



# Microbiome dynamics of soils covered by plastic and bioplastic mulches

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## Abstract

In recent decades, the use of plastic mulch in agriculture has largely increased to meet the growing demand for food. Despite their potential benefits, it is still unknown the long-term impact of mulches on soil microbiome. In this study, we compared at a mesocosm level the effects of polyethylene (Plastic) and Mater-bi® (Bioplastic) mulches on the soil physico-chemical (i.e., pH, soil water content, Fourier transform infrared-attenuated total reflection-FTIR), microbiological, and biochemical (i.e., microbial respiration, enzymatic activities, abundances and composition of bacterial, fungal and microarthropod communities) properties after 6 and 12 months. The analysis of the microbiome revealed an increase in bacterial richness and diversity in the 12-month-treated bioplastic soils. Members of *Solirubrobacterales*, *Vicinamibacterales*, *Nitrososphaerales*, *Crenarchaeota*, and *Clostridiales* were the most abundant following the bioplastic treatment. While the fungal and microarthropod communities varied over time, neither of them was affected by the type of mulching. Further longer-term research is needed to clarify the impact of bioplastic and plastic mulches on the soil microbiome, including microarthropods, and its dynamics over time.

**Keywords** Soil community · Plastics · Bioplastics

## Introduction

The use of plastics in agriculture has increased considerably in recent decades because the application of plastic mulches contributes to weed growth prevention, soil erosion and higher soil temperature (Zhang et al. 2013; Blaise et al.

2021). Despite these potential advantages, the widespread and long-term use of plastic mulches combined with a lack of systematic collection and management may cause their accumulation in soils (Steinmetz et al. 2016). Plastic weathering causes the release of macro- (> 5mm) and microplastics (100µm < MPs < 5mm) that negatively impact the soil quality (de Souza Machado et al. 2018; Buks and Kauptenjohnann 2020; Pathan et al. 2020; Ren et al. 2020; Wang et al. 2021). Enzyme activities are sensitive to environmental changes and may affect soil C and N cycles (Adetunji et al. 2017; Feng et al. 2019). Moreover, although enzyme activities represent some stepwise processes of complex biogeochemical cycles (Nannipieri et al. 2018), they are widely used as indicators for organic matter turnover.

Polyethylene is the non-degradable plastic material conventionally used for agricultural mulch (Hayes et al. 2012) and thus it is the major source of microplastics (MPs) in agricultural soils (Kasirajan and Ngouajio 2012; Blasting and Amelung 2018; Wang et al. 2021). The addition of polyethylene to soil reduces both microbial activity and richness (Fei et al. 2020; Shi et al. 2022). The use of environmentally friendly biodegradable plastics might be a promising

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substitute for conventional non-degradable plastics (Qin et al. 2021). However, fragmentation of bioplastic mulches can also occur under a range of environmental conditions with the subsequent release of micro-bioplastics into agricultural soils (Li et al. 2014; Qin et al. 2021). Micro-bioplastics provide selective niches for soil microorganisms because they can be used as an exogenous carbon source (Brodhagen et al. 2015; Zhou et al. 2021), and as such they can influence soil microbial community composition more than MPs (Qi et al. 2020; Wang et al. 2022).

According to some studies, the effect of biodegradable mulch on the microbial community is minimal and insignificant (Kapanen et al. 2008; Masui et al. 2011; Adhikari et al. 2016; Bandopadhyay et al. 2020; Sintim et al. 2020, 2021). For instance, Masui et al. (2011) did not observe any increase in total bacteria abundance or any change in bacteria community composition where poly-lactic acid (PLA) was buried in the soil after its use. Besides, Adhikari et al. (2016) observed no significant differences in soil bacterial biomass and diversity after two years of exposure to PLA. However, it is unknown how conventional plastic and bioplastic mulches affect the activity and diversity of the microbiome and microarthropods in soil.

To date, only two studies report behavioral effects on soil microarthropods due to mulch-derived MPs. Maaß et al. (2017) showed how collemboli (*Folsomia candida* and *Proisotoma minuta*) were able to transport MPs both horizontally and vertically leading to an increase in their dispersion along the soil column. Likewise, this transport/dispersion may be enhanced (Zhu et al. 2018) due to predator-prey relationships (*Hypoaspis aculeifer* and *Folsomia candida*). In contrast, no evidence has been reported on the effects of MPs on soil microarthropod biomass and species richness.

The present study was performed on soils exposed to both conventional and biodegradable mulches over a 1-year period with the aim of shedding light onto the effects of plastic mulching on soil physicochemical properties and enzymatic activities, as well as on the biomass, richness, and composition of soil bacteria, fungi, and microarthropods. The starting hypotheses of this research are as follows: (Hi) mulching, both with polyethylene and bioplastics, differentially changes the abiotic properties of the soil compared to

the control; (Hii) due to these differential changes in soil abiotic properties, the biomass and the functional diversity of microbial community are lower in soils covered by conventional mulches than in control soils and those covered by bioplastic mulches; (Hiii) microarthropods are affected by plastic mulching, although to a lower extent than bacteria and fungi.

## Material and methods

### Experimental setup and sample collection

Limestones of different sizes ( $\emptyset = 1\text{--}4$  cm) taken from a quarry near Caserta (Italy) was put in mesocosms ( $\emptyset = 1$  m). In November 2020, 50 kg of fresh soil (equivalent to approximately 30 kg of dry weight, d.w.) was collected from the respective agricultural sites, thoroughly mixed in order to obtain a representative sample, and placed (height = 30 cm) on top of the limestones (height = 20 cm) of each experimental pot (height = 60 cm). Polyethylene plastic (thickness: 40  $\mu\text{m}$ ) was chosen as conventional mulch (plastic) and Mater-bi® (thickness: 18  $\mu\text{m}$ ) as biodegradable mulch (bioplastic). In January 2021, five pots were treated with a conventional plastic sheet and other five with a bioplastic sheet (40  $\times$  40 cm). Both plastics were placed on the soil surface of each pot as 16 squares (10  $\times$  10 cm). Four mesocosms were left uncovered and referred as control treatments. All experimental pots were left outdoors on the terrace of the Biology Department of the University of Naples Federico II for 12 months without irrigation and fertilization. Table 1 reports of the overview of the monthly rainfall, humidity and average air temperature for the investigated period.

At the beginning of the experiment (0 months: January 2021) and prior to placing the mulching sheets, soil samples were collected from the top 10 cm of each experimental pot by a corer device (10 cm  $\emptyset$ ) and then sieved (2 mm  $\emptyset$ ). After 6 (6 months: July 2021) and 12 (12 months: January 2022) months, soil cores (top 10 cm) were sampled under one of the squares of the respective mulching sheets and at a random from the control pots. The first sampling time was after 6 months because the degradation of bioplastic occurs after

**Table 1** Monthly precipitation (mm), mean values of humidity (%), and air temperature ( $^{\circ}\text{C}$ ) in the months of soil samplings (<http://www.ilmeteo.it/>). Soil temperature ( $^{\circ}\text{C}$ ) and moisture (% d.w.) at the sampling times for the different treatments (control, bioplastic and plastic)

	Rainfall (mm)	Humidity (%)	Air T ( $^{\circ}\text{C}$ )	Soil T ( $^{\circ}\text{C}$ )			Soil Moisture (% d.w.)		
				Control	Bioplastic	Plastic	Control	Bioplastic	Plastic
January 2021	6.70	73.3	9.27	8.50 $\pm$ 0.213	10.3 $\pm$ 0.33	10.6 $\pm$ 0.67	40.8 $\pm$ 0.63	39.9 $\pm$ 0.12	37.7 $\pm$ 1.36
July 2021	0.94	59.6	26.6	27.3 $\pm$ 0.156	27.0 $\pm$ 0.23	28.0 $\pm$ 0.57	38.5 $\pm$ 1.50	36.6 $\pm$ 0.88	37.2 $\pm$ 0.73
January 2022	31.0	65.5	9.00	8.67 $\pm$ 0.012	10.0 $\pm$ 0.57	10.7 $\pm$ 0.14	41.2 $\pm$ 0.96	39.4 $\pm$ 1.12	42.2 $\pm$ 0.74

this period (Nissa et al. 2019). Soil samples were divided into three parts: the first part was stored at room temperature prior to the physicochemical analyses; the second part was kept at 4 °C prior to biological and biochemical analyses, and the third one was kept at −80 °C prior to the molecular analyses. Plastic degradation was assessed as weight loss with respect to the initial (0 months) mass of sheet squares randomly collected from the pots at each sampling time (6 and 12 months).

### Soil Fourier transform infrared-Attenuated total reflection (FTIR-ATR) spectra

The infrared spectra of the control soil and the soils treated with plastic or bioplastic was analysed by FTIR-ATR spectra, in the 4000–700  $\text{cm}^{-1}$  range, recorded by solid phase by JASCO FT/IR –4700 Spectrometer using HATR crystal zinc selenide (Flat Plate, PN 022–2020–45) using PIKE Technologies. Each spectrum was measured 128 times, at resolution 4  $\text{cm}^{-1}$ .

### Soil physico-chemical, biological, and biochemical analyses

Soil pH was measured by an electrometric method using a distilled water suspension (1:2.5, volume:volume). Water content (WC) was determined gravimetrically by drying fresh soil at 105 °C until constant weight. Organic total C ( $C_{\text{org}}$ ) was measured by a CNS Analyzer (Thermo Finnigan, Italy) after treatment of soil samples with HCl (10%) to remove carbonates. Total C and N concentrations were measured by CNS Analyzer (Thermo Finnigan, Italy) using oven-dried (105 °C) and grounded (Fritsch Analysette Spartan 3 Pulverisette 0) soil samples.

Microbial respiration (Resp) was assessed using the MicroResp® assays (Macaulay Scientific Consulting, Aberdeen, UK) (Campbell et al. 2003). Five technical replicates of each biological soil sample (circa 0.3 g dry weight) were incubated in a 96-deep well microplate (Fisher Scientific E39199, Illkirch France) (Santini et al. 2022).

Fluorescein diacetate (FDA) activity was determined as reported by Adam and Duncan (2001) and dehydrogenase activity (DHA) as reported by Memoli et al. (2018);  $\beta$ -glucosidase activity ( $\beta$ -glu) was determined as reported by Tabatabai (1982) and urease activity (Ure) as indicated by Kandeler and Gerber (1988).

Phytotoxicological assays were performed according to EPA (1996) using *Sorghum saccharatum* L. and *Lepidium sativum* L. as test organisms and assessed using fresh and sieved (2 mm  $\varnothing$ ) soil samples. Ten seeds were placed in Petri dishes containing fresh soil equivalent to 10 g of oven-dried soil (d.w.); subsequently the soil was saturated with water. Standard soil (OECD 1984) and  $\text{K}_2\text{Cr}_2\text{O}_7$  were used as

negative and positive controls, respectively. After incubation under darkness (72 h, at 25 °C), the number of germinated seeds and the root elongation were measured as described by Santini et al. (2022).

### DNA extraction and qPCR analyses

DNA was extracted from 0.5 g of soil using FastDNA™ SPIN Kit for Soil (MP Biomedicals) as reported by Ceccherini et al. (2007). The yield ( $\text{ng DNA g}^{-1}$  soil) and the purity of the extracted DNA were determined using spectrophotometry (Nanodrop™); DNA quality was assessed by agarose gel electrophoresis.

