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Effects of plastic mulch film residues on wheat rhizosphere and soil properties

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Effects of plastic mulch film residues on wheat rhizosphere and soil properties

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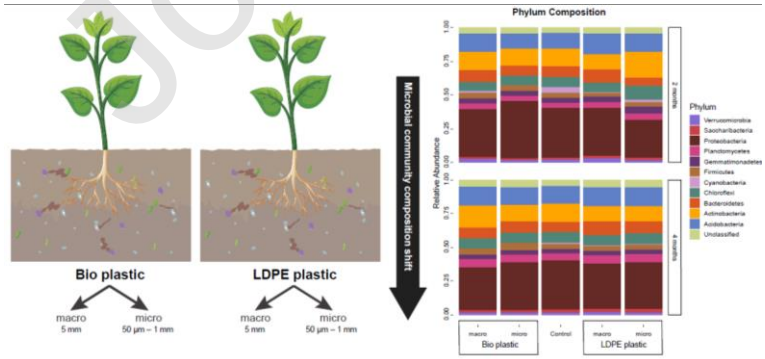
Graphical abstract



Highlights

- Effects of microplastics on wheat rhizosphere bacterial communities were determined.
- The effects of microplastics on volatile profiles and soil properties were significant.
- High amount of dodecanal was detected in treatment with biodegradable microplastics.
- Both LDPE and biodegradable microplastics affected soil stoichiometric C:N ratio.

Abstract

Plastic residues could accumulate in soils as a consequence of using plastic mulching, which results in a serious environmental concern for agroecosystems. As an alternative, biodegradable plastic films stand as promising products to minimize plastic debris accumulation and reduce soil pollution. However, the effects of residues from traditional and biodegradable plastic films on the soil-plant system are not well studied. In this study, we used a controlled pot experiment to investigate the effects of macro- and micro-sized residues of low-density polyethylene and biodegradable plastic mulch films on the rhizosphere bacterial communities, rhizosphere volatile profiles and soil chemical properties. Interestingly, we identified significant effects of biodegradable plastic residues on the rhizosphere bacterial communities and on the blend of volatiles emitted in the rhizosphere. For example, in treatments with biodegradable plastics, bacteria genera like *Bacillus* and *Variovorax* were present in higher relative abundances and volatile compounds like dodecanal were exclusively produced in treatment with biodegradable microplastics. Furthermore, significant differences in soil pH, electrical conductivity and C:N ratio were observed across treatments. Our study provides evidence for both biotic and abiotic impacts of plastic residues on the soil-plant system, suggesting the urgent need for more research examining their environmental impacts on agroecosystems.

Keywords: microplastics; biodegradable plastics; rhizosphere microbiome; volatile organic compounds; soil properties

1. Introduction

Microbial communities are essential for ecosystem functions and services including the decomposition of organic matter, toxin removal, nutrient cycling, plant growth promotion and soil-borne diseases suppression (Brussaard et al., 2007). These functions and services are the results of a multitude of interactions within distinct soil/rhizosphere microbial taxa and between microbial communities and plants (Bakker et al., 2014). Secondary metabolites (i.e. volatiles and non-volatiles) play important roles in belowground microbe-microbe and plant-microbe interactions (van Dam and Bouwmeester, 2016; Weisskopf et al., 2016). For example, plants have the ability to recruit specific soil microorganisms from a distance via root-emitted volatiles (Schulz-Bohm et al., 2018; van Dam et al., 2016). However, relatively little is known about the extent to which anthropogenic pollution such as microplastics (MPs) can affect belowground plant-microbe interactions and volatile profiles.

MPs (defined as plastic particles < 5 mm in diameter) are recognised as an emerging threat to both aquatic and terrestrial ecosystems (Cole et al., 2011; Rillig, 2012). However, the environmental impacts of MPs on terrestrial ecosystems remain largely unknown (Machado et al., 2018a; Ng et al., 2018). According to the recent literatures, farmlands may store more MPs than oceans (Nizzetto et al., 2016a; Nizzetto et al., 2016b; Van Sebille et al., 2015). The use of plastic mulch films is considered to be the main human activity that is contributing to microplastic pollution in agroecosystems (Machado et al., 2018a; Ng et al., 2018). Plastic mulch films have been used increasingly worldwide due to the well-known short-term benefits (e.g., maintaining soil moisture and temperature, preventing weeds, limiting soil erosion), all of which ultimately contribute to the enhancement of crop productivity (Gao et al., 2019; Steinmetz et al., 2016). However, the threats posed by plastic debris accumulating in soil have only been pointed out in recent years (Liu et al., 2014; Yan et al., 2014). Studies have shown that the accumulation of plastic film residues can significantly affect soil quality and crop growth in a negative way (Dong et al., 2015; Qi et al., 2018)

