was used to extract the DNA from plastic pieces.

Amplicon sequencing and microbial community analysis

The amplicon library preparation and sequencing was carried out at the McGill University and Genome Québec Innovation Centre (Montréal, Canada). The PCRs of the bacterial 16S rRNA gene V3-V4 region were performed with the primer set 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCRs of the fungal rDNA gene ITS region was performed with the primer set ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and 58A2R (5'-CTGCGTTCTTCATCGAT-3'). Sequencing was carried out on an Illumina MiSeq platform with 4 biological replicates per treatment).

Adapter sequences were removed using cutadapt 2.10 (Martin, 2011) and the quality of reads was evaluated using FastQC 0.11.9 (Andrews, 2015). All the subsequent work on sequencing data was performed in an R (v 4.0.1) environment using packages specified further. Amplicon sequencing variants (ASVs) were constructed using dada2 1.16.0 (Benjamin J Callahan et al., 2016, p. 2) and taxonomically classified based on the SILVA v132 database for bacterial 16S genes or the UNITE v8 database for fungal ITS sequences. Read counts were rarefied for further analysis using a Vegan 2.5-6 package. Analyses of alpha diversity and differential abundance were performed using phyloseq 1.32.0 and DESeq2 1.28.1 packages and visualized using Ampvis2 2.6.0 and ggplot2 3.3.2. The differences in Shannon indexes were assessed using one-way ANOVA, $p < 0.05$.

Results

Effect of plastic residues on the level of disease suppressiveness, plant biomass and plant nutrient status

A significant effect on plant biomass was observed only for shoot biomass in the treatment Bio_Mi as compared to controls without plastic additions (Fig. 1). No significant difference in root biomass was observed.

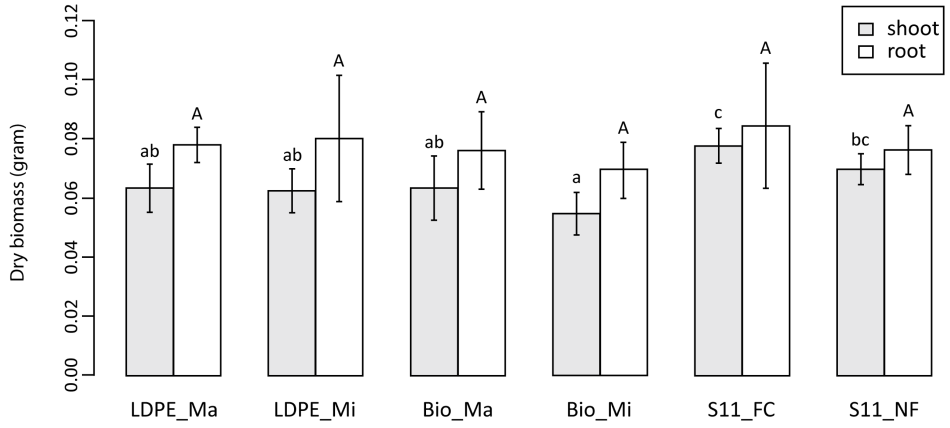


Fig. 1. Shoot and root biomass of wheat in suppressive soil with and without plastic residues. The bars indicate the mean values of each treatment, with the error bars representing the standard deviation. Letters above the bars represent statistically significant differences based on ANOVA, $p < 0.05$.

The results of testing the impact of plastic residues on soil suppressiveness are presented in Fig. 2. It showed that the pathogen was infectious when comparing controls BS_FC to BS_NF and that the soil S11 was suppressive and could significantly reduce disease symptoms (controls BS_FC vs. S11_FC). The presence of plastic residues in suppressive soil did not significantly affect the level of suppressiveness (Fig. 2).