Specific primers were used to quantify eubacteria (16S rDNA) (Muyzer et al. 1993; Simmons et al. 2007), fungi (18S rDNA) (Chemidlin Prévost-Bouré et al. 2011), and certain bacterial groups involved in the N-cycle:  $\text{N}_2$ -fixing bacteria (*nifH*) (Poly et al. 2001); ammonia-oxidizing bacteria (*amoA*) (Mintie et al. 2003); ammonia-oxidizing archaea (*archamoA*) (Francis et al. 2005); denitrifying bacteria (*nirK*) (Henry et al. 2004). qPCR was performed in 25- $\mu\text{L}$  reactions containing 2X SYBR Green qPCR mix (low rox) (GDSBio, Guangzhou, China), 10  $\mu\text{M}$  each forward and reverse primers, 40 ng of template DNA and sterile  $\text{ddH}_2\text{O}$  to reach the appropriate volume. Sterile  $\text{ddH}_2\text{O}$  was used as a non-template control. Each sample was assayed by AriaMx Real-time PCR System (Agilent Research Laboratories, CA, USA). The run efficiencies ranged from 79.7 to 130.1% with  $R^2$  values ranging from 0.961 to 0.996. In addition, melting curves were performed: temperature ranged from 55 to 95 °C with increments of 0.5 °C; the temperature conditions were kept for 5 s. Nanograms of the target gene were normalized to dry grams of soil in order to compare the different functional groups under the mulching treatments.

### NovaSeq sequencing and bioinformatics pipeline

To characterize the bacterial and fungal microbial communities, the 16S V4 and ITS2 gene regions, respectively, were sequenced from each soil sample's DNA extract by Novogene (Cambridge, UK). The primer sets 515F/806R (Caporaso et al. 2011) and ITS3/ITS4 (Bellemain et al. 2010) were used for bacteria and fungi, respectively. Sequencing was performed on a NovaSeq 6000 instrument using PE250 (250 bp  $\times$  2 paired-end) approach. Sequencing data were deposited in the SRA under project number PRJNA868341.

Sequences were demultiplexed and trimmed off adapters, barcodes and primers by Novogene. A table of amplicon sequence variants (ASV) was obtained using DADA2 pipeline (version 1.18, Callahan et al. 2016) according to the standard protocol provided by the developers on GitHub (version 1.16). Firstly, sequences with ambiguous base pairs and those sequences exceeding an expected error rate of >

2 (maxEE) were removed from the dataset. Error models were predicted from randomly selected samples from the run and both forward and reverse reads were curated based on the error profiles. Sequences were then merged; chimeric sequences were removed. For fungi, reads < 220 bp were removed from the dataset. For each dataset, bacteria and fungi, a frequency ASV table was generated giving the relative read abundances of ASVs in each sample. For bacterial ASVs, their taxonomic assignment was obtained from SILVA reference database v.138.1 by using the assignTaxonomy function in DADA2, which implements RDP naive Bayesian classifier (Quast et al. 2013). Both bacterial and fungal sequences that were not annotated at phylum level (NAs) were discarded. Sequences annotated to the phylum Eukaryota were also removed. For fungal ASVs, their taxonomy was assigned based on the UNITE reference database (version 10.05.2021). A total of 12,095 bacterial ASVs and 2769 fungal ASVs were detected across all samples. For bacteria, the sequencing depth ( $35,000 \pm 14,000$  sequences) did not differ with regards to the experimental factors (time:  $F_{1,40} = 1.927$ ;  $p = 0.173$ ; treatment:  $F_{2,39} = 2.991$ ;  $p = 0.0619$ ). For fungi, the sequencing depth significantly differed over time ( $F_{1,31} = 4.987$ ;  $p = 0.0329$ ; 0 months:  $26,000 \pm 7,500$ ; 6 months:  $48,000 \pm 11,000$ ; 12 months:  $31,000 \pm 12,000$  sequences). Microbial community analysis was performed using the R package vegan (2.6-2; Oksanen et al. 2019). Richness and  $\alpha$ -diversity were estimated as the number of observed ASVs and Shannon index (1948), respectively.  $\beta$ -diversity was estimated by non-metric multidimensional scaling (NMDS) on Bray-Curtis distances in order to evaluate the differences in bacterial and fungal compositions between mulch types over time. The correlation between the experimental factors (mulch types and time) and environmental variables, respectively, and the microbial community composition was analyzed using Adonis2 (Permutational Multivariate Analysis of Variance Using Distance Matrices). Environmental variables were plotted on the NMDS by using envfit command. Permutational models were selected by variable addition following previous knowledge; only models with exclusively significant variables in the linear predictor were interpreted.

For the following analysis, only ASVs detected in three out of four control samples and in four out of five soil samples treated with either plastic or bioplastic mulching were considered. Venn diagrams were generated in order to visualize the number of ASVs shared among sample groups or unique to a sample group (Hulsen et al. 2008). Indicator species were identified by calculating a generalized linear model using ALDEx2 (Fernandes et al. 2013). Principal Component Analysis (PCA) was performed by a singular value decomposition of the centred and scaled data matrix so as to evaluate the differences in the soil properties with regards to the treatment and time. Univariate variables were

analyzed using ANOVA and the significant differences among pairwise sample groups were determined using post-hoc Tukey HSD test. ANOVA assumptions of normality and homogeneity of variances were checked using residual diagnostics. Colours were chosen from RColorBrewer (Neuwirth 2014). All statistical analyses were performed by R 4.2.1 (R Core Team 2022).

## Microarthropod sampling and analyses

Microarthropod analyses were done on soil cores (10-cm depth and 10 cm  $\emptyset$ ) at the beginning (0 months: January 2021) and after 12 months (January 2022) of the trial from each experimental pot, in order to determine the animals to adapt to the new environment. The microarthropods were extracted using the MacFadyen method over a one-week period, sorted using a dissecting stereomicroscope, counted, and identified according to the class or order. The results of each group were reported in terms of density (i.e., individual number  $m^{-2}$  soil), taxa richness and percentage of relative abundance of each taxon. On the basis of the microarthropod abundance and diversity, the Shannon (1948) and Pielou (1969) indices were calculated. For each microarthropod taxon, the trophic preferences (detritivores, predators) were attributed as already reported (Dindal 1990; Brussaard 1997; Lavelle and Spain 2001; Parisi et al. 2005) and showed as the relative abundances (expressed as percentage of the total abundance) of microarthropod trophic preferences.

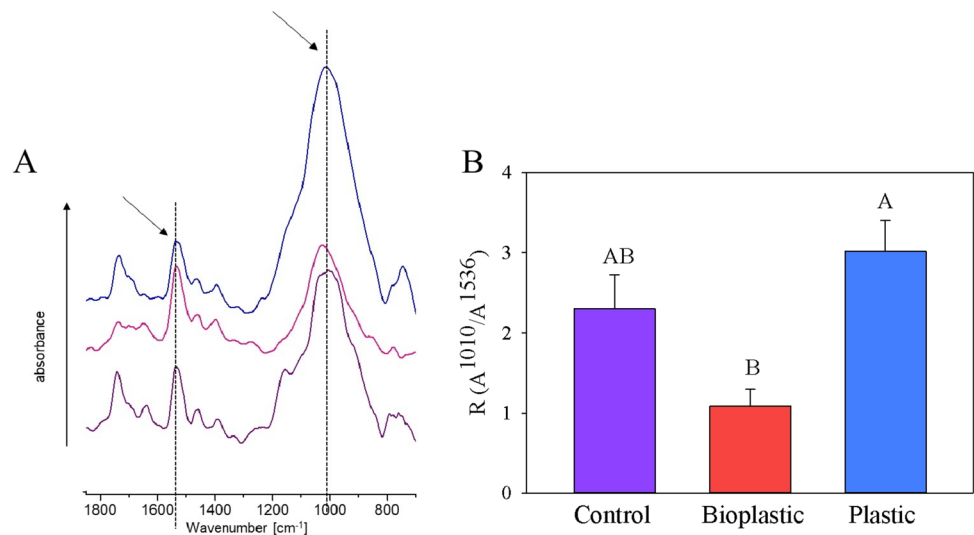
## Results

### Physicochemical properties, microbial abundances, and activities over time and among treatments

The FTIR-ATR spectra revealed differences in the position and the intensity of specific bands due to plastic and bioplastic treatments (Fig. 1A). Those changes occurred in the peak intensity relative to the 1000–1010  $cm^{-1}$  region, which is assigned to C-O-C bending of carbohydrates and/or polysaccharides. In particular, the plastic-treated soil showed a significant higher average ratio of R-areas ( $A^{1010}/A^{1536} = 4$ ) (Fig. 1B) as compared to control and bioplastic-treated soils with ratios of 2 and 3, respectively.

The weight loss of the conventional plastic sheet was 0.1% ( $\pm 0.2$  s.d.) and 6.9% ( $\pm 2.0$  s.d.), and that of the bioplastic sheet was 1.7% ( $\pm 1.2$  s.d.) and 52.9% ( $\pm 9.9$  s.d.), respectively at 6 and 12 months. Irrespective of the treatment, the soil samples were characterized by a water content of around 40% (Table 1) and a slightly alkaline pH (Table 2) over the course of the trial. The microbial abundances at each sampling time and treatment are reported in Table 2. DNA yield after 12 months increased significantly in the

**Fig. 1.** **A** ATR infrared absorbance spectra, in the 1850–700  $\text{cm}^{-1}$  region, of plastic (blue line), control (purple line), bioplastic (pink line) treated soil. **B** Mean ( $\pm$  s.e.) ratios  $A^{1010}/A^{1536}$  for control (violet), bioplastic (coral), and plastic (light blue) treated soil; Capital letters indicate statistically significant (ANOVA test;  $P < 0.05$ ) in each treatment



control, moderately plastic-treated soils and remained almost constant over time in the bioplastic-treated soils (Table 2). The bacterial (EUB) and fungal (Fungi) abundances significantly decreased after 6 months for control, bioplastic and plastic, respectively and increased after 12 months in all treated soils (Table 2). N fixers abundances decreased after 6 months and then increased after 12 months in all the soils. AOA abundance increased after 12 months in all the soils with values 4-, 11-, and 7-fold greater than at the beginning of the trial in the control, bioplastic-treated, and plastic-treated soils, respectively. AOB decreased after 6 months in all the soils and then remained almost constant in the control and plastic-treated soils, while increased in the bioplastic-treated soils after 12 months. Den abundance decreased after 6 months in all the soils and then it decreased in the control soils, increased in the bioplastic-treated soils and remained almost constant in the plastic-treated soils after 12 months (Table 2). Microbial respiration approximately doubled after 6 months and then remained almost constant in all soils (Table 2). HA activity decreased after 6 months and then increased at the end of the experiment in all soils; DHA activity reached higher values after 12 months in the control soils, while it decreased after 6 months and increased after 12 months in both bioplastic- and plastic-treated soils without reaching the initial values. Ure activity increased after 6 months in all soils;  $\beta$ -glu activity peaked after 6 months in both treated soils (Table 2).

PCA plot revealed that the soil samples were primarily grouped according to the exposure time rather than by the mulch treatment (Fig. 2). Soil pH, urease activity, and the abundances of EUB and AOA were the variables that correlated most strongly with the first component (PC1) (Fig. 2). Total N,  $C_{\text{org}}$ ,  $\beta$ -glucosidase activity, respiration, and AOB abundance appeared to be strongly correlated with PC2 (loading scores  $> 0.3$ ) (Fig. 2 and Table S1).

The comparison of the investigated soil properties among treatments highlighted that EUB, AOA, AOB and Den abundances specifically differed after a 12-month exposure (Table 2 and Table 3) with values lower in the control soils. Instead, Resp was lower in the plastic-treated soils compared to the other treatments.