Biodegradable (Bio) plastic mulch films are expected to degrade completely after being tilled into the soil (Brodhagen et al., 2017; Kasirajan and Ngouajio, 2012). However, the short- and long-term ecological impacts of Bio plastic mulch films on agroecosystem remain largely unknown (Bandopadhyay et al., 2018; Sintim and Flury, 2017). In general, knowledge concerning the

degradation or persistence of MPs in soils is scarce, mostly due to the lack of established quantitative and qualitative analytical methods. Thus, so far, most of our knowledge is based on sporadic field surveys that examine MPs in terrestrial ecosystems (Liu et al., 2018; Scheurer and Bigalke, 2018; Zhang and Liu, 2018; Zhou et al., 2018). Furthermore, it is still unclear how plants respond to the presence of MPs in soil and how MPs affect plant-microbe interactions and the overall composition and function of the rhizosphere microbiome (Qi et al., 2018; Rillig et al., 2019b). The rhizosphere is the critical interface between plant roots and the soil matrix where beneficial and harmful interactions between plants and microorganisms take place (Mendes et al., 2013). Moreover, it is important to realize that macro- (Ma) and micro- (Mi) sized plastic residues may affect plant-microbe interactions and the rhizosphere microbiome in a different manner. This is likely to occur due to differences in the physicochemical properties and surface/volume ratios of different sized residues (Brodhagen et al., 2017; Machado et al., 2018a).

Here we conducted a well-controlled pot experiment using wheat plants to test the effect of Ma and Mi sized low-density polyethylene (LDPE) and Bio plastic residues on the assembly of rhizosphere bacterial communities, emission of volatile organic compounds and soil properties. To this end, we used an environmentally relevant concentration of plastic residues (i.e., 1%, w/w) (Fuller and Gautam, 2016; Machado et al., 2018b; Qi et al., 2018). We hypothesized that plastic residues affect the soil chemistry and biology, and these effects vary according to plastic types and sizes.

2. Materials and Methods

2.1 Experimental design and soil sampling

The experimental design comprised two types of plastic mulch films (LDPE and Bio) and two sizes of plastic residues (Ma and Mi). The Ma size residues were manually cut in rectangular pieces with side length ranging from 4 mm to 10 mm, and the Mi size residues were frozen ground powders with size ranging from 50 μm to 1 mm. Additional information on these plastic materials are provided in Figure S1 and reported in a previous study (Qi et al., 2018). Control treatment without plastic residues was also included. In total, fifty pots were used to grow wheat (*Triticum aestivum*). They were divided into five treatments, as follows: (*i*) LDPE-Ma: addition of 1% (w/w) LDPE macroplastics; (*ii*) LDPE-Mi: addition

of 1% (w/w) LDPE MPs; (*iii*) Bio-Ma: addition of 1% (w/w) Bio macroplastics; (*iv*) Bio-Mi: addition of 1% (w/w) Bio MPs; (*v*) Control.

The experiment was conducted in a climate chamber at Unifarm, Wageningen University & Research (WUR), the Netherlands (March ~ August, 2017). Our test soil was a sandy soil collected by Unifarm, WUR from the agricultural land in Wageningen, the Netherlands. Further information of the soil were presented in Figure S2 and reported elsewhere (Qi et al., 2018). To make 1% (w/w) plastic residues mixture, we spiked 15 g of the respective plastic material in 1500 g test soil for each pot. Wheat seeds were sowed in the 2 L plastic pots and cultivated under the temperature and light controlled conditions. The temperature was set at 22 °C during the day and 17 °C during the night, day/night photoperiod (14/10 h) with a light intensity of 300 µmol m^{-2} s^{-1}. Details of the materials and the cultivation of plants followed the same protocols as previously described (Qi et al., 2018). The experiment was harvested at two plant growth stages, i.e. 61st day (2 months) when the flag leaf appeared and 139th day (4 months) after seeds were sowed when the mature grains developed, representing for vegetative and reproductive growth. At each time point, five pots were harvested and plants were completely removed from the pots. Rhizosphere soil samples were collected after gently shaken the roots to remove the loosely adhered soil and they were immediately stored at -20°C for further analysis. Bulk soil was sampled from pots without plants, air-dried and stored at room temperature.