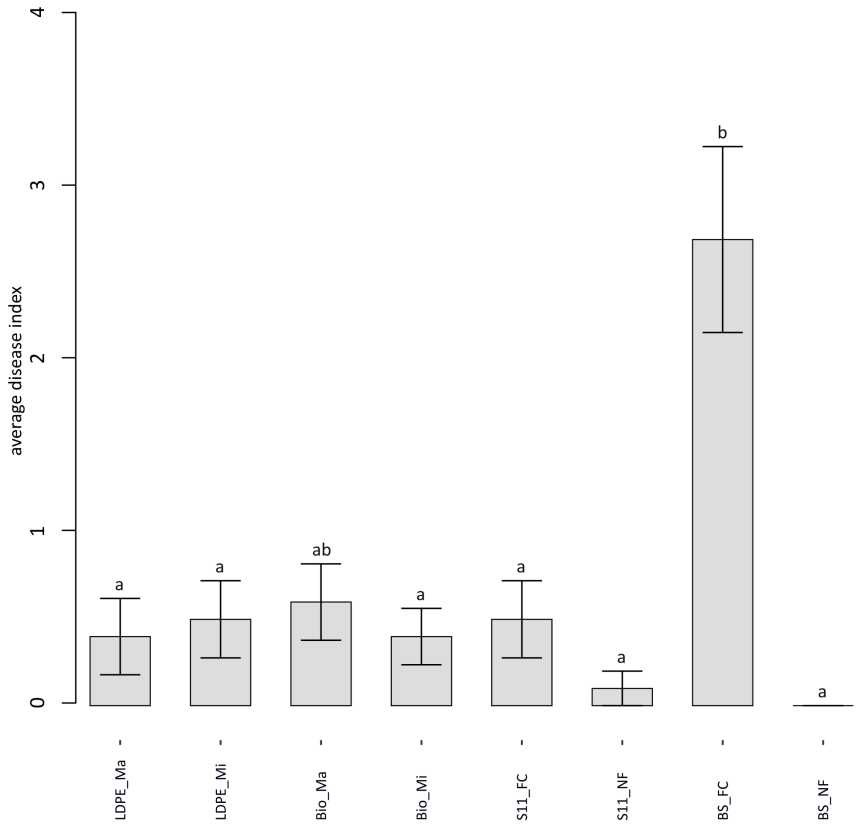


Fig. 2. Disease symptoms observed in wheat inoculated with F. culmorum grown in substrates with plastic and without plastic as controls. The bars indicate the average of the disease symptoms index, with the error bars representing the standard error. Letters above the bars represent significance levels based on the chi-square test.

In addition, we performed an analysis of the mineral nutrient content of shoots and roots, defined as plant ionome. The Principal Component Analysis (PCA) performed on the macronutrient (Mg, K, Ca) content of the shoots revealed a clear separation between LDPE_Ma and LDPE_Mi (Fig. 3a). The PCA performed on the micronutrient (Mn, Fe, Zn, Mo, Cu) content of the shoots revealed separation between LDPE_Ma and LDPE_Mi samples (Fig. 3b). In the shoots, we observed a significant difference in K content between treatments LDPE_Ma and LDPE_Mi and the Mn contents of both treatments were higher than the controls (Table S2). The PCA revealed different macro- and micro- nutrient composition in the roots of the treatment LDPE_Mi (Fig. 3c and 3d). Treatment LDPE_Mi showed higher Cu content in root tissues (Table S3).

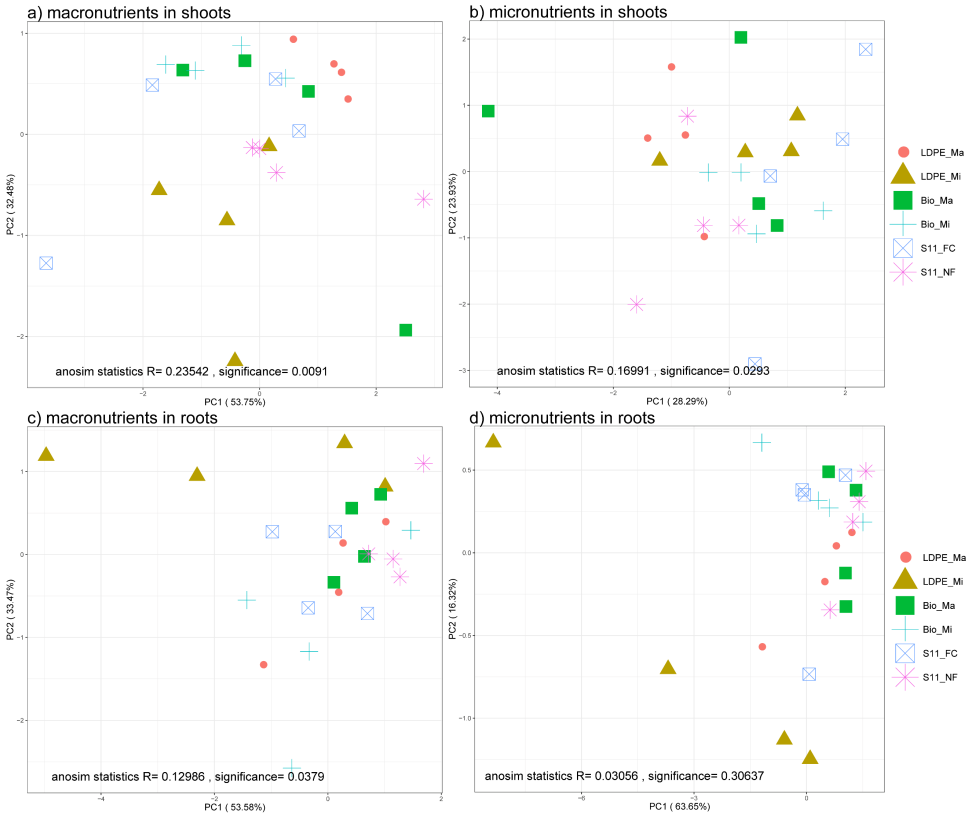


Fig. 3 Principal component analysis (PCA) of the macronutrient and micronutrient content in shoots and roots of wheat. ANOSIM statistics is indicated in the left-bottom corner of each plot.