### Bacterial communities in bioplastic-treated soils diverged from plastic and control ones over time

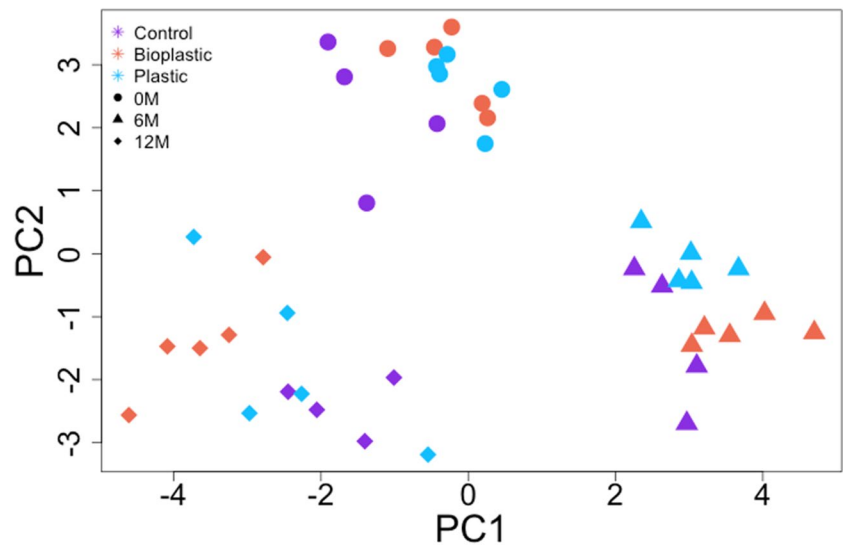
*Actinobacteriota* and *Proteobacteria* were the dominant phyla across all samples (Fig. 3A) accounting for 38.5% and 27.5% of all reads, respectively. *Acidobacteriota* and *Firmicutes* accounted for 7.8% and 6.4% of the bacterial community composition (Fig. 3A). Other phyla, such as *Gemmatimonadota*, *Crenarchaeota*, *Chloroflexi*, *Bacteroidota*, *Myxococcota*, and *Verrucomicrobiota* contributed with a lower percentage ( $< 4\%$ ) to the overall composition. On a class level, members of *Thermoleophilia* and *Actinobacteria* were the most representative of the phylum *Actinobacteriota*, accounted for 22.8% and 12.1% for all samples. Among *Firmicutes*, *Bacilli* represented the most dominant class accounting for 5.5% of the total reads, whereas among *Proteobacteria* the dominant bacterial classes were *Alpha- and Gamma-proteobacteria* (16.5% and 11.4% of the total reads, respectively) (Table S2).

Beta-diversity of the bacterial communities of the bioplastic-treated soil collected after 12 months differed from bacterial communities of the other sample groups (Fig. 3B) ( $p = 0.02$ , interaction = 22% variance). This clustering depended on the abundances (Table 4) of EUB and AOA measured via qPCR, which were higher in the bioplastic-treated samples than in the control and plastic treatments at the end of the trial (Table 2). Likewise, the highest values of bacterial richness and diversity were recorded in the

**Table 2** Mean values ( $\pm$  s.e.) of soil properties including pH, abundances of Eubacteria (EUB), ammonia-oxidizing bacteria and archaea (AOA and AOB), denitrifiers (Den), microbial respiration (Resp), bacteria richness, Shannon index, FDA hydrolysis (HA), dehydrogenase activity (DHA), urease activity (Ure),  $\beta$ -glucosidase activity ( $\beta$ -glu) phytotoxicity of *Sorghum saccharatum* L. (*Sorghum*), and *Lepidium sativum* L. (*Lepidium*) in the control and the different plastic mulch treatments (bioplastic and plastic) at the beginning of the experiment (0M), after six and twelve months of exposure (6M and 12M respectively)

	0M			6M			12M		
	Control	Bioplastic	Plastic	Control	Bioplastic	Plastic	Control	Bioplastic	Plastic
pH	7.03 $\pm$ 0.07	7.41 $\pm$ 0.03	7.58 $\pm$ 0.01	8.03 $\pm$ 0.04	8.12 $\pm$ 0.02	8.13 $\pm$ 0.09	7.23 $\pm$ 0.06	7.23 $\pm$ 0.01	7.35 $\pm$ 0.04
DNA.Yield (ng g <sup>-1</sup> d.w.)	7.58 10 <sup>3</sup> $\pm$ 8.91	7.90 10 <sup>3</sup> $\pm$ 5.66	8.22 10 <sup>3</sup> $\pm$ 6.30	7.88 10 <sup>3</sup> $\pm$ 9.47	8.09 10 <sup>3</sup> $\pm$ 9.13	7.94 10 <sup>3</sup> $\pm$ 7.03	1.24 10 <sup>4</sup> $\pm$ 7.71	7.86 10 <sup>3</sup> $\pm$ 5.37	9.22 10 <sup>3</sup> $\pm$ 1.04 10 <sup>1</sup>
EUB_qPCR (ng g <sup>-1</sup> d.w.)	1.01 10 <sup>3</sup> $\pm$ 3.16	9.00 10 <sup>2</sup> $\pm$ 2.85	9.36 10 <sup>2</sup> $\pm$ 3.38	8.16 10 <sup>1</sup> $\pm$ 1.02	7.37 10 <sup>1</sup> $\pm$ 7.91 10 <sup>-1</sup>	7.90 10 <sup>1</sup> $\pm$ 6.97 10 <sup>-1</sup>	1.69 10 <sup>3</sup> $\pm$ 4.04	3.58 10 <sup>3</sup> $\pm$ 3.55	2.89 10 <sup>3</sup> $\pm$ 6.82
Fungi_qPCR (ng g <sup>-1</sup> d.w.)	5.28 10 <sup>3</sup> $\pm$ 7.08 10 <sup>-3</sup>	6.41 10 <sup>3</sup> $\pm$ 4.39 10 <sup>3</sup>	5.72 10 <sup>3</sup> $\pm$ 4.29 10 <sup>3</sup>	5.05 10 <sup>3</sup> $\pm$ 8.56 10 <sup>3</sup>	3.95 10 <sup>3</sup> $\pm$ 6.98 10 <sup>3</sup>	4.21 10 <sup>3</sup> $\pm$ 5.45 10 <sup>3</sup>	6.65 10 <sup>3</sup> $\pm$ 7.77 10 <sup>3</sup>	7.99 10 <sup>3</sup> $\pm$ 6.06 10 <sup>3</sup>	8.01 10 <sup>3</sup> $\pm$ 7.72 10 <sup>3</sup>
N_fixers_qPCR (ng g <sup>-1</sup> d.w.)	3.36 10 <sup>-4</sup> $\pm$ 2.51 10 <sup>-3</sup>	2.65 10 <sup>-4</sup> $\pm$ 1.64 10 <sup>-3</sup>	2.99 10 <sup>-4</sup> $\pm$ 1.26 10 <sup>-3</sup>	1.64 10 <sup>-4</sup> $\pm$ 1.94 10 <sup>-3</sup>	1.14 10 <sup>-4</sup> $\pm$ 1.23 10 <sup>-3</sup>	9.65 10 <sup>-5</sup> $\pm$ 1.55 10 <sup>-3</sup>	1.81 10 <sup>-4</sup> $\pm$ 1.40 10 <sup>-3</sup>	3.05 10 <sup>-4</sup> $\pm$ 1.32 10 <sup>-3</sup>	2.34 10 <sup>-4</sup> $\pm$ 1.53 10 <sup>-3</sup>
AOA_qPCR (ng g <sup>-1</sup> d.w.)	5.59 10 <sup>3</sup> $\pm$ 1.32 10 <sup>-2</sup>	6.01 10 <sup>3</sup> $\pm$ 8.11 10 <sup>-3</sup>	6.33 10 <sup>3</sup> $\pm$ 4.66 10 <sup>-3</sup>	7.98 10 <sup>3</sup> $\pm$ 8.00 10 <sup>-3</sup>	5.80 10 <sup>3</sup> $\pm$ 8.59 10 <sup>-3</sup>	6.57 10 <sup>3</sup> $\pm$ 8.73 10 <sup>-3</sup>	1.84 10 <sup>-2</sup> $\pm$ 1.74 10 <sup>-2</sup>	6.88 10 <sup>-2</sup> $\pm$ 1.72 10 <sup>-2</sup>	4.35 10 <sup>-2</sup> $\pm$ 2.91 10 <sup>-2</sup>
AOB_qPCR (ng g <sup>-1</sup> d.w.)	3.17 10 <sup>-2</sup> $\pm$ 2.04 10 <sup>-2</sup>	2.60 10 <sup>-1</sup> $\pm$ 1.31 10 <sup>-2</sup>	3.17 10 <sup>-2</sup> $\pm$ 1.44 10 <sup>-2</sup>	1.92 10 <sup>-2</sup> $\pm$ 1.55 10 <sup>-2</sup>	1.26 10 <sup>-2</sup> $\pm$ 9.94 10 <sup>-3</sup>	2.13 10 <sup>-2</sup> $\pm$ 1.18 10 <sup>-2</sup>	1.93 10 <sup>-2</sup> $\pm$ 1.63 10 <sup>-2</sup>	2.36 10 <sup>-2</sup> $\pm$ 1.10 10 <sup>-2</sup>	2.41 10 <sup>-2</sup> $\pm$ 1.57 10 <sup>-2</sup>
Den_qPCR (ng g <sup>-1</sup> d.w.)	3.37 10 <sup>-2</sup> $\pm$ 2.13 10 <sup>-2</sup>	3.00 10 <sup>-2</sup> $\pm$ 2.33 10 <sup>-2</sup>	2.91 10 <sup>-2</sup> $\pm$ 1.78 10 <sup>-2</sup>	1.32 10 <sup>-2</sup> $\pm$ 1.76 10 <sup>-2</sup>	1.57 10 <sup>-2</sup> $\pm$ 1.63 10 <sup>-2</sup>	1.60 10 <sup>-2</sup> $\pm$ 2.01 10 <sup>-2</sup>	5.20 10 <sup>-3</sup> $\pm$ 9.39 10 <sup>-3</sup>	3.37 10 <sup>-2</sup> $\pm$ 2.15 10 <sup>-2</sup>	1.60 10 <sup>-2</sup> $\pm$ 1.92 10 <sup>-2</sup>
Resp (mg CO <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )	0.13 $\pm$ <0.01	0.14 $\pm$ <0.01	0.13 $\pm$ <0.01	0.27 $\pm$ <0.01	0.26 $\pm$ <0.01	0.23 $\pm$ <0.01	0.27 $\pm$ 0.02	0.26 $\pm$ <0.01	0.23 $\pm$ <0.01
Bacterial Richness	1142 $\pm$ 46.7	1130 $\pm$ 56.0	1095 $\pm$ 37.2	1036 $\pm$ 81.5	1121 $\pm$ 37.6	1079 $\pm$ 36.2	987 $\pm$ 65.2	1357 $\pm$ 67.3	997 $\pm$ 86.7
Bacterial Shannon Index	6.56 $\pm$ 0.03	6.53 $\pm$ 0.03	6.50 $\pm$ 0.03	6.38 $\pm$ 0.07	6.52 $\pm$ 0.02	6.48 $\pm$ 0.02	6.40 $\pm$ 0.06	6.65 $\pm$ 0.03	6.41 $\pm$ 0.05
HA (mmol of FDA min <sup>-1</sup> g <sup>-1</sup> d.w.)	6.38 $\pm$ 0.44	4.52 $\pm$ 0.41	5.54 $\pm$ 0.57	3.18 $\pm$ 0.11	3.67 $\pm$ 0.20	3.30 $\pm$ 0.13	5.17 $\pm$ 0.39	6.71 $\pm$ 0.93	4.90 $\pm$ 0.67
DHA (mmol of TPF min <sup>-1</sup> g <sup>-1</sup> d.w.)	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01	0.06 $\pm$ 0.03	0.02 $\pm$ <0.01	0.01 $\pm$ <0.01	0.008 $\pm$ <0.01	0.05 $\pm$ 0.02	0.02 $\pm$ <0.01	0.02 $\pm$ <0.01
Ure (mmol of NH <sub>4</sub> <sup>+</sup> min <sup>-1</sup> g <sup>-1</sup> d.w.)	2.72 10 <sup>-5</sup> $\pm$ 1.21 10 <sup>-6</sup>	2.92 10 <sup>-5</sup> $\pm$ 2.33 10 <sup>-6</sup>	2.41 10 <sup>-5</sup> $\pm$ 9.45 10 <sup>-7</sup>	4.33 10 <sup>-5</sup> $\pm$ 4.33 10 <sup>-6</sup>	5.10 10 <sup>-5</sup> $\pm$ 2.65 10 <sup>-5</sup>	4.73 10 <sup>-5</sup> $\pm$ 1.75 10 <sup>-6</sup>	5.30 10 <sup>-6</sup> $\pm$ 1.29 10 <sup>-6</sup>	3.83 10 <sup>-6</sup> $\pm$ 7.50 10 <sup>-7</sup>	8.74 10 <sup>-6</sup> $\pm$ 1.19 10 <sup>-6</sup>
$\beta$ -glu (mmol of PNP min <sup>-1</sup> g <sup>-1</sup> d.w.)	4.31 $\pm$ 0.19	3.91 $\pm$ 0.31	4.01 $\pm$ 0.21	6.18 $\pm$ 0.36	6.90 $\pm$ 0.12	5.73 $\pm$ 0.05	6.17 $\pm$ 0.40	5.42 $\pm$ 0.67	5.81 $\pm$ 0.18
<i>L. sativum</i> L. (% effect)	-26.0 $\pm$ <0.01	-1.20 $\pm$ 0.96	-31.5 $\pm$ 3.97	-32.2 $\pm$ 3.21	-37.2 $\pm$ 3.23	-32.1 $\pm$ 2.18	9.21 $\pm$ 5.07	-6.41 $\pm$ 5.62	-9.42 $\pm$ 3.16
<i>S. saccharatum</i> L. (% effect)	10.0 $\pm$ 4.04	26.7 $\pm$ 2.53	18.8 $\pm$ 1.31	6.60 $\pm$ 1.93	12.3 $\pm$ 1.91	16.3 $\pm$ 2.21	21.4 $\pm$ 4.93	10.9 $\pm$ 6.87	14.0 $\pm$ 1.71