2.2 Measurements of soil properties and plant biomass

The soil pH, electrical conductivity (EC) and C:N ratio are fundamental soil properties which are closely related to soil chemistry and biology, and therefore they were measured for the collected bulk soil samples. Before the experiment started, soil pH, EC and C:N ratio of test soil were measured as the initial values. To determine the pH and EC, a SenTix meter and a conductivity cell TetraCon 325 was used with a soil-to-water ratio of 1:5. For the C:N ratio measurements, five to six milligrams of ground soil were filled in a small tin cup, gently folded into a solid ball and analyzed by FlashEA 1112 series NC Analyzer (Thermo Fisher Scientific, CA, USA). For both sampling points, plant shoot and root biomasses were obtained after drying the plant materials at 70°C.

Statistical analyses were performed by IBM SPSS Statistics 23 and R version 3.5.0. Comparisons across treatments for soil properties were conducted by independent one-way analysis of variance (ANOVA), followed by Tukey HSD test. The level of significance was established at $p < 0.05$.

2.3 DNA extraction, Illumina sequencing and bioinformatics analysis

Soil DNA was extracted using the QIAGEN DNeasy PowerSoil Kits (Qiagen Benelux B.V., Venlo, the Netherlands), following the manufacturer's protocol. The quantity and quality of extracted DNA samples were determined using a Nanodrop ND-2000 (Thermo Fisher Scientific, CA, USA), and the DNA integrity was checked by electrophoresis on agarose gel (1% w/v). The PCRs of bacterial 16S rRNA gene V3-V4 region was performed with the primer set 341F (5'-CCTACGGGNGGCWGCAG -3') and 785R (5'- GACTACHVGGGTATCTAATCC -3'). Sequencing was carried out on a single lane of Illumina MiSeq platform at BaseClear B.V. (Leiden, Netherlands).

The raw FASTQ files of bacterial sequences were analyzed using the Hydra pipeline (DOI: 10.5281/zenodo.597131). In brief, sequences were quality trimmed and chimeric sequences were removed. After the Hydra pipeline, sequences with ≥97% similarity were clustered into operational taxonomic units (OTUs). Taxonomic information of the OTUs representative sequences was assigned using the SILVA database. Prior to statistical analyses, samples were normalized using the cumulative sum scaling (CSS) method. To improve the normality and homogeneity of the variances, the OTUs table was *z*-score transformed. Predicted OTUs that significantly segregated across treatments were identified by random forest analysis using the Boruta feature selection (Breiman, 2001; Kursa and Rudnicki, 2010). All statistical inferences and data plotting were done in R version 3.5.0.

2.4 Volatile trapping and measurement

For the collection of volatiles, polydimethylsiloxane (PDMS)-silicone tubes were conditioned and buried in the wheat rhizosphere for 20 min before final harvest, as described by Huerta Lwanga et al. (2018). The PDMS tubes were stored at -20°C before analyzed by GC-Q-TOF (Agilent 7890B GC and the Agilent 7200A QTOF, USA). The measuring conditions and parameters were previously described by Huerta Lwanga et al. (2018). The acquired mass spectra data were processed with MZmine 2.14.2

(Pluskal et al., 2010), in a similar way as described by Schulz-Bohm et al. (2015). The identification of volatile compounds was evaluated using the software AMDIS 2.72. The retention indexes were calculated and compared with those in the NIST 2014 database and using an available *in-house* database. The statistical analysis was performed using MetaboAnalyst V4.0 (http://www.metaboanalyst.ca).

3. Results and Discussion

3.1 Effect of plastic residues on rhizosphere bacterial community

To study the rhizosphere bacterial community, rhizosphere soils were sampled and examined by high throughput 16S rRNA amplicon sequencing at 2 and 4 months of wheat growth. The relative abundance of bacterial OTUs in the wheat rhizosphere at the phylum level varied among treatments (Figure 1). Across treatments, the bacterial community at the phylum level was dominated by Proteobacteria (35.9% of the total on average) followed by Actinobacteria (14.0%) and Acidobacteria (13.4%) (Figure 1). This pattern in phyla composition aligns with what others have described for the wheat rhizosphere (Donn et al., 2015; Fan et al., 2018), which most likely occurs because these phyla also constitute the dominant strains found in soils on the global scale (Delgado-Baquerizo et al., 2018).