Effect of plastic residues on the rhizosphere bacterial and fungal communities

The analysis of bacterial and fungal rhizosphere communities was based on 16S rRNA and ITS amplicons sequencing. The effect of the addition of the plastic residues may be seen as a change between treatments (LDPE_Ma, LDPE_Mi, Bio_Ma, Bio_Mi) and control S11_FC – all with pathogenic fungus added.

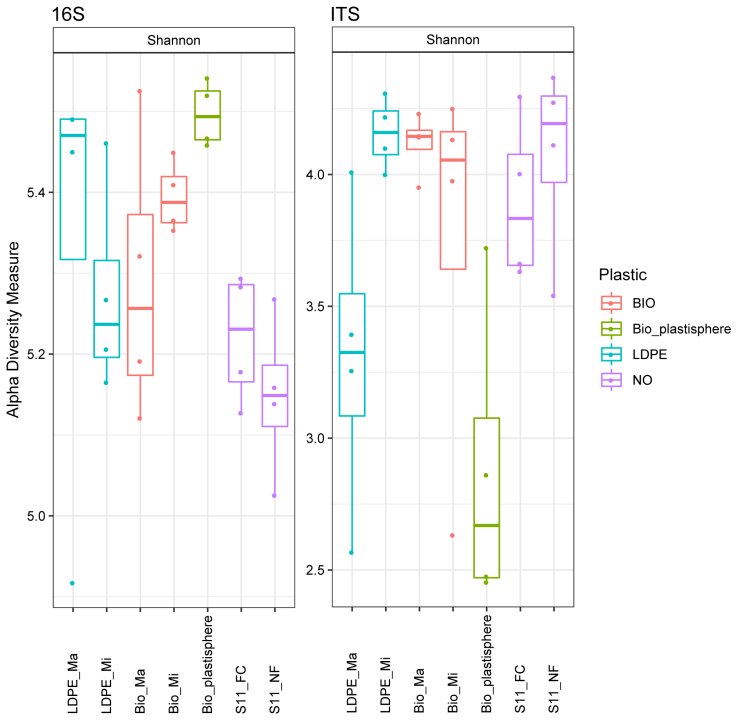


Fig. 4. Alpha diversity of bacterial (16S) and fungal (ITS) communities based on ASVs presented as Shannon index.

The diversity of bacterial and fungal community based on Shannon index revealed that, as compared to control (S11_FC), there was no statistically significant change due to the addition of plastic (Fig. 4, Table S4 and S5). Moreover, looking at the abundance of the major bacteria phyla and fungal genera (Fig.5 and 6 respectively), there was only a small significant change in the abundance *Chloroflexi* between LDPE_Ma treatment and control. Altogether, the addition of LDPE and Bio plastic residues to soil did not have a direct impact on bacterial and fungal rhizosphere community. These results are also supported by PCA analysis (Fig. S1).

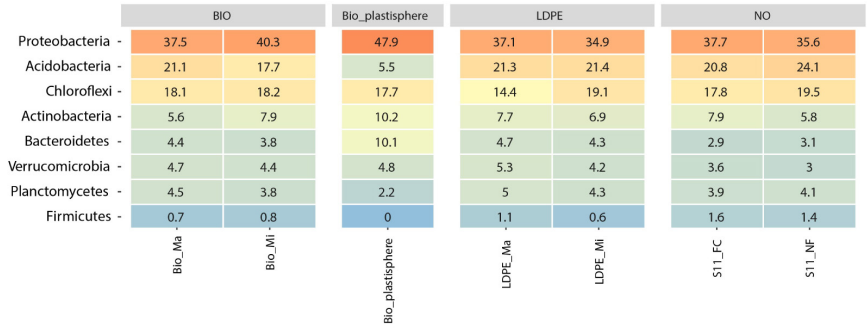


Fig. 5. Heatmap showing the average relative abundance of the top eight bacteria phyla across the samples. The headers of columns indicate the type of plastic used, "NO" indicates controls without the addition of plastic.