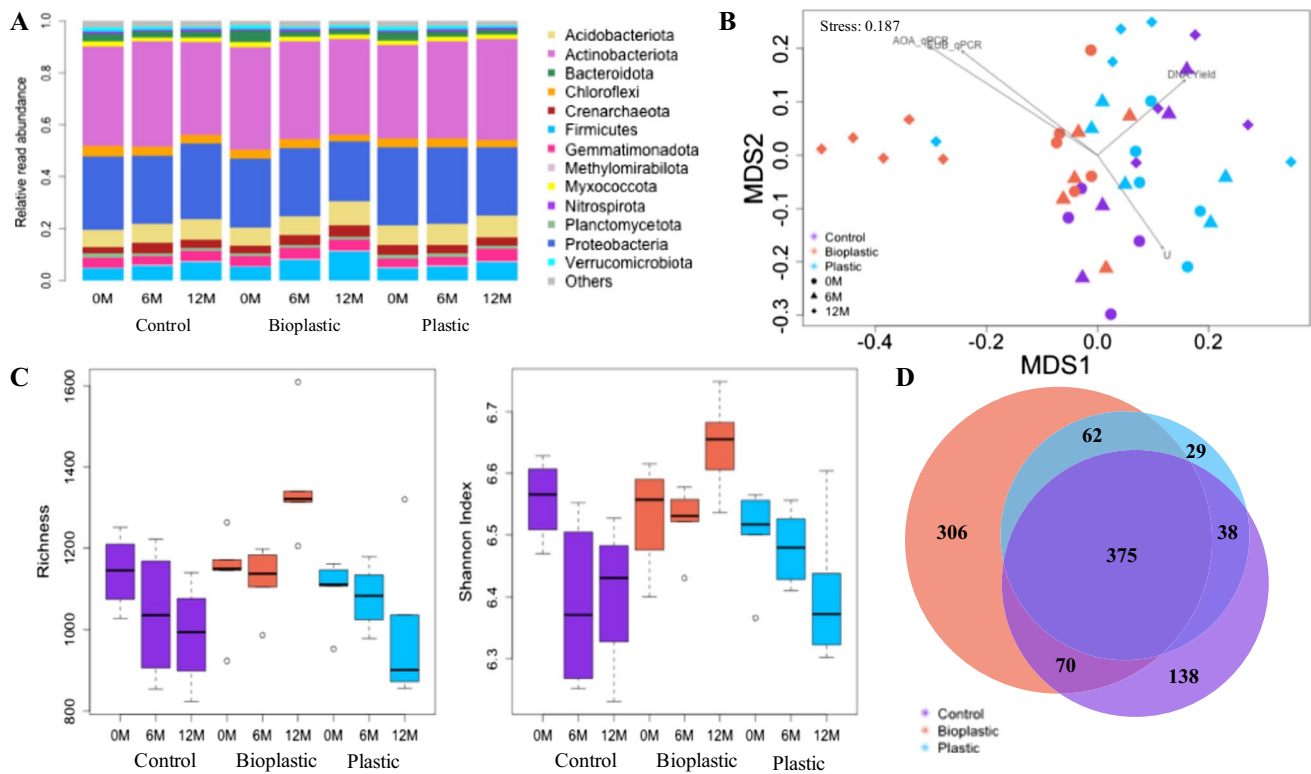
**Fig. 2** Graphical display of the first two axes (PC1: 34.5%, PC2: 23.8 %) of the principal component analysis on the soil physico-chemical, biological and ecotoxicological properties (control: violet stars, bioplastic: coral stars, plastic: light blue stars) at the beginning of the experiment (0M: circle), after six (6M: triangle) and twelve (12M: square) months of the experiment



**Table 3** Two-way ANOVA for the soil properties measured in the control and the two plastic mulch treatments (bioplastic and plastic) at the beginning of the experiment, after 6 and 12 months of exposure. The effect (“Effect”) is the factors percentage of sum of squares. Water content (WC), organic C, abundances of eubacteria (EUB), ammonia-oxidizing bacteria and archaea (AOA and AOB),

denitrifiers (Den), microbial respiration (Resp), FDA hydrolysis activity (HA), dehydrogenase activity (DHA), urease activity (Ure), β-glucosidase activity (β-glu), bacterial richness and Shannon index, phytotoxicity of *Sorghum saccharatum* L. (Sorghum), and *Lepidium sativum* L. (Lepidium)

	P-value			Effect		
	Time	Treatments	Time vs treatments	Time	Treatments	Time vs treatments
pH	2.18 10 <sup>-23</sup>	8.97 10 <sup>-8</sup>	1.01 10 <sup>-5</sup>	85.0	6.29	4.93
WC (% d.w.)	4.22 10 <sup>-4</sup>	0.175	0.130	30.9	5.72	11.9
Total C (% d.w.)	6.94 10 <sup>-2</sup>	6.52 10 <sup>-2</sup>	5.87 10 <sup>-4</sup>	8.20	8.41	36.6
Total N (% d.w.)	2.50 10 <sup>-6</sup>	0.218	0.185	47.7	3.90	8.05
C.N (% d.w.)	1.24 10 <sup>-7</sup>	0.401	0.240	56.8	1.99	6.15
C <sub>org</sub> (% d.w.)	2.94 10 <sup>-6</sup>	0.177	4.96 10 <sup>-2</sup>	44.8	4.26	12.4
DNA Yield (ng g <sup>-1</sup> d.w.)	6.08 10 <sup>-3</sup>	8.35 10 <sup>-2</sup>	8.89 10 <sup>-3</sup>	17.9	8.06	24.3
EUB_qPCR (ng g <sup>-1</sup> d.w.)	1.36 10 <sup>-16</sup>	6.25 10 <sup>-3</sup>	2.93 10 <sup>-4</sup>	78.5	3.47	8.35
Fungi_qPCR (ng g <sup>-1</sup> d.w.)	1.66 10 <sup>-9</sup>	0.468	5.79 10 <sup>-2</sup>	63.9	1.25	8.20
N.fixers_qPCR (ng g <sup>-1</sup> d.w.)	9.69 10 <sup>-9</sup>	0.642	8.86 10 <sup>-3</sup>	57.6	0.76	13.7
AOA_qPCR (ng g <sup>-1</sup> d.w.)	2.91 10 <sup>-15</sup>	5.24 10 <sup>-5</sup>	4.31 10 <sup>-7</sup>	64.5	7.98	17.8
AOB_qPCR (ng g <sup>-1</sup> d.w.)	2.20 10 <sup>-7</sup>	1.88 10 <sup>-2</sup>	8.60 10 <sup>-2</sup>	49.8	8.86	8.83
Den_qPCR (ng g <sup>-1</sup> d.w.)	1.85 10 <sup>-4</sup>	3.75 10 <sup>-2</sup>	7.26 10 <sup>-3</sup>	28.3	9.12	21.2
Resp (mg CO <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> )	1.44 10 <sup>-23</sup>	2.42 10 <sup>-3</sup>	2.75 10 <sup>-2</sup>	92.7	1.76	1.51
HA (mmol of FDA min <sup>-1</sup> g <sup>-1</sup> d.w.)	1.06 10 <sup>-4</sup>	0.698	7.31 10 <sup>-2</sup>	36.2	1.08	13.9
DHA (mmol of TPF min <sup>-1</sup> g <sup>-1</sup> d.w.)	4.61 10 <sup>-2</sup>	0.795	0.120	14.05	0.96	16.5
Ure (mmol of NH <sub>4</sub> <sup>+</sup> min <sup>-1</sup> g <sup>-1</sup> d.w.)	6.99 10 <sup>-24</sup>	0.199	1.69 10 <sup>-2</sup>	94.1	0.39	1.65
β-glu (mmol of PNP min <sup>-1</sup> g <sup>-1</sup> d.w.)	3.12 10 <sup>-7</sup>	0.550	0.277	55.2	1.38	6.05
Bacterial richness	0.642	4.81 10 <sup>-3</sup>	1.39 10 <sup>-2</sup>	1.47	20.6	24.0
Bacterial Shannon Index	0.275	4.09 10 <sup>-3</sup>	2.05 10 <sup>-2</sup>	4.32	21.0	21.6
<i>L. sativum</i> L. (% effect)	2.31 10 <sup>-11</sup>	4.65 10 <sup>-3</sup>	5.61 10 <sup>-6</sup>	55.0	6.21	22.6
<i>S. saccharatum</i> L. (% effect)	4.60 10 <sup>-2</sup>	0.310	4.29 10 <sup>-3</sup>	11.1	3.99	30.6



**Fig. 3.** Soil bacteriome overview exposed to different treatments (control: violet, bioplastic: coral, plastic: light blue) at the beginning (0M: circle), after six (6M: triangle) and twelve (12M: roar) months of the experiment. **A** Distribution of taxa across time course and soil samples. Amplicon sequence variants (ASVs) detected by Illumina Novaseq sequencing of the 16S V4 were summarized based on their phylum annotation. **B** Non-metric multidimensional scaling (NMDS)

based on Bray Curtis dissimilarities between the ASV compositions of the samples. The correlation of soil physico-chemical, biological, and ecotoxicological properties and ASV composition was plotted based on Adonis analysis. Lowest stress was 0.177. The iteration converged after 20 tries. **C** Richness and diversity across soil samples. **D** Venn diagram of soil exposed to bioplastic after 12 months

**Table 4** Fit of environmental variables onto the NMDS ordination as calculated by permutational analysis. The  $R^2$  gives the goodness of fit statistic (squared correlation coefficient).  $P$ -values are based on

	NMDS1	NMDS2	$R^2$	Pr (> r)
DNA.Yield (ng g <sup>-1</sup> d.w.)	0.746	0.665	0.199	0.016 *
EUB_qPCR (ng g <sup>-1</sup> d.w.)	- 0.782	0.622	0.435	0.001 ***
AOA_qPCR (ng g <sup>-1</sup> d.w.)	- 0.830	0.577	0.589	0.001 ***
Ure (mmol of NH <sub>4</sub> <sup>+</sup> min <sup>-1</sup> g <sup>-1</sup> d.w.)	0.559	- 0.828	0.199	0.011*