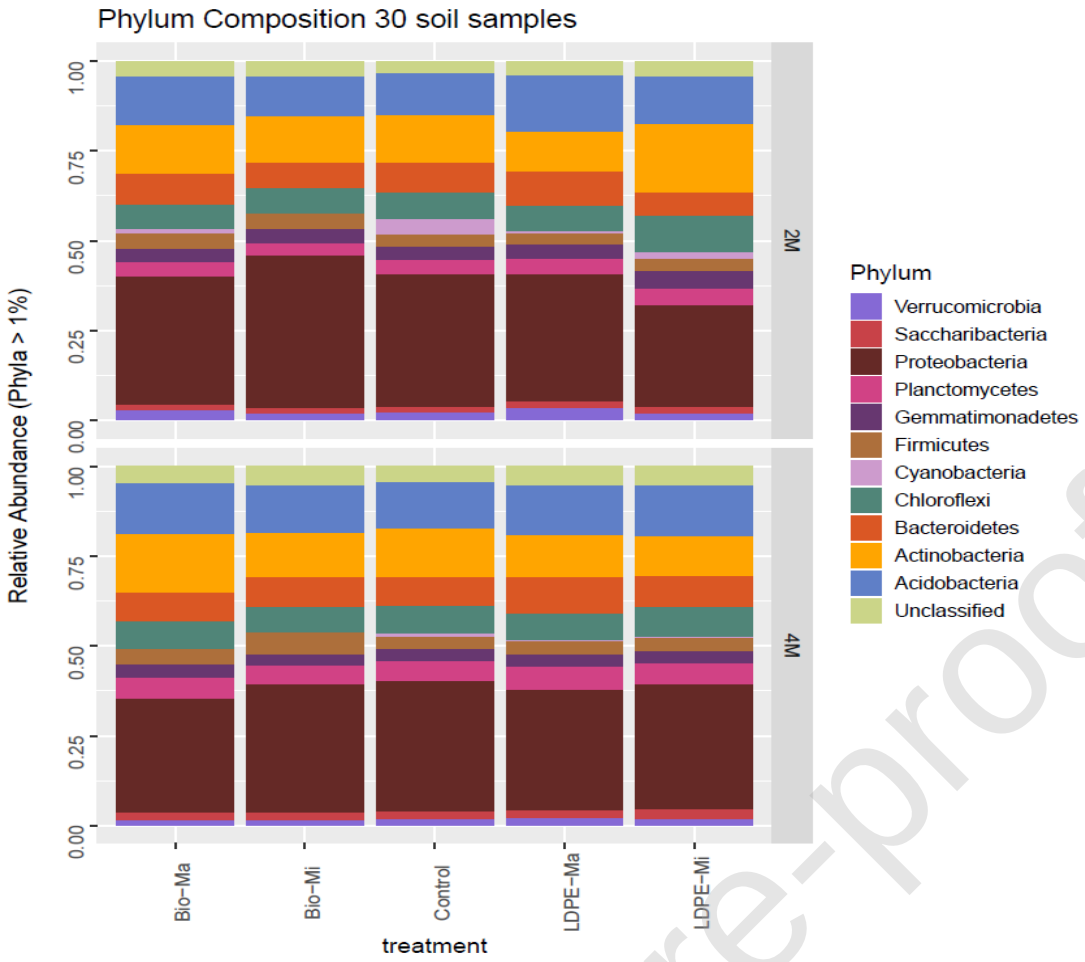


Figure 1. Bar charts displaying the most abundant bacterial phyla (phyla relative abundance > 1%) in the community structure of each individual treatment at 2 and 4 months.

The total plant biomass was significantly reduced with the addition of plastic residues at both time points and the treatments Bio-Ma and Bio-Mi revealed the strongest negative effect (Figure 2A). The negative effects of plastic residues on wheat development during the growth process were previously discussed and reported (Qi et al., 2018). Beta-diversity analysis based on Bray-Curtis distances were conducted to examine the separation among bacterial communities across treatments. The first principal coordinate axis showed that the rhizosphere soil at 2 and 4 months had distinct bacterial community structures (Figure 2B). The different treatments were clearly separated along the second principal coordinate axis and treatments exposed to plastic residues had significantly different bacterial communities compared to the Control, thus indicating that the presence of plastic residues in the soil had significant effects on the wheat rhizosphere bacterial communities (Figure 2B).

