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Analysis of microbial populations in plastic–soil systems after exposure to high poly(butylene succinate-co-adipate) load using high-resolution molecular technique

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Abstract

Background: Bio-based and biodegradable plastics are considered as plastics of the future owing to their ability to decompose under various environmental conditions. However, their effects on the soil microbiome are poorly characterised. In this study, we aimed to investigate the effects of an important bio-based and biodegradable plastic, polybutylene succinate-co-adipate (PBSA), on soil microbial diversity and community composition using high-resolution molecular technique (Illumina sequencing) targeting all three microbial domains: archaea, bacteria, and fungi.

Results: Adding high load of PBSA to soil (6% (w/w)) caused a significant decline in archaeal (13%) and fungal (45%) richness and substantial changes in both bacterial (Proteobacteria, Actinobacteria, and Acidobacteria) and fungal (Eurotiomycetes, Sordariomycetes, Leotiomycetes, and Dothideomycetes) community composition compared with no PBSA addition to soil. The combined effects of PBSA and $(NH_4)_2SO_4$ fertilisation on the soil microbiome were much greater than the effects of PBSA alone. We only detected opportunistic human pathogens in low abundance on PBSA and in the surrounding soil. However, some plant pathogenic fungi were detected and/or enriched on the PBSA films and in surrounding soil. Apart from plant pathogens, many potential microbial control agents and plant growth-promoting microorganisms were also detected/enriched owing to PBSA addition. Adding high load of PBSA together with $(NH_4)_2SO_4$ fertilisation can either eliminate some plant pathogens or enrich specific pathogens, especially *Fusarium solani*, which is economically important.

Conclusions: We conclude that high load of bio-based and biodegradable PBSA plastic may negatively affect soil microbiome.

Keywords: PBSA, Metabarcoding, Plant health, Human health, Plant growth-promoting microbes, N-fixing bacteria, Biological control agents, Plant pathogens

Background

Biodegradable plastics can be produced by both bio-based and petroleum-based raw materials [1]. Biodegradability of plastics is not directly linked to the origin of raw materials. On the one hand, many bio-based plastics (e.g. lignin-based plastics, bio-polyethylene, bio-polyethylene terephthalate) have only limit biodegradability and

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some of them are classified as non-biodegradable [2, 3]. On the other hand, some petroleum-based plastics are biodegradable [1]. Nowadays, many petroleum-based and biodegradable plastics (including, polybutylene succinate (PBS) and polybutylene succinate-co-adipate (PBSA)) [1] can be produced by bio-based raw materials and considered as bio-based and biodegradable plastics. Bio-based and biodegradable plastics are considered as eco-friendly plastics because they decompose under various environmental conditions and are less toxic to ecosystems [4]. Moreover, their production not only reduces the use of non-renewable resources, particularly fossil fuels, but also the emission of greenhouse gases, which are the main drivers of anthropogenic climate change, compared with petroleum-based plastics [5]. Because of the negative effects of petrochemical plastics on environmentally sensitive marine and terrestrial ecosystems, the European Union issued a law banning single-use plastics by 2021 and introduced taxes on other countries, such as China, India, and the United Kingdom [5]. Owing to these constraints, the demand for biodegradable plastics has increased globally [4]. Currently, polylactic acid (PLA), PBS, and its derivatives, i.e. PBSA, polyhydroxyalkanoate (PHA), and polybutylene adipate terephthalate (PBAT), are commercially available bio-based and biodegradable plastics that lead in market share [4]. However, PLA should be considered as bio-based and compostable plastic because it requires a high temperature during composting to be degraded due to its high glass transition temperature (60 °C) [6]. The concentration of microbes is also a factor that accelerates the degradation of PLA [6, 7]. By contrast, PBS, PBSA, PHA, and PBAT are degraded in soils at ambient or lower temperature [8]. Among them, PBS and PBSA are promising materials and commercially available [9]. PBS and its composites have mechanical and physical properties, which closely resemble those of conventional plastics, such as polyethylene (PE) and polypropylene (PP) [9–11]. The mechanical flexibility of PBS can also be improved by blending with other bioplastics [11]. They are commercially used in agriculture, fishery, food packaging, durable construction, and medicine [11]. For instance, PBS and PBSA are among the commonly used biodegradable mulch films in agricultural fields to preserve moisture in agricultural soil and regulate soil temperature [12]. They are also used as carriers for drug delivery and in medical materials for drug encapsulation systems [13].