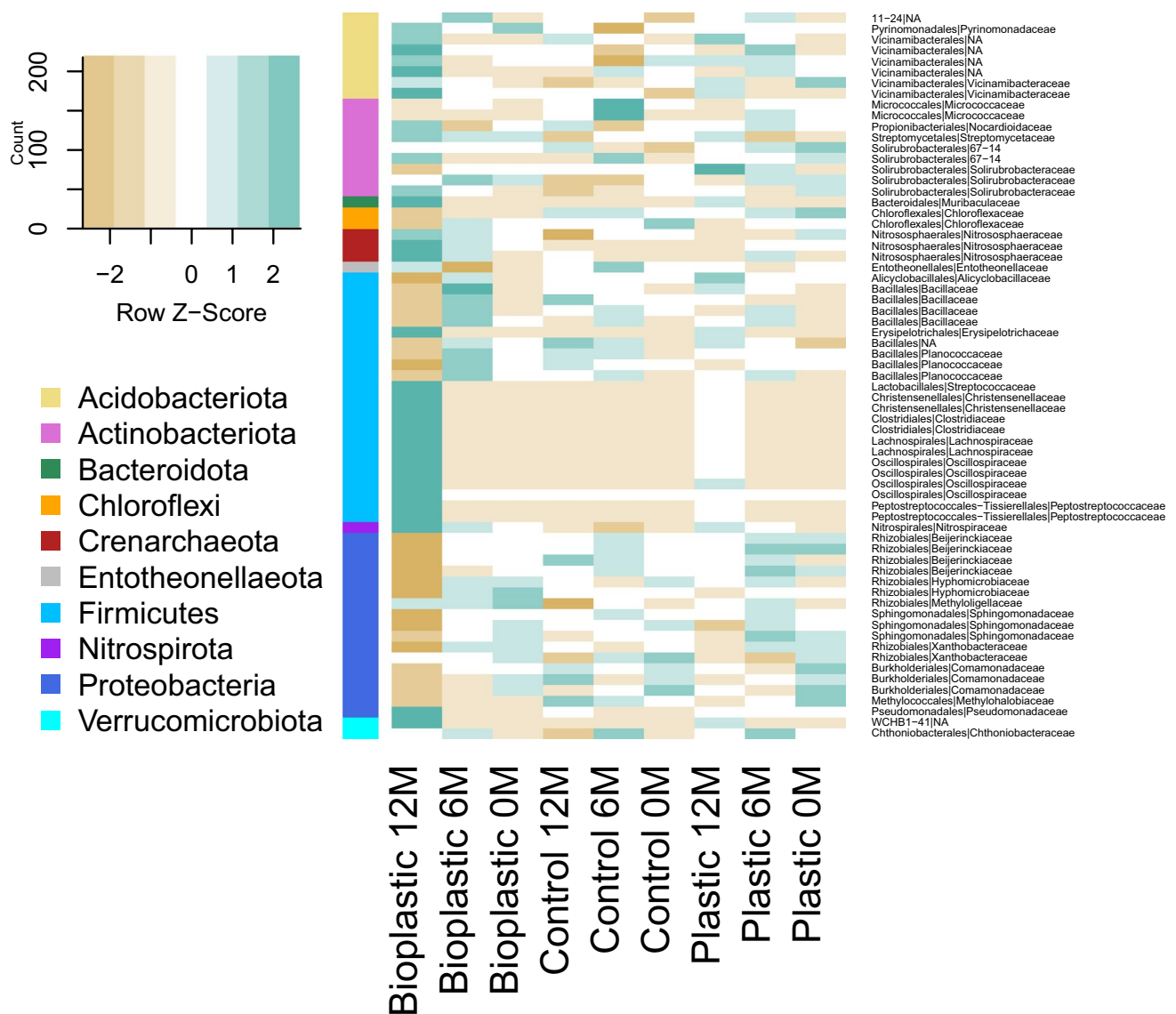
bioplastic mulch treatment after 12 months of exposure (Fig. 3C; Table 2). This increased richness was also reflected by a high number of unique ASVs detected exclusively in the bioplastic-treated soil samples at the end of the experiment (Fig. 3D). A total of 306 ASVs were exclusively found in the bioplastic treatment, whilst a 138 ASVs were detected in the control (138 ASVs) and 29 ASVs in the plastic treatments.

The bacterial core across the three treatments consisted of a total of 375 ASVs (Fig. 3D). According to Aldex2 analysis, several ASVs were indicative for the 12 months

999 permutations urease activity (Ure), total soil DNA amount: DNA Yield and abundances of eubacteria (EUB) and ammonia-oxidizing archaea (AOA)

bioplastic-treated sample group (Fig. 4), and included members of the orders *Streptomycetales*, *Solirubrobacterales* (phylum *Actinobacteriota*), *Pyrinomonadales*, and *Vicinamibacterales* (phylum *Acidobacteria*). Within the phylum *Firmicutes*, seven orders including *Erysipelotrichales*, *Lactobacillales*, *Christensenellales*, *Clostridiales*, *Lachnospirales*, *Oscillopirales*, and *Peptostreptococcales* were also listed as indicator species of the bioplastic mulch soil samples collected after 12 months of exposure. Members of the orders *Bacteroidales* (phylum *Bacteroidota*) and





**Fig. 4** Heatmap illustrating the relative abundance of ASVs indicative for sample groups. Indicator ASVs were identified using AIdex2 and were ASVs with significant differences among sample groups ( $p < 0.001$ ). The relative abundances of these indicator ASVs were cal-

culated for all sample groups and illustrated in a heatmap. In the heatmap, data were scaled by rows, thereby allowing the comparison of ASV relative abundances among sample groups, but not among ASVs

*Nitrososphaerales* (phylum *Crenarchaeota*) also appeared as indicator ASVs of this sample group (Fig. 4).

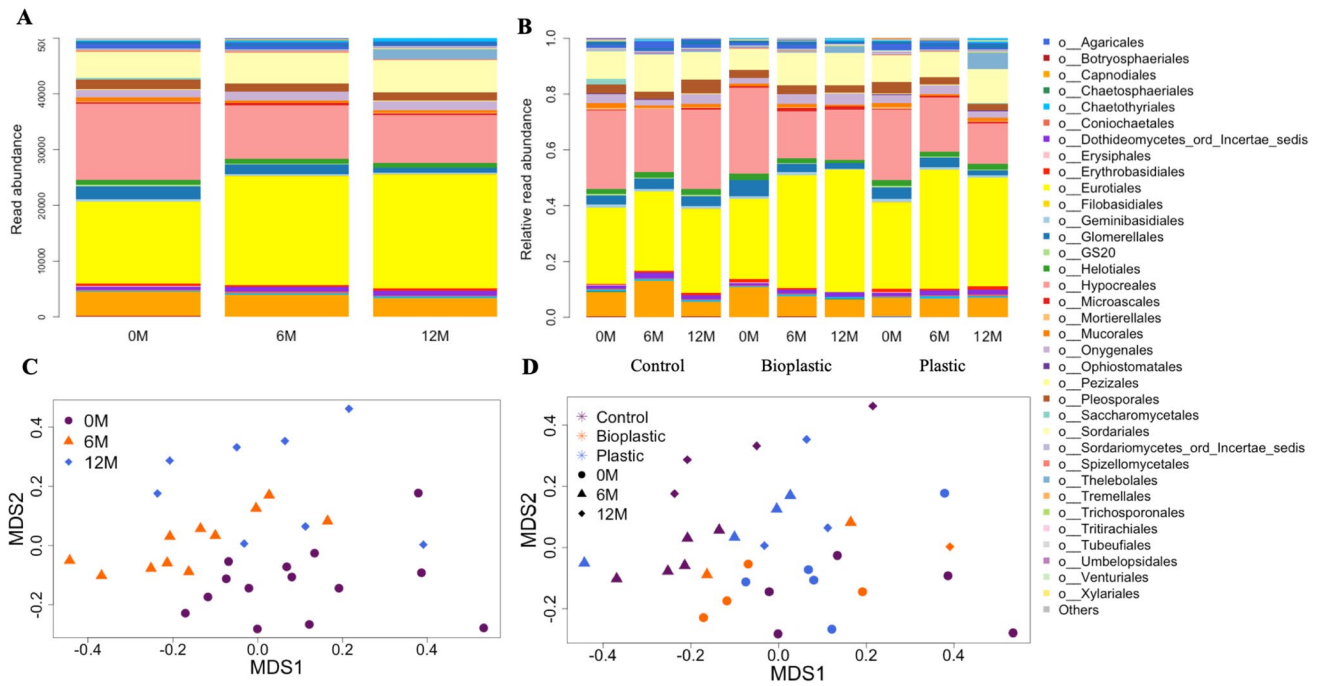
**The composition of fungal communities was only influenced by time**

Ascomycota was the dominant fungal phylum accounting for 96% of the fungal reads. The orders *Eurotiales* (37.6%), *Hypocreales* (21.6%), and *Sordariales* (11%) were the most abundant in all soil samples (Fig. 5A, B). Orders such as *Capnodiales* (7.9%), *Glomerellales* (3.4%), *Pleosporales* (3.1%), and *Onygenales* (3%) were present in low percentages (Table S3). Contrarily to bacteria, the soil

fungal communities solely clustered by time (15% variance,  $pAdonis = 0.001$ ) (Fig. 5C, D). pH was the only variable that correlated with fungal composition ( $p = 0.002$ ,  $R^2 = 0.133$ ).

**Microarthropods community were slightly influenced by time**

At the beginning of the experiment (0 months), the microarthropod density ranged from 815 to 3185  $org\ m^{-1}$  and, at 12 months between 382 and 1019  $org\ m^{-1}$  (Table 5). The Shannon and Pilon's evenness indexes ranged from 0.11 to 1.67 and from 0.81 to 1 over the 12-month period, respectively (Table 5). None of the targeted microarthropod



**Fig. 5** **A** Distribution of fungal community composition across time course. **B** Distribution of fungal community composition across time course and soil samples. Amplicon sequence variants (ASVs) detected by Illumina Novaseq sequencing of the 18S were summa-

rized based on their orders annotation. **C, D** Non-metric multidimensional scaling (NMDS) based on Bray Curtis dissimilarities between the ASV compositions of the samples. Lowest stress was 0.177. The iteration converged after 20 tries

**Table 5** Mean values ( $\pm$  s.e.) of the soil microarthropod density, richness, Shannon and evenness indices, and the relative abundance of Collembola, Acarina, Coleoptera larvae, and Pauropoa and the trophic preferences (detritivores and predators) measured in the con-

trol and the different plastic mulch treatments (bioplastic and plastic) at the beginning of the experiment (0M), and after twelve months of exposure (12M)

	0M			12M		
	Control	Bioplastic	Plastic	Control	Bioplastic	Plastic
Density_MA (n. org. m <sup>-2</sup> )	3184 $\pm$ 1359	815 $\pm$ 345	1019 $\pm$ 426	382 $\pm$ 218	407 $\pm$ 297	1019 $\pm$ 227
Richness_MA	1.75 $\pm$ 0.23	1.00 $\pm$ 0.31	1.00 $\pm$ 0.31	0.75 $\pm$ 0.41	0.8 $\pm$ 0.38	1.20 $\pm$ 0.20
Shannon_MA	0.44 $\pm$ 0.03	0.11 $\pm$ 0.0.2	0.14 $\pm$ 0.01	1.67 $\pm$ 0.15	0.22 $\pm$ 0.11	0.13 $\pm$ 0.07
Evenness_MA	0.98 $\pm$ 0.01	0.81 $\pm$ 0.01	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	0.92 $\pm$ 0.02
Collembola	72.5 $\pm$ 14.3	93.7 $\pm$ 8.94	87.5 $\pm$ 5.63	25.0 $\pm$ 15.0	50.0 $\pm$ 12.0	73.3 $\pm$ 21.0
Acarina	10.0 $\pm$ 3.20	6.30 $\pm$ 2.4	12.5 $\pm$ 1.64	75.0 $\pm$ 15.0	16.7 $\pm$ 10.1	26.0 $\pm$ 11.0
Carabidae Larvae	17.5 $\pm$ 2.10	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	16.7 $\pm$ 10.5	0.00 $\pm$ 0.00
Pauropoda	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	16.7 $\pm$ 10.5	0.00 $\pm$ 0.00
Detritivores	82.5 $\pm$ 4.50	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	83.33 $\pm$ 8.30	100 $\pm$ 0.00
Predators	17.5 $\pm$ 2.30	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	16.67 $\pm$ 9.05	0.00 $\pm$ 0.00

groups differed among treatments in terms of abundance; and the time of exposure had a significant impact on the abundance of *Acarina* reaching higher values in the control, bioplastic, and plastic treatments at the end of the experiment (Tables 5 and 6).