Furthermore, differential abundance analysis using random forest revealed that specific bacterial genera (e.g. *Bacillus*, *Variovorax*, *Comamonadaceae*, etc.) were present in higher relative abundances in treatments with Bio plastics, while some specific genera (e.g. *Bradyrhizobium*, *Cellvibrio*, etc.) significantly increased in relative abundances in the treatment Bio-Mi (Figure 2C). Collectively, these results indicate that plastic residues can impose selective pressure on distinct microbial taxa as anthropogenic substrates. In line with that, the presence of LDPE residues also had an effect on the assembly of the rhizosphere bacterial community. For instance, bacteria taxa affiliated with the genus *Saccharibacteria* were higher in relative abundance in the treatments with LDPE plastics (Figure 2C).

Regarding the effect of the sizes of plastic residues, bacterial community structures in the treatments Bio-Mi and Bio-Ma were clearly separated, as shown in the non-metric multidimensional scaling (NMDS) plot (Figure 2B). This suggests that different sizes of plastic residues may exert different influences on the rhizosphere microbiome. Plausibly, the physicochemical surface properties of plastic residues may play specific roles in their effects. Comparable results were reported for an aquatic ecosystem where the shape of plastic debris (i.e. plastic sheet and dolly rope) significantly affected the bacterial community composition of the biofilm formed on the plastic debris (De Tender et al., 2017). Considering numerous types, sizes and shapes of MPs in the ecosystem (Cole et al., 2011), it is critical to further study how the physical and chemical properties of plastic residues influence their environmental effects.