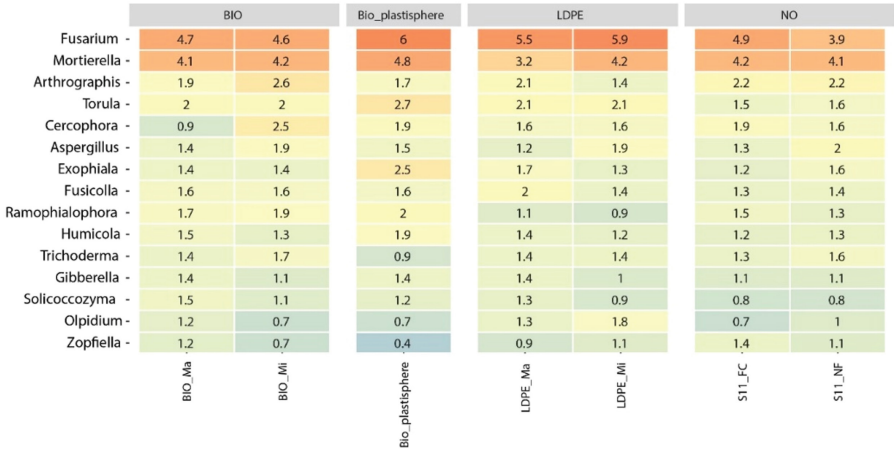


Fig. 6. Heatmap showing the average relative abundance of the top fifteen fungal genera across the samples. The headers of columns indicate the type of plastic used, "NO" indicates controls without the addition of plastic.

Microbial communities in the plastisphere

We compared the bacterial and fungal communities inhabiting the surface of Bio_Ma plastic residues and compared them to the rhizosphere communities of plants from which these residues were extracted. The analysis of bacterial community in the Bio_plastisphere compared to Bio_Ma rhizosphere showed an increase in diversity in "plastisphere" (Fig. 4) and a significantly higher relative abundance of bacteria phyla Actinobacteria, Bacteroidetes, and Proteobacteria and a lower relative abundance of Acidobacteria and Planctomycetes (Fig.7).

We found that the diversity of fungal community (Shannon index) in “plastisphere” was significantly lower comparing to rhizosphere (Fig. 4). The “plastisphere” was vastly dominated by three fungal genera *Rhizoctonia*, *Arthrobotrys* and *Fusarium* (on average around 50% of relative abundance) where the first two genera were significantly enriched compared to the rhizosphere community (Fig. 8). The results of differential abundance comparison revealed also statistically significant higher relative abundance of fungal genera *Torula* and *Exophiala* and lower abundance of *Zoptelia*. Significantly, a higher relative abundance of the fungal genera *Rhizoctonia* and *Arthrobotrys* were measured in the Bio_plastisphere (Fig. 8).

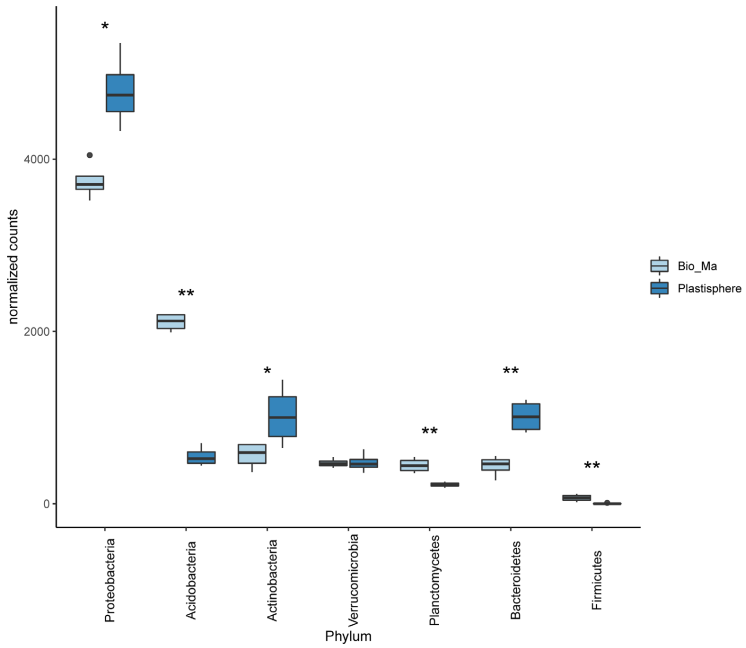


Fig. 7. Boxplots displaying the relative abundance of bacteria phyla between bioplastic plastisphere and the rhizosphere of wheat grown in soil with the addition of bioplastic. Statistically significant differences based on deseq2 analysis are marked with a single asterisk (p<0.05) or with a double asterisk (p<0.01).