### Discussion

The present study focused on the impact of plastic and bioplastic mulches on soil properties, with a special emphasis on the biological properties which are more sensitive

**Table 6** *P*-values (two-way ANOVA) and effect of microarthropod density, richness, Shannon, and evenness indices, and the relative abundances of Collembola, Acarina, Coleoptera larvae, Pauropoda, and the trophic preferences (detritivores and predators) for the main factors (time, treatment, and their interaction)

	<i>P</i> -value			Effect		
	Time	Treatment	Time vs Treatment	Time	Treatment	Time vs Treatment
Density_MA (n. org. m <sup>-2</sup> )	0.067	0.184	0.07	10.5	10.4	16.5
Richness_MA	0.40	0.703	0.367	2.87	2.80	8.20
Shannon index_MA	0.776	0.609	0.583	0.34	4.19	4.56
Evenness_MA	0.398	0.284	0.202	5.07	30.0	62.2
Collembola	0.052	0.296	0.633	3.68	1.19	1.95
Acarina	0.045	0.226	0.245	4.78	1.65	1.55
Coleoptera Larvae	0.962	0.331	0.106	< 0.01	1.19	2.61
Pauropoda	0.069	0.052	0.052	3.84	3.63	3.63
Detritivores	0.959	0.286	0.069	< 0.01	1.35	3.17
Predators	0.959	0.286	0.069	< 0.01	1.35	3.17

to environmental changes than the soil physicochemical properties (Barnard et al. 2013). To date, previous studies on such effects have mostly covered a period of time shorter than a year (Ruthi et al. 2020). Our findings indicated that the time of exposure had a larger impact than the plastic treatment on most of the investigated soil properties; however, the presence of both plastics and bioplastics cannot be overlooked as it positively affected the abundances of EUB, AOA, AOB and Den.

It is plausible that the effects related to the time of exposure were mediated by seasonal changes over the 12-month period. Particularly, seasonality influenced the abundances of EUB, N-fixers and AOB showed the same temporal trend with a progressive decrease after 6 months of exposure followed by an increase until 12 months. However, it cannot be excluded the influence of bioplastics in enhancing EUB abundance compared to the other treatments. Our findings agree with those reported by Ma et al. (2016), who observed a higher soil bacterial abundance in soils covered by bioplastic mulches than by conventional plastic, probably due to the higher content of available organic C as a result of the release of biodegradable plastic residues into soil (Bandopadhyay et al. 2018). In the bioplastic-treated soils, the input of organic C associated to the airflow, produced by microbial degradation of mulches (de Souza Machado et al. 2018), could be responsible for the highest abundance of AOA, involved in nitrification (Rillig 2018; Brust 2019). This hypothesis is corroborated by the separation of 12 months bioplastic-treated soils in the beta-diversity analysis. The presence of conventional plastic mulches, acting as a barrier on the soil surface, modified the soil microclimate and might have caused an overall stress condition responsible for the lowest microbial respiration detected (Bandopadhyay et al. 2018). Moreover, both plastic and bioplastic mulches may cause N losses from soil (Qin et al. 2015; Nan et al. 2016), owing to changes in soil structure and porosity (Ingraffia et al. 2022). The fact that

after 12 months the plastic and bioplastic-treated soils showed higher abundances of denitrifying bacteria than those in the control soils could partly support the occurrence of greater N-losses in response to mulching.

As occurred for the soil abiotic properties, the composition of bacterial communities was similar across the sample groups over the course of the trial. *Proteobacteriota* and *Actinobacteriota* were the dominant phyla and they were reported to be involved in plastic degradation (Wu et al. 2022). *Proteobacteria* are widespread in soil environments being involved in the turnover of various nutrients such as C, N, and S cycling (Castañeda and Barbosa 2017); particularly, *Alpha-* and *Gamma-proteobacteria* use organic C (Zhao et al. 2018). Similarly, some *Actinobacteria* can degrade mulch sheets (Abraham et al. 2017; Huang et al. 2019; Zhang et al. 2019; Singh and Singh 2022), potentially leading to the presence of microplastics in plastic-treated soils (Ren et al. 2020; Singh and Singh 2022).

Despite the abovementioned similarities in community composition on a phylum level, beta-diversity analysis showed a clear separation of the bioplastic-treated soils collected after 12 months from the plastic-treated and control soils, probably because bioplastics, as exogenous carbon source provided selective niches for soil microorganisms (Zhou et al. 2021). It is also likely that the bioplastics might have stimulated nutrient turnover and bacterial growth over time (Sun et al. 2022), as suggested by the highest bacterial abundance in the bioplastic-treated soils.

In line with previous research (Seeley et al. 2020; Ju et al. 2021; Schöpfer et al. 2022), both bacterial richness and diversity showed higher values in the bioplastic-treated soils at 12 months of exposure than in the respective control and plastic-treated soils. The presence of bioplastic mulches could have created favorable microclimatic conditions and optimized soil water storage, thereby increasing the bacterial diversity (Li et al. 2022). Indeed, the Venn diagram showed

a higher number of unique ASVs in the 12-month bioplastic-treated soil. According to the Aldex2 analysis, members of *Streptomycetales* and *Solirubrobacterales* (affiliated to *Actinobacteriota*), in line with their plastic degrading capability (Debroas et al. 2017; Auta et al. 2017; Frere et al. 2018), were dominant in these soils. Among the specific members of the bioplastic-treated soils at 12 months, *Solirubrobacterales* are involved in organic C metabolism, secondary metabolite production and organic nitrogen metabolism (Wang et al. 2019; Ren et al. 2020). Other bacterial indicators of the 12-month bioplastic cluster referred to *Vicinamibacterales* (*Acidobacteriota*) which can utilize carbohydrates, carboxylic acids, and amino acids (Liu et al. 2022).

Members of the order *Nitrososphaerales* (*Crenarchaeota*), which are involved in ammonium oxidation, were also identified as indicator species for soils covered with bioplastic for 12 months (Lehtovirta-Morley 2018). Some authors report a niche differentiation between AOA and AOB (Di et al. 2010; Schleper and Nicol 2010), but the relative current knowledge is still scarce (Yin et al. 2022); whereas, other bacterial orders such as *Christensenellales*, *Clostridiales*, *Lachnospirales*, *Lactobacillales*, *Oscillospirales*, and *Peptostreptococcales*, all of them belonging to Firmicutes were indicators of the presence of biodegradable plastics. *Clostridiales* have been found to be associated to the presence of polyethylene microplastics (Li et al. 2020); whereas *Bacteroidales*, *Oscillospirales*, and *Lachnospirales* changed the structure of microplastics in the gut of medaka navy (Usman et al. 2022).

Previous research on the impact of microplastics on soil microbial communities have primarily focused on bacteria (Li et al. 2022; Lian et al. 2022; Wang et al. 2022; Sun et al. 2022; Huang et al. 2023), whilst only few studies have dealt with fungal communities (Accinelli et al. 2020; Wang et al. 2020; Zhou et al. 2022). More than 90% of the fungal taxa detected in the investigated treatments were affiliated to the phylum *Ascomycota*, and in contrast to bacteria, changes in the composition of fungal communities were driven by the time of exposure rather than by the mulch type. The stronger impact of bioplastic mulching on the bacterial community might be due to their higher turnover rates compared to fungi (Zhou et al. 2013; Glassman et al. 2018), which make them more sensitive to changing conditions. Consequently, they react more quickly than fungi to the physical and chemical changes as bioplastic degradation progresses.

As occurred for fungi, neither the plastic nor the bioplastic mulch sheets affected the abundance and diversity of microarthropod community. This finding does not concur with previous research reporting the positive role of plastic mulch on microarthropods (Agustina et al. 2019). Plastic mulch is expected to protect the soil from the adverse abiotic environment, preserving soil temperature

variations (Mahmudi and Rianto 2017) and protecting soil nutrient drifting (Fahrurrozi 2009). Nevertheless, the sensitivity of microarthropods to mulches and their debris is not clear yet, as studies performed in microcosms adding polyester and polypropylene microfibers showed no impact on the abundance of microarthropods (Barreto et al. 2020) despite polyester fibers have been found to negatively affect them in short-term laboratory assays (Selonen et al. 2019). In the present study, regardless of the mulching treatment, we found *Acarina* as the only group influenced by time with an increased abundance at the end of the trial. The variation in *Acarina* abundances could be due to both the organism life cycle and the different climatic conditions (Zhu et al. 2010; Santorufo et al. 2014). Taken together, more research is necessary to further evaluate whether and how the microarthropod community reacts to plastic and bioplastic mulch types depending on the time of exposure.

## Conclusions

This mesocosm study reveals that (i) based on soil physicochemical and biological properties, temporal variations exceeded the effect of both plastic and bioplastic mulching; (ii) after 12 months, the presence of plastic and bioplastic mulch sheets enhanced the abundances of eubacteria, ammonium-oxidizing archaea and bacteria, and denitrifying bacteria. Only after 12 months, the microbiome composition of soils covered by bioplastic mulches significantly differed from those of the other treatments with *Solirubrobacterales*, *Vicinamibacterales*, *Nitrososphaerales*, *Crenarchaeota*, and *Clostridiales* being indicators of the bioplastic treatment. Contrarily to bacteria, fungi and micro-arthropods were only sensitive to changes over time. Altogether, this research provided innovative results about the one-year impact of conventional and biodegradable plastic sheets on soil microbial and microarthropod communities. However, further studies need to evaluate longer-term impact than that of this study and to delineate changes in both microbiome and the trophic chains that can affect the microarthropod community.