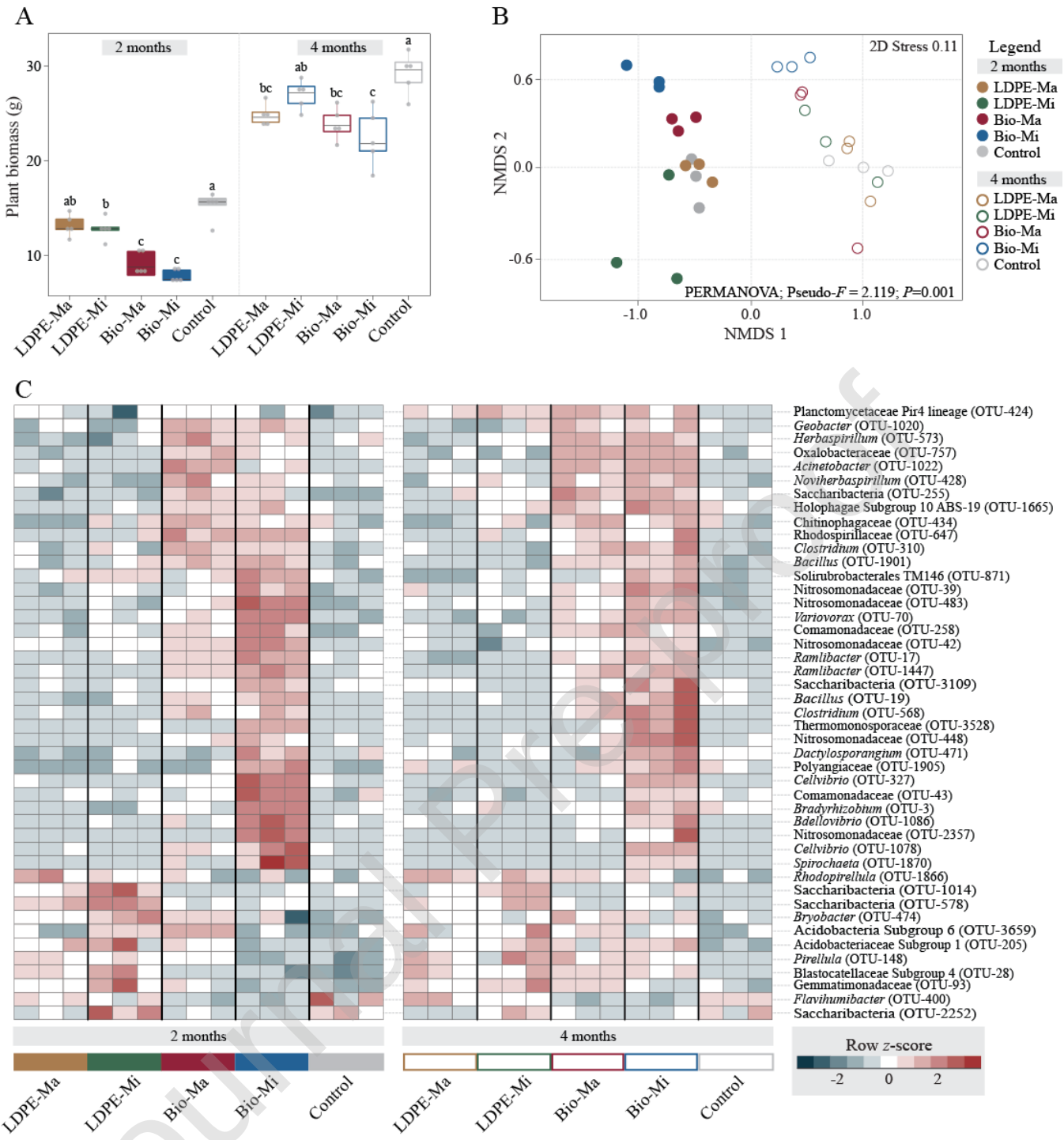


Figure 2. (A) Total biomass of wheat in each treatment for samples collected after 2 and 4 months. (B) Beta-diversity biplot of bacterial communities displayed by non-metric multidimensional scaling (NMDS); (C) Relative abundance of significantly different OTUs across treatments identified by random forest analysis.

Overall, our results clearly revealed that Bio plastics had stronger effects on the composition of the wheat rhizosphere bacterial communities. One possible explanation is that the chemical composition

of LDPE and Bio plastic are very different. The Bio plastics used in this study consisted mainly of pullulan, polyethylene terephthalate and polybutylene terephthalate, while the LDPE mulch film is a linear hydrocarbon polymer consisting of ethylene monomers. On the other hand, LDPE is a polymer resistant to degradation with remarkable chemical inertness (Restrepo-Flórez et al., 2014). Another possible explanation is the fact that our experiment was restricted to four months, so it is plausible that Bio plastics had a quick and abrupt effect on the soil microbial community and activity, especially the smaller sized Bio plastic residues (Mi, 50 µm – 1 mm in this study) (Bandopadhyay et al., 2018; Haider et al., 2019).

Moreover, soil bacteria are known to be attracted to easily degradable root exudates and mucilage present in plant roots and in soil (Lugtenberg and Kamilova, 2009). Following this line of reasoning, it is also possible to speculate that the presence of Bio plastic residues in soil may also attract and/or favour specific bacterial taxa and interfere with belowground plant-microbe interactions. Although only bacterial communities were investigated in this study, it is likely that other organismal taxa (e.g. fungi, archaea and protists) within this system would also be affected, thus resulting in more complex impacts on biological interactions in the rhizosphere. (Fan et al., 2018). We propose that the negative effects on plant growth are – at least in part – caused by the influence of plastic residues on the rhizosphere microbiome and the potential disruption of beneficial plant-microbe interactions.

3.2 Effect of plastic residues on rhizosphere volatile organic compounds

Secondary metabolites (both volatile and non-volatile) play important roles in plant-microbe interactions. More specifically, the chemical composition of volatile metabolites in the rhizosphere is crucial for soil below-ground interactions (Massalha et al., 2017). In this study, we collected and analysed the volatiles emitted in the rhizosphere of wheat at the end of the experiment. Our results revealed that the addition of plastic residues significantly affected the blend of volatiles emitted in the rhizosphere (Figure 3). The PLS-DA score plots showed that the treatments Bio-Ma and Bio-Mi had significantly different blends of volatile compounds compared to the LDPE and Control treatments (Figure 3A). The heatmap clearly displays that some compounds were exclusively produced in treatments with Bio plastics. Furthermore, differences in volatile profiles were observed between the treatments with different plastic sizes (Figure

3B). Interestingly, distinct volatiles were found only in the Bio-Mi treatment (Table S1), such as high amounts of dodecanal. Dodecanal is known to be produced by bacteria and have negative effects on both fungal and plant growth (Kai et al., 2007; Vespermann et al., 2007). In addition, a recent study indicated that some volatiles are the by-products of bacterial MPs decay in soil (Huerta Lwanga et al., 2018). Several studies conducted in the past decades have indicated that volatile compounds can have plant growth-inducing or growth-inhibiting effects, e.g. through the modulation of plant hormonal balance, metabolism, and nutrient acquisition (Fincheira and Quiroz, 2018). Although the mechanisms of differential volatile emissions in the rhizosphere remain largely unknown, the variations observed for volatiles in the presence of plastic residues might be – at least in part – another reason accounting for the observed negative effects of plastic residues on wheat growth.