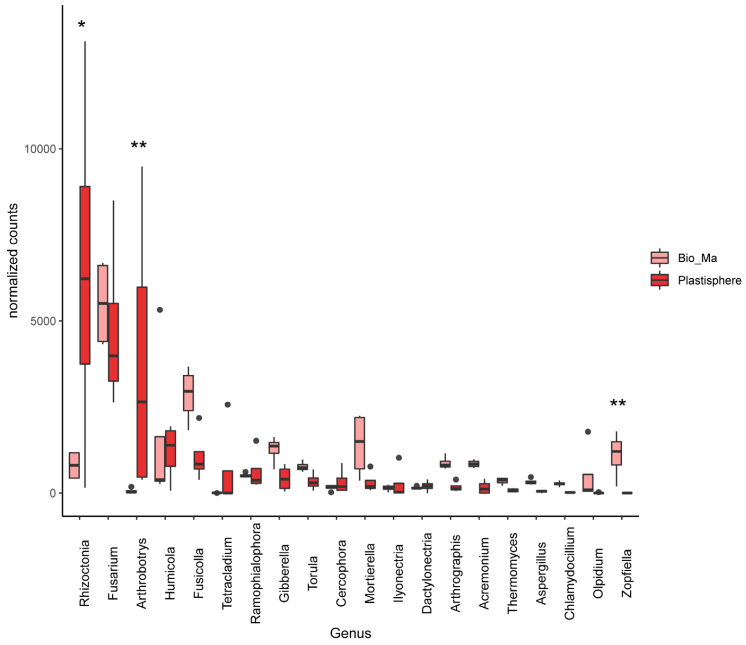


Fig. 8. Boxplots displaying the relative abundance of fungal genera between bioplastic plastisphere and the rhizosphere of wheat grown in soil with the addition of bioplastic. Statistically significant differences based on deseq2 analysis are marked with a single asterisk ($p<0.05$) or with a double asterisk ($p<0.01$).

Discussion

One of the greatest challenges of our generation is to consolidate or even increase current agricultural yields and nutritional quality while reducing the input of fertilizers and pesticides. As a critical agricultural tool, plastic mulch films have made significant contributions to food security and modern agricultural development (Espí et al., 2006; Kasirajan and Ngouajio, 2012). However, we tend to ignore the impact of plastic mulch films residues as pollutants. Plastic residues, especially microplastics, may represent a hidden danger for agriculture affecting soil functions, plants and crop yield (Rillig and Lehmann, 2020; Dan Zhang et al., 2020). Recently, Fuller & Gautam (2016) suggested that background concentrations of microplastics may range from 0.03% to 6.7% in agricultural and industrial soils (Fuller and Gautam, 2016). A promising approach to overcome the accumulation of residual polyethylene mulch films in soils is to use biodegradable mulch films composed of polymers designed to be degraded by soil microorganisms. Biodegradable plastics are a family of various polymers, such as starch blends, poly(lactic acid), poly(butylene adipate terephthalate), polyhydroxyalkonates, etc. In addition, they contain substantial amounts of chemical additives, such as plasticizers, which could be physically and chemically hazardous

to soil (micro)organisms and hence, disturb soil functioning (Zimmermann et al., 2020). Our work is the first to explore the impact of plastic mulch film residues (both LDPE and Biodegradable plastics) on the level of soil disease suppressiveness and plant nutrient status. The results of our study did not reveal major short-term effect of plastic residues on the level of soil suppressiveness against *F. culmorum*.

A significant effect, however, was observed on plant shoot biomass in the treatment Bio_Mi but not in the other treatments, indicating that different types and sizes of plastic residues may cause different effects. For example, the addition of LDPE_Mi affected the macro- and micronutrients composition in the roots. The mineral nutrient content profile of a plant can be considered to be a signature of the nutrient status of plants under stressed conditions (Martín-Sánchez et al., 2020; Pii et al., 2015). Our findings are based on a short-term experiment (two weeks of activation and three weeks of infection) however, it is plausible that in the long-term, the effects on the plant biomass, plant nutrient content and level of soil suppressiveness could be stronger. For example, in our previous study, we observed a significant adverse effect on plant biomass after 2 and 4 months (Qi et al., 2018).

Both soil and plants depend heavily on their microbiome for specific functions and traits (Berg, 2009; Liu et al., 2020). Rhizosphere, the narrow zone surrounding and influencing plant roots, is considered to be one of the most dynamic interfaces on earth (Philippot et al., 2013). Since large parts of the soil have limited nutrient access, the rhizosphere represents an oasis for soil microorganisms due to the release of rhizodeposits by plant roots. These rhizodeposits, defined as the easily available organic nutrients and signaling compounds, include root exudates, border cells and mucilage (D. L. Jones et al., 2009; Philippot et al., 2013; Raaijmakers et al., 2009). We recently observed that the addition of microplastics could have strong effects on the rhizosphere bacterial community (Qi et al., 2020b). In the current study, we did not observe significant changes in bacterial or fungal rhizosphere communities (diversity and assembly) among rhizosphere soil samples. Nonetheless, bacterial and fungal communities that were closely attached to the Bio_Ma "plastisphere" were very different from the rhizosphere communities in the Bio_Ma treatment. The Bio_Ma plastispheres revealed a high abundance of specific bacteria phyla (Actinobacteria, Bacteroidetes, and Proteobacteria) and fungal genera (*Rhizoctonia* and *Arthrobotrys*). By providing a new niche for soil microorganisms, the "plastisphere" could alter the structure and function of soil and rhizosphere microbial community. Despite the increasing interest in "plastisphere", very few studies have been conducted on this topic and they are focused only on aquatic ecosystems (Amaral-Zettler et al., 2020; Yang et al., 2019). In our study, we were able to obtain high-quality DNA only from the plastisphere of the Bio_Ma treatments for amplicon sequencing. However, it would be of great interest to further study the "plastispheres" formed around different types and sizes of plastic. The addition of microplastics to soil could be a source of nutrients and extra surfaces attractive