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## Declarations

**Conflict of interest** The authors declare no competing interests.

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## References

- Abraham J, Ghosh E, Mukherjee P, Gajendiran A (2017) Microbial degradation of low-density polyethylene. *Environ Prog Sustain* 36:147–154. <https://doi.org/10.1002/ep.12467>
- Accinelli C, Abbas HK, Bruno V, Nissena L, Vicaria A, Bellaloui N, Little NS, Shiere WT (2020) Persistence in soil of microplastic films from ultra-thin compostable plastic bags and implications on soil *Aspergillus flavus* population. *J Waste Manag* 113:312–318. <https://doi.org/10.1016/j.wasman.2020.06.011>
- Adam G, Duncan H (2001) Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol Biochem* 33:943–951. [https://doi.org/10.1016/S0038-0717\(00\)00244-3](https://doi.org/10.1016/S0038-0717(00)00244-3)
- Adetunji FB, Lewu AT, Mulidzi R, Ncube B (2017) The biological activities of  $\beta$ -glucosidase, phosphatase and urease as soil quality indicators: a review. *J Soil Sci Plant Nutr* 13:794–807. <https://doi.org/10.4067/S0718-95162017000300018>
- Adhikari D, Mukai M, Kubota K, Kai T, Kaneko N, Araki KS, Kubo M (2016) Degradation of bioplastics in soil and their degradation effects on environmental microorganisms. *J Agric Food Chem* 5:23–34. <https://doi.org/10.4236/jacen.2016.51003>
- Agustina D, Tarwojto U, Rahadian R (2019) The effectiveness of plastic mulch for maintaining the potato farmland in Dieng Plateau using soil biological quality index. *Biosaintifika* 11(1):125–131. <https://doi.org/10.15294/biosaintifika.v11i1.17804>
- Auta HS, Emenike CU, Fauziah SH (2017) Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. *Environ Int* 102:165–176
- Bandopadhyay S, Martin-Closas L, Pelacho AM, DeBruyn JM (2018) Biodegradable plastic mulch films: impacts on soil microbial communities and ecosystem functions. *Front Microbiol* 9:819. <https://doi.org/10.3389/fmicb.2018.00819>
- Bandopadhyay S, Sintim HY, DeBruyn JM (2020) Effects of biodegradable plastic film mulching on soil microbial communities in two agroecosystems. *Microbiol PeerJ* 8:9015. <https://doi.org/10.7717/peerj.9015>
- Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *ISME J* 7:2229–2241
- Barreto C, Rillig M, Lindo Z (2020) Addition of polyester in soil affects litter decomposition rates but not microarthropod communities. *Soil Org* 92:109–119
- Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kausserud H (2010) ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiol* 10:189. <https://doi.org/10.1186/1471-2180-10-189>
- Blaise D, Manikandan A, Desouza ND, Bhargavi B, Somasundaram J (2021) Intercropping and mulching in rain-dependent cotton can improve soil structure and reduce erosion. *Adv Environ Sci* 4:100068
- Blasting M, Amelung W (2018) Plastics in soil: analytical methods and possible sources. *Sci Total Environ* 612:422–435
- Brodhagen M, Peyron M, Miles C, Inglis DA (2015) Biodegradable plastic agricultural mulches and key features of microbial degradation. *Appl Microbiol Biotechnol* 99:1039–1056. <https://doi.org/10.1007/s00253-014-6267-5>
- Brussaard L (1997) Biodiversity and Ecosystem Functioning in Soil. *Ambio* 26:563–570
- Brust GE (2019) Management strategies for organic vegetable fertility. In: Biswas D, Micallef SA (eds) Safety and practice for organic food. Academic Press, London, pp 193–212. <https://doi.org/10.1016/B978-0-12-812060-6.00009-X>
- Buks F, Kaupenjohann M (2020) Global concentrations of microplastics in soils—a review. *Soil* 6:649–662. <https://doi.org/10.5194/soil-6-649-2020>
- Callahan BJ, McMurdie PJ, Rosen MJ, Han MW, Johnson AJA, Holmes SP (2016) Dada2: high-resolution sample inference from Illumina amplicon data. *Nat Meth* 13:581–583
- Campbell CD, Chapman SJ, Cameron CM, Davidson MS, Potts JM (2003) A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Appl Environ Microb* 69:3593–3599
- Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci* 108:4516–4522
- Castañeda LE, Barbosa O (2017) Metagenomic analysis exploring taxonomic and functional diversity of soil microbial communities in Chilean vineyards and surrounding native forests. *PeerJ* 5:3098. <https://doi.org/10.7717/peerj.3098>
- Ceccherini MT, Ascher J, Pietramellara G, Mocali S, Viti C, Nannipieri P (2007) The effect of pharmaceutical waste-fungal biomass, treated to degrade DNA, on the composition of eubacterial and ammonia oxidizing populations of soil. *Biol Fert Soils* 44:299–306
- Chemidlin Prévost-Bouré N, Christen R, Dequiedt S, Mougél C, Lelièvre M, Jolivet C, Reza Shahbazkia H, Guillou L, Arrouays D, Ranjard L (2011) Validation and application of a PCR primer set to quantify fungal communities in the soil environment by real-time quantitative PCR. *PLoS One* 6:e24166. <https://doi.org/10.1371/journal.pone.0024166>
- de Souza Machado AA, Lau CW, Till J, Kloas W, Lehmann A, Becker R, Rillig MC (2018) Impacts of microplastics on the soil biophysical environment. *Environ Sci Technol* 52:9656–9665
- Debroas D, Monea A, Halle AT (2017) Plastics in the North Atlantic garbage patch: a boat-microbe for hitchhikers and plastic degraders. *Sci Total Environ* 599-600:1222–1232. <https://doi.org/10.1016/j.scitotenv.2017.05.059>
- Di HJ, Cameron KC, Shen J, Winefield CS, O'Callaghan M, Bowatte S, He J (2010) Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiol Ecol* 72:386–394. <https://doi.org/10.1111/j.1574-6941.2010.00861.x>
- Dindal DL (1990) *Soil Biology Guide*. Wiley, Interscience, New York
- U.S. Environmental Protection Agency (EPA) (1996) Ecological effects test guidelines (OPPTS850.4200): Seed Germination/Root Elongation Toxicity Test.
- Fahrurrozi (2009) Fakta ilmiah dibalik penggunaan mulsa plastik hitam perak dalam produksi tanaman sayuran. *Orasi Ilmiah Pada Dies*

- Natalis & Wisuda Sarjana I. <http://unib.ac.id/blog/fahrurrozi/2009/03/16/mulsa-pla-sik-hitam/perak/>
- Fei YF, Huang SY, Zhang HB, Tong YZ, Wen DS, Xia XY, Wang H, Luo YM, Barcelo D (2020) Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil. *Sci Total Environ* 707:135634
- Feng C, Ma Y, Jin X, Wang Z, Ma Y, Fu S, Chen HYH (2019) Soil enzyme activities increase following restoration of degraded subtropical forests. *Geoderma* 351:180–187. <https://doi.org/10.1016/j.geoderma.2019.05.006>
- Fernandes AD, Macklaim JM, Linn TG, Reid G, Gloor GB (2013) ANOVA-lik differential expression (ALDEx) analysis for mixed population RNA-seq. *PLoS One* 8:67019
- Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *PNAS* 102:14683–14688
- Frere L, Maignien L, Chalopin M, Huvet A, Rinnert E, Morrison H, Kerninon S, Cassone A, Lambert C, Reveillaud J, Paul-Pont I (2018) Microplastic bacterial communities in the Bay of Brest: influence of polymer type and size. *Env Pol* 242:614–625
- Glassman SI, Weihe C, Li J, Albright MBN, Looby CI, Martiny AC, Treseder KK, Allison SD, Martiny JB (2018) Decomposition responses to climate depend on microbial community composition. *PNAS* 115:11994–11999
- Hayes DG, Dharmalingam S, Wadsworth LC, Leonas KK, Miles C, Inglis DA (2012) Biodegradable agricultural mulches derived from biopolymers. In: Khemani KC, Scholz C (eds) *Degradable polymers and materials, principles and practice*, 2nd edn. ACS Symposium Series, 1114, American Chemical Society, Washington, DC
- Henry S, Baudouin E, Lopez-Gutierrez JC, Martin-Laurent F, Brauman A, Philippot L (2004) Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *J Microbiol Meth* 59:327–335
- Huang F, Liu Z, Mou H, Li J, Zhang P, Ji Z (2019) Impact of farmland mulching practices on the soil bacterial community structure in the semiarid area of the loess plateau in China. *Eur J Soil Biol* 92:8–15. <https://doi.org/10.1016/j.ejsobi.2019.04.001>
- Huang M, Zhu Y, Chen Y, Liang Y (2023) Microplastics in soil ecosystems: soil fauna responses to field applications of conventional and biodegradable microplastics. *J Hazard Mater* 5(441):129943. <https://doi.org/10.1016/j.jhazmat.2022.12994>
- Hulsen T, de Vlieg J, Alkema W (2008) BioVenn – a web application for the comparison and visualisation of biological lists using area-proportional Venn diagrams. *BMC Genomics* 9:488
- Ingraffia R, Amato G, Iovino M, Rillig MC, Giambalvo D, Frenda AS (2022) Polyester microplastic fibers in soil increase nitrogen loss via leaching and decrease plant biomass production and N uptake. *Environ Res Lett* 17:054012. <https://doi.org/10.1088/1748-9326/ac652d>
- Ju Z, Du X, Feng K, Li S, Gu S, Jin D, Deng Y (2021) The succession of bacterial community attached on biodegradable plastic mulches during the degradation in soil. *Front Microbiol* 12:785737
- Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol Fertil Soils* 6:68–72
- Kapanen A, Schettini E, Vox G, Itävaara M (2008) Performance and environmental impact of biodegradable films in agriculture: a field study on protected cultivation. *J Polym Environ* 16:109–122. <https://doi.org/10.1007/s10924-008-0091-x>
- Kasirajan S, Ngouajio M (2012) Polyethylene and biodegradable mulches for agricultural applications: a review. *Agron Sustain Dev* 32:501–529
- Lavelle P, Spain AV (2001) *Soil Ecology*. Kluwer Scientific, Amsterdam
- Lehtovirta-Morley LE (2018) Ammonia-oxidation. Ecology, physiology, biochemistry and why they must all come together. *FEMS Microbiol Lett* 365:fny058:1–9
- Li B, Ding Y, Cheng X, Sheng D, Xu Z, Rong Q, Wu Y, Zhao H, Ji X, Zhang Y (2020) Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. *Chemosphere* 244:125492. <https://doi.org/10.1016/j.chemosphere.2019.125492>
- Li C, Moore-Kucera J, Lee J, Corbin A, Brodhagen M, Miles C, Inglis D (2014) Effects of biodegradable mulch on soil quality. *Appl Soil Ecol* 79:59–69
- Li S, Ding F, Flury M, Wang Z, Xu L, Li S, Jones DL, Wang J (2022) Macro- and microplastic accumulation in soil after 32 years of plastic film mulching. *Env Pol* 300:118945. <https://doi.org/10.1016/j.envpol.2022.118945>
- Lian Y, Liu W, Shi R, Zeb A, Wang Q, Li J, Zheng Z, Tang J (2022) Effects of polyethylene and polylactic acid microplastics on plant growth and bacterial community in the soil. *J Hazard Mater* 435:129057. <https://doi.org/10.1016/j.jhazmat.2022.129057>
- Liu A, Wang W, Chen X, Zheng X, Fu W, Wang G, Ji J, Guan C (2022) Phytoremediation of DEHP and heavy metals co-contaminated soil by rice assisted with a PGPR consortium: Insights into the regulation of ion homeostasis, improvement of photosynthesis and enrichment of beneficial bacteria in rhizosphere soil. *Env Pol* 314:120303
- Ma Z, Ma Y, Qin L, Liu J, Su H (2016) Preparation and characteristics of biodegradable mulching films based on fermentation industry wastes. *Int Biodeterior* 111:54–61. <https://doi.org/10.1016/j.ibiod.2016.04.024>
- Maaß S, Daphi D, Lehmann A, Rillig MC (2017) Transport of microplastics by two collembolan species. *Env Pol* 225:456–459. <https://doi.org/10.1016/j.envpol.2017.03.009>
- Mahmudi S, Rianto H, Historiawati (2017) Pengaruh mulsa plastik hitam perak dan jarak tanam pada hasil bawang merah (*Allium cepa* fa. *ascalonicum*, L.) varietas biru lancor. *Jurnal Ilmu Pertanian Tropika Dan Subtropika* 2:60–62.
- Masui A, Ikawa S, Fujiwara N, Hirai H (2011) Influence for soil environment by continuing use of biodegradable plastic. *J Polym Environ* 19:622–627. <https://doi.org/10.1007/s10924-011-0314-4>
- Memoli V, Eymar E, García-Delgado C, Esposito F, Panico SC, De Marco A, Barile R, Maisto G (2018) Soil element fractions affect phytotoxicity, microbial biomass and activity in volcanic areas. *Sci Tot Environ* 636:1099–1108. <https://doi.org/10.1016/j.scitotenv.2018.04.327>
- Mintie AT, Heichen RS, Cromack K, JrDD M, Bottomley PJ (2003) Ammonia-oxidizing bacteria along meadow-to-forest transects in the Oregon Cascade mountains. *Appl Environ Microbiol* 69:3129–3136
- Muyzer G, De Waal E, Uitterlinden A (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16s rRNA. *Appl Environ Microbiol* 59:695–700
- Nan W, Shan-chao Y, Hai-zhou H, Shi-qing L, Yu-fang S (2016) Effects of plastic film mulching on soil greenhouse gases (CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O) concentration within soil profiles in maize fields on the Loess Plateau, China. *J Integr Agric* 15:451–464
- Nannipieri P, Trasar-Cepeda C, Dick RP (2018) Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biol Fertil Soils* 54:11–19
- Neuwirth E (2014) RColorBrewer: ColorBrewer Palettes. R package version 1:1–2
- Nissa RC, Fikriyyah AK, Abdullah AHD, Pudjiharti S (2019) Preliminary study of biodegradability of starch-based bioplastics using ASTM G21-70, dip-hanging, and Soil Burial Test methods. *IOP Conf Ser Earth Environ* 277:012007