Currently, almost all plastics widely used in various sectors have been intentionally and unintentionally disposed of in both terrestrial and aquatic ecosystems [14, 15]. Excessive usage of food packaging is responsible for 40% of plastic waste globally [7, 16], while plastic mulching film is the major source of plastic pollution in terrestrial soil ecosystems [17, 18]. Food packaging plastics are unintentionally released into terrestrial soils, are transported by rivers, and end up as important sources of plastic pollution to oceans [19, 20]. In agriculture, after harvesting, plastic residues may be left in the field despite removal efforts. In some cases, farmers may leave residues on their fields and till them into soil [14, 18]. Moreover, during degradation, PBS and PBSA can release CO_2, H_2O, monomers (e.g. succinic acid, 1,4-butanediol, and adipic acid), and low-molecular mass oligomers, which are water-soluble [21]. Monomers, such as succinic acid and 1,4-butanediol, can be used by soil microbes in the tricarboxylic acid or Krebs cycle as precursors to produce cellular energy [22]. During its decomposition period, PBSA remains present in soils for at least one year under natural conditions; thus, it can interact with soil microbes and the environment [8, 23]. Plastic residues in soils may host microorganisms, including various pathogens and beneficial microbes [1, 24], thus colonisation of plastics by pathogens can elevate disease risks as the pathogens are facultative decomposers which can use plastic residues to grow and reproduce. However, only few observations of beneficial microbes associated with PBS, PBSA, and other bio-based and biodegradable plastics are reported [12, 24, 25]. Although previous studies have shown that the decomposition of PBS and PBSA has no negative effect on the soil ecosystem and soil microbial community, these assumptions are based on very limited numbers of studies using low-resolution microbiological approaches [4, 12, 25, 26]. High-resolution molecular approaches, such as next-generation sequencing should be applied to investigate the effect of bio-based and biodegradable plastics on microbial community composition and diversity [27].

In this study, we focus on the positive and negative aspects of bio-based and biodegradable plastic in agricultural soil systems. Specifically, we aimed to test the effects of PBSA on soil microbial diversity and community composition using high-resolution molecular technique (Illumina sequencing) targeting three microbial domains: archaea, bacteria, and fungi. We also tested the effects of PBSA addition along with nitrogen (N) fertilisation. Fertilisation is a common practice in agricultural sector that strongly affects soil microbial community composition and diversity [28]. We hypothesised that as a single factor, high load of PBSA does not significantly affect archaeal, bacterial, and fungal community composition and diversity; however, when combined with a second factor [here, synthetic N fertiliser, $(NH_4)_2SO_4$], PBSA might reduce microbial richness and significantly change the microbial community that favour nitrophilic fungi and bacteria.