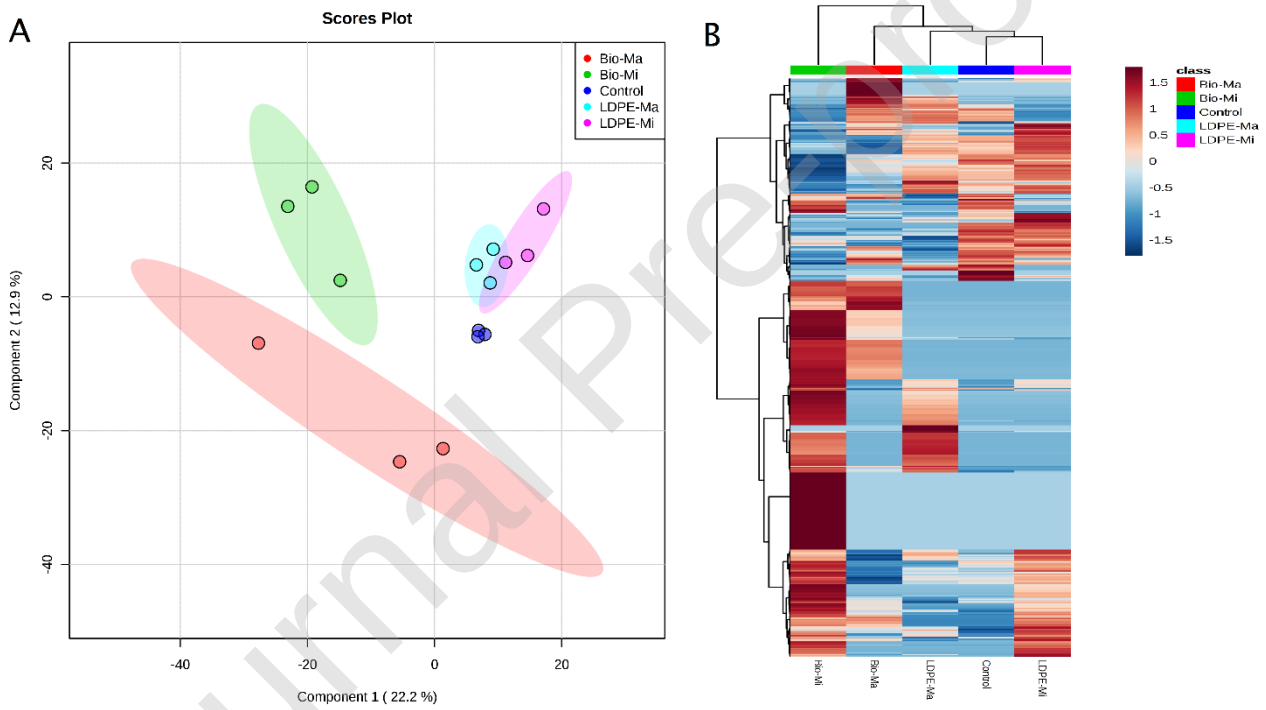


Figure 3. (A) Score plot based on partial least square-discriminant analysis (PLS-DA) of volatile profiles emitted in the rhizosphere of wheat at 4 months; (B) Heatmap displaying the volatile profiles in the rhizosphere of wheat. Each column represents three collated replicate measurements per treatment. Coloured cells on the map correspond to the concentration value per compound (blue: low abundance; red: high abundance).

3.3 Effect of plastic residues on soil chemical properties

In order to gain a comprehensive biogeochemical understanding of plastic residues in soil, there has been an increasing emphasis placed on examining the potential impacts of microplastic pollution on soil physicochemical properties (Machado et al., 2018b). Here, we specifically tested for variations in soil pH, EC and C:N ratio in our experimental system. For all treatments, an increase in pH and decrease in EC were observed as compared to the initial values (Table 1). For both time points, LDPE-Mi had the highest EC (390 ± 119.39 µS/cm at 2 months, 179 ± 76.73 µS/cm at 4 months) and Bio-Mi had the lowest EC (130 ± 48.42 µS/cm at 2 months, 75 ± 15.58 µS/cm at 4 months) (Table 1). Although soil acidification and the decrease in EC are well-known challenges for sustainable agriculture (Miao et al., 2010), both soil pH and EC are influenced by many factors and they should not be directly correlated with crop growth (Atkinson et al., 2010; Humphreys et al., 2005). In addition, Dong et al. (2015) studied large sizes of plastic mulch film residues (0 - 200 cm^2) in cotton fields with the density gradient ranging from 250 to 2000 kg hm^{-2} and found that the increase of residual mulch films impacted soil quality, e.g. increased pH, decreased organic matter, and negatively affected the overall nutrient availability. In that study, they proposed that the distinct tolerance to plastic residues of two varieties of cotton may be caused by their different root systems (Dong et al., 2015).