- OECD (1984) Test No. 207: earthworm, acute toxicity tests. OECD Guidelines for the Testing of Chemicals, 2, OECD Publishing, Paris. <https://doi.org/10.1787/9789264070042-en>
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Szoecs E, Wagner H (2019) Vegan: Community Ecology Package. R package Version 2:5–6
- Parisi V, Menta C, Gardi C, Jacomini C, Mozzanica E (2005) Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. *Agric Ecosyst Environ* 105:323–333
- Pathan SI, Arfaioi P, Bardelli T, Ceccherini MT, Nannipieri P, Pietramellara G (2020) Soil pollution from micro- and nanoplastic debris: a hidden and unknown biohazard. *Sustainability* 12:7255. <https://doi.org/10.3390/su12187255>
- Pielou EC (1969) *An Introduction to Mathematical Ecology*. Wiley, New York
- Poly F, Ranjard L, Nazaret S, Gourbière F, Monrozier LJ (2001) Comparison of nifH gene pools in soils and soil microenvironments with contrasting properties. *Appl Environ Microbiol* 67:2255–2262. <https://doi.org/10.1128/AEM.67.5.2255-2262.2001>
- Qi R, Jones DL, Li Z, Liu Q, Yan C (2020) Behavior of microplastics and plastic film residues in the soil environment: a critical review. *Sci Tot Environ* 703:134722. <https://doi.org/10.1016/j.scitotenv.2019.134722>
- Qin M, Chen C, Song B, Shen M, Cao W, Yang H, Zeng G, Gong J (2021) A review of biodegradable plastics to biodegradable microplastics: another ecological threat to soil environments? *J Clean Prod* 312:127816. <https://doi.org/10.1016/j.jclepro.2021.127816>
- Qin W, Hu C, Oenema O (2015) Soil mulching significantly enhances yields and water and nitrogen use efficiencies of maize and wheat: a meta-analysis. *Sci Rep* 5:16210. <https://doi.org/10.1038/srep16210>
- Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41:D590–D596
- R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Ren XW, Tang JC, Liu XM, Liu QL (2020) Effects of microplastics on greenhouse gas emissions and the microbial community in fertilized soil. *Env Pol* 256:113347
- Rillig MC (2018) Microplastic digesting as soil carbon storage. *Environ Sci Technol* 52:6079–6080
- Ruthi J, Bolsterli D, Pardi-Comensoli L, Brunner I, Frey B (2020) The “plastisphere” of biodegradable plastics is characterized by specific microbial taxa of alpine and arctic soils. *Front Environ Sci* 8:173
- Santini G, Acconcia S, Napoletano M, Memoli V, Santorufo L, Maisto G (2022) Un-biodegradable and biodegradable plastic sheets modify the soil properties after six months since their applications. *Env Poll* 306:119608. <https://doi.org/10.1016/j.envpol.2022.119608>
- Santorufo L, Van Gestel CAM, Maisto G (2014) Sampling season affects conclusions on soil arthropod community structure responses to metal pollution in Mediterranean urban soils. *Geoderma* 226–227:47–53. <https://doi.org/10.1016/j.geoderma.2014.02.001>
- Schleper C, Nicol GW (2010) Ammonia-oxidising archaea – physiology, ecology and evolution. *Adv Microb Physiol* 51:1–41. <https://doi.org/10.1016/B978-0-12-381045-8.00001-1>
- Schöpfer L, Schnepf U, Marhan S, Brümmer F, Kandeler E, Pagel H (2022) Hydrolyzable microplastics in soil—low biodegradation but formation of a specific microbial habitat? *Biol Fertil Soils* 58:471–486. <https://doi.org/10.1007/s00374-022-01638-9>
- Seeley ME, Song B, Passie R, Hale RC (2020) Microplastics affect sedimentary microbial communities and nitrogen cycling. *Nat Commun* 11:2372
- Selonen S, Dolar A, Kokalj AJ, Skalar T, Parramon L, Hurley R, Gestel CAMV (2019) Exploring the impacts of plastics in soil – the effects of polyester textile fibers. *Sci Total Environ* 700:134451. <https://doi.org/10.1016/j.scitotenv.2019.134451>
- Shannon CE (1948) A mathematical theory of communication. *Bell Syst Tech J* 27:379–423. <https://doi.org/10.1002/j.1538-7305.1948.tb01338.x>
- Shi J, Sun YZ, Wang X, Wang J (2022) Microplastics reduce soil microbial network complexity and ecological deterministic selection. *Environ Microbiol* 24:2157–2169. <https://doi.org/10.1111/1462-2920.15955>
- Simmons SL, Bazylnski DA, Edwards KJ (2007) Population dynamics of marine magnetotactic bacteria in a meromictic salt pond described with qPCR. *Environ Microbiol* 9(9):2162–2174
- Singh B, Singh K (2022) Microplastics contamination in soil affects growth and root nodulation of fenugreek (*Trigonella foenum-graecum* L.) and 16 s rRNA sequencing of rhizosphere soil. *J Haz Mat* 7:100146. <https://doi.org/10.1016/j.hazadv.2022.100146>
- Sintim HY, Bandopadhyay S, English ME, Bar A, Liquet González JE, DeBruyn JM, Schaeffer SM, Miles CA, Flury M (2021) Four years of continuous use of soil-biodegradable plastic mulch: impact on soil and groundwater quality. *Geoderma* 381:114665. <https://doi.org/10.1016/j.geoderma.2020.114665>
- Sintim HY, Bary AI, Hayes DG, Wadsworth LC, Anunciado MB, English ME, Bandopadhyay S, Schaeffer SM, DeBruyn JM, Miles CA, Reganold JP, Flury M (2020) In situ degradation of biodegradable plastic mulch films in compost and agricultural soils. *Sci Total Environ* 727:138668. <https://doi.org/10.1016/j.scitotenv.2020.138668>
- Steinmetz Z, Wollmann C, Schaefer M, Buchmann C, David J, Tröger J, Munoz K, Fror O, Schaumann GE (2016) Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Sci Total Environ* 550:690–705. <https://doi.org/10.1016/j.scitotenv.2016.01.153>
- Sun Y, Duan C, Cao N, Li X, Li X, Chen Y, Huang Y, Wang J (2022) Effects of microplastics on soil microbiome: the impacts of polymer type, shape, a concentration. *Sci Total Environ* 806:150516. <https://doi.org/10.1016/j.scitotenv.2021.150516>
- Tabatabai MA (1982) Soil Enzymes. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis*. E-Publishing Inc., Madison, WI, pp 903–947
- Usman S, Razis AFA, Shaari K, Azmai MNA, Saad MZ, Isa NM, Nazarudin MF (2022) Polystyrene microplastics induce gut microbiome and metabolome changes in Javanese medaka fish (*Oryzias javanicus* Bleeker, 1854). *Toxicol Rep* 9:1369–1379
- Wang F, Wang Q, Adams CA, Sun Y, Zhang S (2022) Effects of microplastics on soil properties: current knowledge and future perspectives. *J Haz Mat* 424:127531. <https://doi.org/10.1016/j.jhazmat.2021.127531>
- Wang Q, Adams CA, Wang F, Sun Y (2021) Zhang S (2021) Interactions between microplastics and soil fauna: a critical review. *Crit Rev Environ Sci Technol* 10(1080/10643389):1915035
- Wang W, Ge J, Yu X, Li H (2020) Environmental fate and impacts of microplastics in soil ecosystems: progress and perspective. *Sci Total Environ* 708:13484. <https://doi.org/10.1016/j.scitotenv.2019.134841>
- Wang Z, Zhang Q, Staley C, Gao H, Ishii S, Wei X, Liu J, Cheng J, Hao M, Sadowsky MJ (2019) Impact of long-term grazing

- exclusion on soil microbial community composition and nutrient availability. *Biol Fertil Soils* 55:121–134. <https://doi.org/10.1007/s00374-018-01336-5>
- Wu XJ, Rensing C, Han DF, Xiao KQ, Dai YX, Tang ZX, Liesack W, Peng JJ, Cui ZL, Zhang FS (2022) Genome-resolved metagenomics reveals distinct phosphorus acquisition strategies between soil microbiomes. *mSystems* 7:01107–01121
- Yin C, Tan C, Chen H, Ye M, Fan X, Zheng W, Gao Z, Peng H, Liang Y (2022) Insight into the role of competition in niche differentiation between ammonia-oxidizing archaea and bacteria in ammonium-rich alkaline soil: a network-based study. *Soil Biol Biochem* 168:108638. <https://doi.org/10.1016/j.soilbio.2022.108638>
- Zhang B, Wu X, Tai X, Sun L, Wu M, Zhang W, Chen X, Zhang G, Chen T, Liu G, Dyson P (2019) Variation in actinobacterial community composition and potential function in different soil ecosystems belonging to the Arid Heihe River Basin of Northwest China. *Front Microbiol*. <https://doi.org/10.3389/fmicb.2019.02209>
- Zhang G, Zhang X, Xuebai H (2013) Runoff and soil erosion as affected by plastic mulch patterns in vegetable field at Dianchi lake's catchment, China. *Agric Water Manag* 122:20–27
- Zhao Z, Luo J, Jin B, Zhang J, Li B, Ma B, An X, Zhang S, Shan B (2018) Analysis of bacterial communities in partial nitrification and conventional nitrification systems for nitrogen removal. *Sci Rep* 8:12930. <https://doi.org/10.1038/s41598-018-30532-4>
- Zhou J, Gui H, Banfield CC, Wen Y, Zang H, Dippold MA, Charlton A, Jones DL (2021) The microplastisphere: Biodegradable microplastics addition alters soil microbial community structure and function. *Soil Biol and Biochem* 156:108211. <https://doi.org/10.1016/j.soilbio.2021.108211>
- Zhou J, Sun X, Jiao J, Liu M, Hu F, Li H (2013) Dynamic changes of bacterial community under the influence of bacterial-feeding nematodes grazing in prometryne contaminated soil. *Appl Soil Ecol* 64:70–76
- Zhou Y, Sun Y, Liu J, Ren X, Zhang Z, Wang Q (2022) Effects of microplastics on humification and fungal community during cow manure composting. *Sci Tot Environ* 803:150029. <https://doi.org/10.1016/j.scitotenv.2021.150029>
- Zhu D, Bi Q, Xiang Q, Chen Q, Christie P, Ke X, Wu LW, Zhu YG (2018) Trophic predator-prey relationships promote transport of microplastics compared with the single *Hypoaspis aculeifer* and *Folsomia candida*. *Env Pol* 235:150–154. <https://doi.org/10.1016/j.envpol.2017.12.058>
- Zhu X, Gao B, Yuan S, Hu Y (2010) Community structure and seasonal variation of soil arthropods in the forest-steppe ecotone of the mountainous region in Northern Hebei, China. *J Mt Sci* 7:187–196. <https://doi.org/10.1007/s11629-010-0198-0>

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