Materials and methods

Experimental procedure: soil, PBSA, and experimental conditions

To demonstrate the effects of PBSA on soil microbial diversity and community composition, we set up a PBSA decomposition experiment in the laboratory using soil from a plot under conventional farming and ambient climate treatment at the Global Change Experimental Facility [29], Bad Lauchstädt, Central Germany (51°22'60 N, 11°50'60 E, 118 m a.s.l.). The soil was classified as a Chernozem (water holding capacity = 35%, total organic C = 2%, C:N ratio = 10, pH = 7.5). The conventional farming treatment at the study site integrates crop rotation (winter rape, winter wheat, and winter barley) and application of common fertilisers and pesticides, as described elsewhere [29]. Briefly, for winter rape, N fertiliser was applied three times (before seeding (40 kg/ha N as calcium ammonium nitrate together with P, K and S fertilisers); at the start of the growing season (100 kg/ha N as calcium ammonium nitrate together with S fertiliser); and at the start of shoot elongation (60 kg/ha N as calcium ammonium nitrate)). For winter wheat and winter barley, 60 kg/ha N and 40 kg/ha N as calcium ammonium nitrate were applied at the start of the growing season and before shoot elongation, respectively [29]. Soil from winter barley was sampled, homogenised and subdivided in this study for four treatments: (1) control soil (soil without PBSA was incubated for 90 days in sterile glass jars which was used as controls); (2) control soil with $(NH_4)_2SO_4$ addition and without PBSA (control SN); (3) PBSA–soil (PS) treatment (soil with PBSA addition was incubated for 90 days in sterile glass jars), and (4) PBSA–soil–N (PSN) treatment (soil with PBSA and $(NH_4)_2SO_4$ addition was incubated for 90 days in sterile glass jars). Initial soil was also analysed along with soil samples from these four treatments and used as a reference. For PS, and PSN treatments, PBSA films (BioPBS FD92, PTT MCC Biochem Company Limited, Thailand; in the form of double-layer thin film with 50 µm thickness, percent bio-based carbon = 35%) were treated with 70% ethanol to sterilise the PBSA surface, cut into pieces (2–5 mm × 2–5 mm), 1 g of which was weighed, and buried in a 100 ml sterile glass jar containing 19 g soil (accounting for 15.68 g soil dry weight) from one of the two treatments: normal field soil (PS treatment) and field soil with $(NH_4)_2SO_4$ addition (PSN treatment), with five replicates for each treatment and PBSA: dried soil = 6% w/w. Initial PBSA chemical and physical properties are available in Additional file 1: Appendix 1. In PSN treatment, $(NH_4)_2SO_4$ was directly added to the soil [1.4 mL of 1.42 M $(NH_4)_2SO_4$, 0.055 g N, equivalent to 280 kg N per hectare] to make N immediately available to soil microbes and to mimic fertilisation in agricultural systems. This N fertilisation rate is comparable to the highest rate of N fertilisation used in agroecosystems [30, 31], and it maintains a 10:1 C:N ratio in the soil–PBSA system. For PS and control treatments, 1.4 mL sterile Milli-Q water was added to achieve a soil water content equivalent to that in PSN treatment (17.5%, accounting for 50% of the water holding capacity), which was considered to be at the field capacity of this soil under actual field conditions. We maintained the soil water contents of all treatments at 17.5% and air temperature at 22 °C during the 90-day experiment, which was conducted in dark. The experiment was conducted for 90 days at a constant soil moisture (17.5% vol/vol) and temperature (22 °C), which was long enough for PBSA to be degraded [8]. The lids of glass jars were manual opened and closed every 2 weeks under laminar flow to avoid anoxic conditions. The PBSA samples were separated from soil by sieving, washed, and dried at 60 °C for 48 h (until constant weight). Then the samples were weighted using five digits balance (Mewes Wägetechnik, Haldensleben, Germany). The plastic degradation was estimated by specific weight loss per cm^2 as compared with original samples (5 replicates). After 90 days, PBSA samples were degraded under PS treatment (overall average mass loss = 13%) and highly degraded under PSN treatment (72% in three out of five samples, overall average mass loss = 60%). Soil pH was investigated along with microbial diversity and community composition and was used as an explanatory variable for microbial responses. The pH of initial soil, control soil, control soil with $(NH_4)_2SO_4$, and soils under PS, and PSN treatments was measured in a 1:5 soil:water suspension.

Analyses of microbiomes in PBSA and soils

PBSA and soil microbiomes were characterised by 16S rRNA gene-based and fungal internal transcribed spacer (ITS)-based amplicon sequencing on the Illumina MiSeq sequencing platform. Samples for DNA extraction from PBSA samples were processed by modifying a previously published protocol [32]. Briefly, we removed loosely adherent soil particles from PBSA samples by vortexing them in sterile phosphate-buffered saline (0.01 M) for 5 min. PBSA samples were then submerged and shaken vigorously in 45 mL sterile Tween (0.1%), and this step was repeated three times. The samples were then washed seven times using sterile water. Microbial biomass attached firmly to the PBSA sample was then subjected to DNA extraction using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) and a Precellys 24 tissue homogeniser (Bertin Instruments, Montigny-le-Bretonneux, France). Soil samples corresponding to PBSA samples were also subjected to DNA extraction using the DNeasy PowerSoil Kit with the same procedure used for PBSA samples,

according to the manufacturer's instructions. The presence and quantity of genomic DNA was checked using NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany), and the extracts were stored at − 20 °C. For construction of the bacterial and archaeal amplicon libraries, the 16S rRNA gene V4 region was amplified using the universal bacterial/archaeal primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′)[33] with Illumina adapter sequences. For establishing fungal amplicon libraries, the fungal ITS2 gene was amplified using the fungal primer pair fITS7 (5′-GTGARTCAT CGAATCTTTG-3′) [34] and ITS4 primer (5′-TCCTCC GCTTATTGATATGC-3′) [35] with Illumina adapter sequences. Amplifications were performed using 20-µL reaction volumes with 5 × HOT FIRE Pol Blend Master Mix (Solis BioDyne, Tartu, Estonia). The amplified products were visualised by gel electrophoresis and purified using an Agencourt AMPure XP kit (Beckman Coulter, Krefeld, Germany). Illumina Nextera XT Indices were added to both ends of the bacterial and fungal amplicons. The products from three technical replicates were then pooled in equimolar concentrations. Paired-end sequencing (2 × 300 bp) was performed on the pooled PCR products using a MiSeq Reagent kit v3 on an Illumina MiSeq system (Illumina Inc., San Diego, CA, United States) at the Department of Soil Ecology, Helmholtz Centre for Environmental Research, Germany. The raw 16S and ITS rRNA gene sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the accession number PRJNA702448.