for certain microbes, and hence, affecting microbial community and function. Taking into consideration that many species belonging to two fungal genera dominating the "plastisphere" (*Rhizoctonia* and *Fusarium*) are pathogenic, we can speculate that this habitat may act as a reservoir of pathogens. Hence, it would be important in the future to study the effect of plastic residues on the abundance of soil borne pathogens.

Conclusions

In the current study, the addition of plastic mulch film residues to suppressive soil, did not reveal significant effects on disease symptoms in wheat inoculated with *F. culmorum*, nor on the plant-associated bacterial and fungal community composition, structure and diversity. However, we observed changes in the plant biomass and mineral nutrient content. Moreover, the analysis of "plastisphere" revealed substantially different bacterial and fungal taxonomic patterns and diversity as compared to the rhizosphere soil. Based on our results, we suggest that the introduction of plastic into the soil would create a new niche "plastisphere" that harbours a distinct microbial community. Such findings highlight the importance to characterize the plastisphere in soil and to unravel its impact on the plant-soil system.

Funding

This research was funded by the Natural Sciences and Engineering Research Council of Canada Discovery grant RGPIN-2014-05274 and Strategic grant for projects STPGP 494702. This work was also supported by the China Scholarship Council (CSC: 201604910510 to Y Qi).

Supplementary Material

Table S1. Detailed information about suppressive soil and Bergharen sand

soil parameters	unit	S11	BS
pH	/	7.28 ± 0.19	7.53 ± 0.05
OM	%	3.48 ± 0.47	0.24 ± 0.04
Fe	mg/kg	0.11	0.19 ± 0.01
K	mg/kg	68.77 ± 1.1	2.02 ± 0.32
Mg	mg/kg	56.43 ± 0.58	0
P	mg/kg	5.43 ± 0.04	0.11 ± 0.03
S	mg/kg	1.17 ± 0.15	0
C	%	1.99 ± 0.88	0.05 ± 0.01
N	%	0.16 ± 0.07	0
C:N	/	12.44	NA

Table S2. Content of some mineral nutrients in shoot of wheat plants. The content of Mg, K and Ca are expressed as mg g^{-1} DW while the content of Mn, Fe, Cu, Zn, Mo are expressed as µg g^{-1} DW. Nutrients displaying significant differences among treatment (p<0.05) are reported in bold.

	LDPE_Ma	LDPE_Mi	Bio_Ma	Bio_Mi	S11_FC	S11_NF
Mg	1.88±0.11	2.06±0.26	1.97±0.51	1.64±0.11	1.77±0.18	2.06±0.23
K	**52.29±1.89 b**	**40.44±2.76a**	**48.38±3.71ab**	**44.12±3.61ab**	**39.98±10.36ab**	**45.51±5.11ab**
Ca	3.67±0.09	3.03±0.31	3.32±0.39	3.20±0.35	3.02±0.58	3.61±0.36
Mn	**19.48±3.90 b**	**21.10±2.05b**	**17.94±2.09ab**	**17.45±4.27ab**	**11.17±1.47a**	**23.54±3.25b**
Fe	154.26±25.42	121.76±40.00	165.48±131.78	95.33±16.73	91.87±17.02	130.03±26.49
Cu	9.15±2.89	6.67±2.47	11.12±7.24	11.59±9.56	8.14±4.13	8.46±2.28
Zn	42.25±1.84	30.45±6.21	37.72±5.16	35.33±2.40	35.05±16.77	42.80±8.15
Mo	11.54±1.90	5.32±0.52	13.68±9.37	9.06±1.24	6.53±2.49	7.03±0.22

Table S3. Content of some mineral nutrients in root of wheat plants. The content of Mg, K and Ca are expressed as mg g^{-1} DW while the content of Mn, Fe, Cu, Zn, Mo are expressed as µg g^{-1} DW. Nutrients displaying significant differences among treatment (p<0.05) are reported in bold.