Table 1. Soil pH, electrical conductivity (EC) and C:N ratio values measured for the bulk soil samples in each treatment collected at 2 and 4 months.

		pH (initial 6.55±0.047)			EC (initial 411±18.33) µS/cm			C:N ratio (initial 16.67±1.008)		
		mean	SD	sig	mean	SD	sig	mean	SD	sig
2M	LDPE-Ma	6.74	0.059	b	250	56.50	bc	16.28	1.015	bc
	LDPE-Mi	6.79	0.137	ab	390	119.39	a	23.32	5.130	a
	Bio-Ma	6.81	0.115	ab	182	86.01	cd	15.56	0.577	c
	Bio-Mi	6.90	0.045	a	130	48.42	d	19.59	3.120	ab
	Control	6.72	0.075	b	339	68.07	ab	15.94	0.579	c
4M	LDPE-Ma	6.86	0.064	B	136	54.05	AB	15.98	0.804	B
	LDPE-Mi	6.91	0.070	AB	179	76.73	A	19.43	2.234	A
	Bio-Ma	6.91	0.041	AB	106	42.05	B	15.72	0.466	B
	Bio-Mi	6.96	0.126	AB	75	15.58	B	18.84	1.485	A
	Control	7.01	0.094	A	103	52.38	B	15.84	0.593	B

2M: 2 months harvest; 4M: 4 months harvest; SD: standard deviation; lowercase letters in column sig mean significant differences at 2 months; uppercase letters in column sig mean significant differences at 4 months.

For the C:N ratio, treatments with Mi size residues (i.e. LDPE-Mi and Bio-Mi) had significantly higher values compared to the Control at both time points (Table 1). The treatment LDPE-Mi had the highest C:N ratio at 2 (23.32 ± 5.130) and 4 months (19.43 ± 2.234) across all treatments (Table 1). Together with the effects of plastic residues on rhizosphere bacterial communities and volatile profiles, our experiment provides strong evidence supporting the biotic and abiotic impacts of plastic residues on the soil-plant system. Recently, researchers also observed that exposing soil to four different types of MPs with concentrations of up to 2% affected microbial activity and soil physical properties (e.g. bulk density and water holding capacity) (Machado et al., 2018b). Although plastic particles have a relatively high content of carbon, most of it is relatively inert, which hinders the decomposition of MPs (Rillig, 2018). Thus, the carbon in plastic residues could affect the carbon cycle and soil microorganisms (Rillig et al., 2019a). Furthermore, it was recently proposed that due to the slow degradation rate, the progressive accumulation of MPs in soils can result in a very wide C:N ratio that leads to microbial immobilization (Rillig et al., 2019b).

4. Conclusion

Here we showed that both LDPE and Bio plastic mulch film residues have strong (albeit different) effects on wheat growth, rhizosphere bacterial community composition and structure, rhizosphere volatile profiles and soil chemical properties. Given the rapidly increasing global accumulation of plastic fragments in soils, a better understanding of the impact of such residues on complex interactions that take place in the soil-plant system is urgently needed. In this sense, this study provides evidence that highlights how plants, soil microbes and chemistry respond to plastic residues under controlled experimental conditions. As such, we advocate for further research efforts aiming at developing prospective experimental designs and field surveys to broaden our understanding of the mechanisms by which conventional and biodegradable plastics affect the soil ecosystem, particularly in agricultural settings.

Declaration of 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.

Declarations of interest: none.

Author Contribution Statement

Yueling Qi: Conceptualization, Investigation, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization. **Adam Ossowicki:** Formal analysis, Software, Writing - Review & Editing, Visualization. **Xiaomei Yang:** Conceptualization, Writing - Review & Editing. **Esperanza Huerta Lwanga:** Conceptualization, Writing - Review & Editing. **Francisco Dini-Andreote:** Formal analysis, Software, Writing - Review & Editing, Visualization. **Violette Geissen:** Conceptualization, Resources, Supervision. **Paolina Garbeva:** Conceptualization, Resources, Writing - Review & Editing, Supervision.

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