The 16S and ITS rDNA sequences corresponding to the forward and reverse primers were trimmed from the demultiplexed raw reads using cutadapt [36]. Paired-end sequences were quality-trimmed, filtered for chimeras, and merged using the DADA2 package [37] through the pipeline dadasnake [38]. Assembled reads fulfilling the following criteria were retained for further analyses: minimum lengths of 200 and 150 nt forward and reverse sequences (bacteria) or 70 nt (fungi), quality scores at least equal to 15 (bacteria) or 9 (fungi) with maximum expected error score of 2 (bacteria) and 5 (fungi) for forward and reverse sequences and no ambiguous nucleotides. Merging was conducted with 2 mismatches allowed and a minimum overlap of 20 nucleotides required for bacterial and fungal sequences, respectively. High-quality reads were clustered into 6514 and 788 amplicon sequence variants (ASVs) for prokaryotes and fungi, respectively, after chimera removal. The SILVA SSU database v. 138 was used for taxonomic classification of the bacterial and archaeal ASVs. Fungal ASVs were classified against the UNITE v7.2 database [39]. Both sets of ASVs were classified using the Bayesian classifier as implemented in the mothur classify.seqs command, with a cut-off of 60. The ASV method is used to infer the biological sequences in the sample, as described previously [40]. Rare ASVs (singletons), which potentially represent artificial sequences, were removed. Finally, we obtained the minimum sequencing depths of 34,856 and 46,400 sequences per sample for the prokaryotic and fungal datasets, respectively. Relative abundance and presence/absence datasets for archaea, bacteria, and fungi were used in the data analyses. Microbial taxonomic and relative abundance information is provided in Additional file 2: Tables S1, S2, and S3. The rarefaction curves of all the samples reached saturation (Additional file 1: Fig. S1); thus, we used the observed richness as a measure of microbial diversity. We checked the taxonomic annotations of the top 40 most abundant fungal ASVs used in this study by conducting BLAST searches against the current version of UNITE (version: 8.2; 2020-01-15), and the UNITE species hypotheses [41] were used for all ASVs. The fungal ecological function of each ASV was determined using FUNGuild [42]. We verified taxonomic annotations at the species level for the 40 most abundant bacterial ASVs from in this study by conducting BLAST searches against the current version of NCBI (version: 2.11.0). Only the top match with 99%–100% identity was included. Potential functions (animal and human pathogen, biological control, denitrifying bacteria, plant growth promotion, N fixing, opportunistic human pathogen, plant pathogen, potential lipase producer, potential PBS and PBSA degrader, saprotroph, and sulphur cycling) of archaea, bacteria, and fungi were manually assigned using publications listed in the Web of Science (ISI Web of Knowledge) [43].

Statistical analysis

The effects of soil treatments on archaeal, bacterial, and fungal ASV richness in soil and PBSA samples were analysed using analysis of variance (ANOVA), incorporating the Jarque–Bera JB test for normality and Levene's test to assess the equality of group variances. The effects of soil treatments on archaeal, bacterial, and fungal community compositions in soil and PBSA samples were visualised and analysed using non-metric multidimensional scaling, permutational multivariate ANOVA (PERMANOVA), and analysis of similarities (ANOSIM), based on presence–absence data and the Jaccard distance measure; for ANOSIM, over 999 permutations were run. As relative abundance data from metabarcoding may contain some biases [44], we analysed the microbial community composition mainly using presence/absence data sets. The effects of soil treatments on soil pH were analysed using the Kruskal–Wallis test. The relationships between

pH and archaeal, bacterial, and fungal richness were analysed using Pearson's correlation coefficient. All statistical analyses were performed using PAST version 2.17. The amounts of variation in archaeal, bacterial, and fungal community compositions, explained by various factors, were estimated through variation partitioning using the Vegan package in R [45].