	LDPE_Ma	LDPE_Mi	Bio_Ma	Bio_Mi	S11_FC	S11_NF
Mg	6.31±3.58	19.51±14.65	5.54±2.16	7.33±5.85	5.19±2.04	3.08±1.41
K	16.71±2.26	15.39±2.23	15.31±1.18	18.42±3.06	16.65±0.94	14.84±1.48

Ca	2.99±0.33	3.96±1.41	2.88±0.21	2.94±0.52	3.40±0.70	2.39±0.19
Mn	51.71±14.81	101.06±69.58	43.80±8.81	58.83±23.78	56.67±8.04	41.27±8.36
Fe	2773.92±1581.59	7516.81±6038.32	2285.71±723.17	3320.64±2228.92	2825.24±728.29	1758.14±633.59
Cu	**18.10±6.61a**	**35.38±7.98b**	**13.94±3.17a**	**13.82±2.40a**	**15.92±5.96a**	**12.45±4.88a**
Zn	46.43±6.40	66.12±19.37	40.40±0.45	49.08±6.25	52.29±6.27	41.44±0.79
Mo	1.28±0.45	0.92±0.31	0.91±0.19	0.87±0.34	0.80±0.37	0.86±0.35

Table S4. One-way ANOVA analysis of bacteria 16S community alpha diversity based on Shannon index.

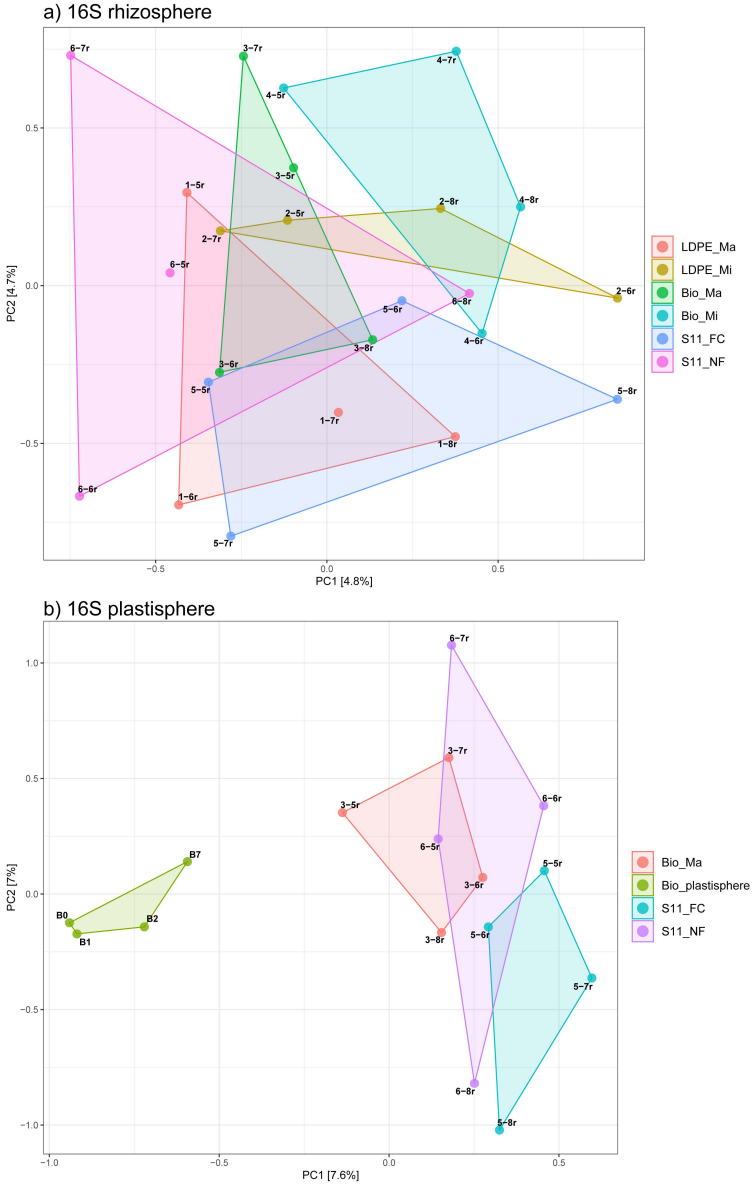
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatments	6	0.3144	0.05240	2.488	0.0561
Residuals	21	0.4422	0.02106		

Table S5. One-way ANOVA and Tuckey posthoc analysis of fungi ITS community alpha diversity based on Shannon index.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatments	6	5.534	0.9224	4.207	0.00621
Residuals	21	4.604	0.2192		