Results

General information regarding microbial communities associated with PBSA degradation

We analysed the archaeal, bacterial, and fungal communities that were adhered to PBSA films in soils with and without $(NH_4)_2SO_4$ addition (PSN and PS treatments, respectively), those present in the soil surrounding the PBSA in the aforementioned treatments, and those present in soil not treated with PBSA (control soil and control SN treatments) after 90 days of incubation; for comparison, we also analysed the communities present in the initial soil without incubation (initial soil treatment). Taxonomic resolution was conducted at the ASV level (Additional file 2: Tables S1, S2, and S3). In total, we detected 27, 6,487, and 788 archaeal, bacterial, and fungal ASVs, respectively, across all PBSA and soil microbial communities (Additional file 2: Tables S1, S2, and S3). The archaeal, bacterial, and fungal ASVs corresponded to 3, 418, and 164 genera, respectively. Among these, we detected 7 (26% of total archaeal ASVs), 2,113 (33% of total bacterial ASVs), and 170 ASVs (22% of total fungal ASVs) for archaea, bacteria, and fungi in PBSA, respectively (Additional file 2: Tables S1, S2, and S3). In soil samples from PS and PSN treatments, we detected 20 (74% of total archaeal ASVs), 5,429 (84% of total bacterial ASVs), and 313 ASVs (40% of total fungal ASVs) for archaea, bacteria, and fungi, respectively (Additional file 2: Tables S1, S2, and S3). The relative archaeal (ASV level), bacterial (phylum level), and fungal (class level) abundances are shown in Fig. 1. Briefly, microbial community composition (at the coarse taxonomic level) of initial soil and control soils were comparable for archaeal (dominated by *Nitrososphaeraceae*), bacteria (dominated by Proteobacteria), and fungi (dominated by Sordariomycetes) (Fig. 1). While pure PBSA addition did not significantly change archaeal community composition, it increased relative abundance of Proteobacteria and strongly reduced abundance of Sordariomycetes (Fig. 1). Adding $(NH_4)_2SO_4$ into PBSA–soil system increased Proteobacteria and Sordariomycetes (Fig. 1). The microbial community composition at coarse taxonomic level of PBSA was significantly different from those of the surrounding soil (Fig. 1). PSN treatment significantly reduced microbial diversity on PBSA.