Treatments	diff	lwr	upr	p adj
LDPE_Mi-LDPE_Ma	0,85	-0,23	1,93	0,19
Bio_Ma-LDPE_Ma	0,81	-0,27	1,89	0,23
Bio_Mi-LDPE_Ma	0,44	-0,63	1,52	0,83
Bio_plastisphere-LDPE_Ma	-0,43	-1,50	0,65	0,85
S11_FC-LDPE_Ma	0,59	-0,48	1,67	0,57
S11_NF-LDPE_Ma	0,77	-0,31	1,84	0,28
Bio_Ma-LDPE_Mi	-0,04	-1,11	1,04	1,00
Bio_Mi-LDPE_Mi	-0,41	-1,48	0,67	0,87
Bio_plastisphere-LDPE_Mi	-1,28	-2,35	-0,20	0,01
S11_FC-LDPE_Mi	-0,26	-1,33	0,82	0,98
S11_NF-LDPE_Mi	-0,08	-1,16	0,99	1,00
Bio_Mi-Bio_Ma	-0,37	-1,45	0,71	0,92
Bio_plastisphere-Bio_Ma	-1,24	-2,32	-0,16	0,02
S11_FC-Bio_Ma	-0,22	-1,30	0,86	0,99
S11_NF-Bio_Ma	-0,04	-1,12	1,03	1,00

Bio_plastisphere-Bio_Mi	-0,87	-1,95	0,21	0,17
S11_FC-Bio_Mi	0,15	-0,93	1,23	1,00
S11_NF-Bio_Mi	0,33	-0,75	1,40	0,95
S11_FC-Bio_plastisphere	1,02	-0,06	2,10	0,07
S11_NF-Bio_plastisphere	1,20	0,12	2,27	0,02
S11_NF-S11_FC	0,18	-0,90	1,25	1,00



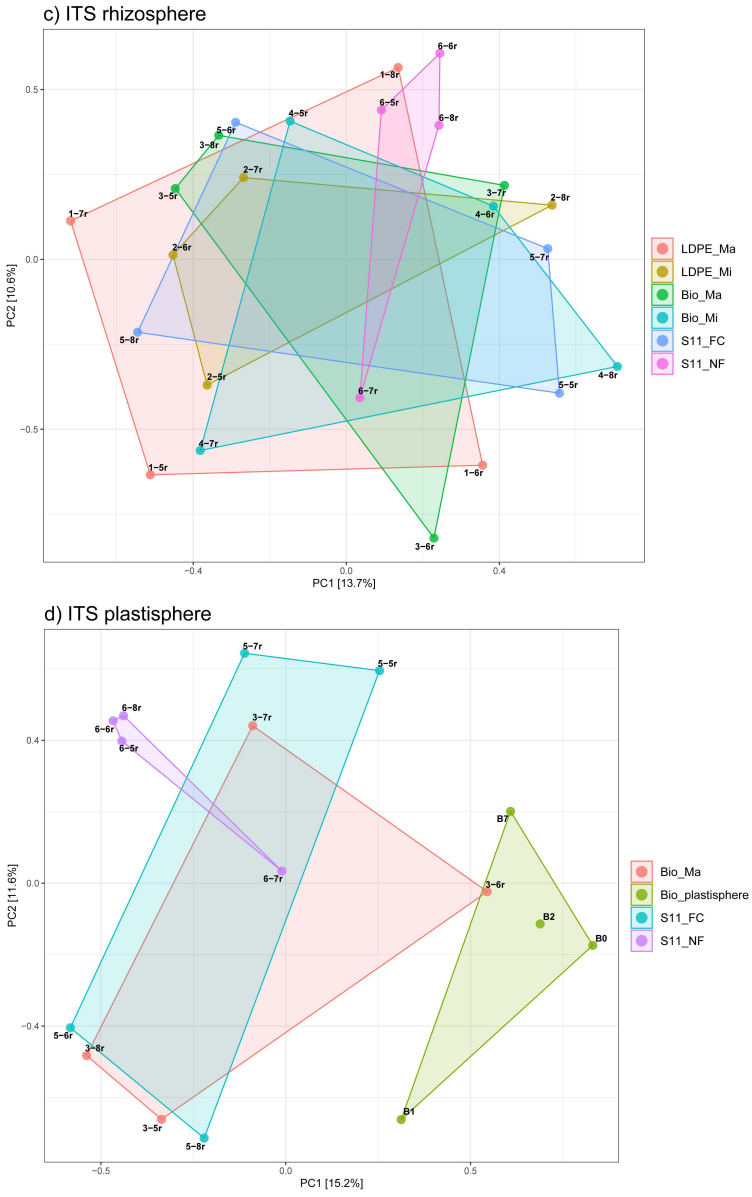


Fig. S1. Principal Component Analysis of bacterial (16S) and fungal (ITS) communities. ANOSIM statistics is indicated in the left-bottom corner of each plot.