Effects of PBSA on microbial ASV richness

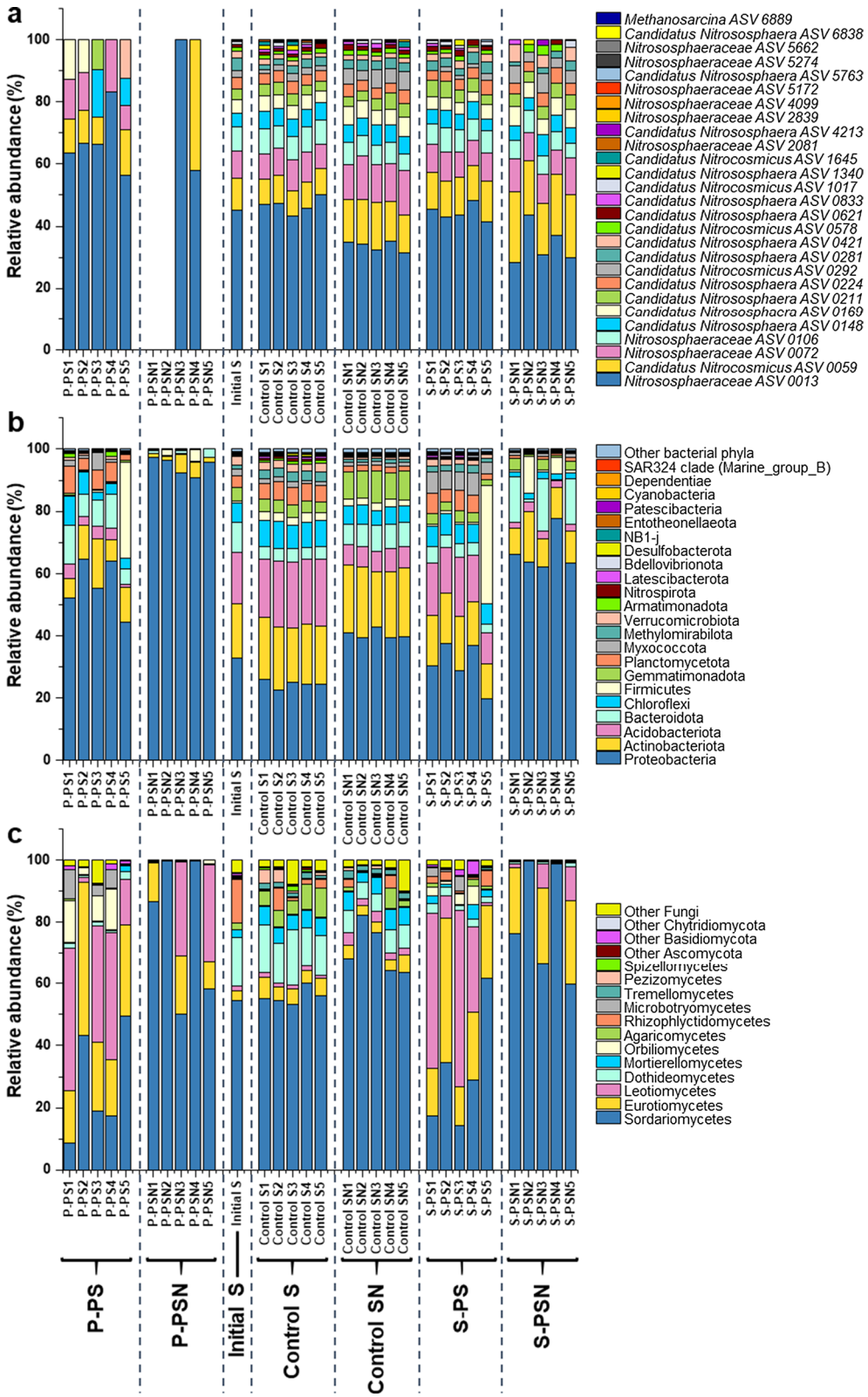
Both prokaryotic (archaeal and bacterial) and fungal ASV richness in the control soil without PBSA (control S) and control soil with $(NH_4)_2SO_4$ addition (control SN) were similar to those in the initial soil (Fig. 2). Bacterial ASV richness in soil under PS treatment was not significantly affected by PBSA, while archaeal ASV richness was slightly decreased (by 13%) and fungal ASV richness was strongly reduced by approximately 45% (Fig. 2a, c, e). $(NH_4)_2SO_4$ as a single factor (control SN treatment) did not significantly affect the microbial richness. Contrarily, adding $(NH_4)_2SO_4$ into PBSA–soil system caused a significant reduction in archaeal, bacterial and fungal ASV richness in soil under PSN treatment by 31%–79%. The richness of bacteria and fungi colonising PBSA in the PS treatment accounted for 60% and 43%, respectively, of the richness of the surrounding soil (Fig. 2). Moreover, bacterial and fungal ASV richness in the communities adhered to PBSA following PSN treatment accounted for only 14% and 38%, respectively, of the corresponding richness of the soil microbiome (Fig. 2).

Effects of PBSA on soil microbial community composition and activity

Both prokaryotic and fungal community compositions in control soil were almost identical to those in the initial soil (Fig. 3a, c, e). We found small but significant changes in bacterial and fungal community compositions in soils under control SN and PS treatment but not in archaeal community composition. After adding PBSA, the relative abundance of Proteobacteria (30.6%, 2.7% contributed by *Bradyrhizobium* ASV 15) in PS-treated soil slightly increased compared with its abundance in control soils, whereas the relative abundance of Actinobacteriota and Acidobacteriota slightly decreased (Fig. 1b). For fungi, there were large changes in the dominant fungal classes in PS-treated soil compared with its abundance in initial

(See figure on next page.)

Fig. 1 Composition of the archaeal (**a**) (ASV level), the bacterial (**b**) (phylum-level, considering only phyla with ≥ 10 ASVs or relative abundances ≥ 1%, the rest of the bacterial phyla were pooled as "others") and the fungal (**c**) (class-level, considering only classes with ≥ 10 ASVs or relative abundances ≥ 1%, the rest of the fungal classes were pooled as "others") communities associated with the degradation of a bio-based and biodegradable poly(butylene succinate-co-adipate) (PBSA) based on relative abundance. Data are presented for initial soil (initial S), control soils (control S), control soils with $(NH_4)_2SO_4$ addition (control SN), soils under PS treatment (S–PS), soils under PSN treatment (S–PSN), PBSA plastics under PS treatment (P–PS), and PBSA plastics under PSN treatment (P–PSN)



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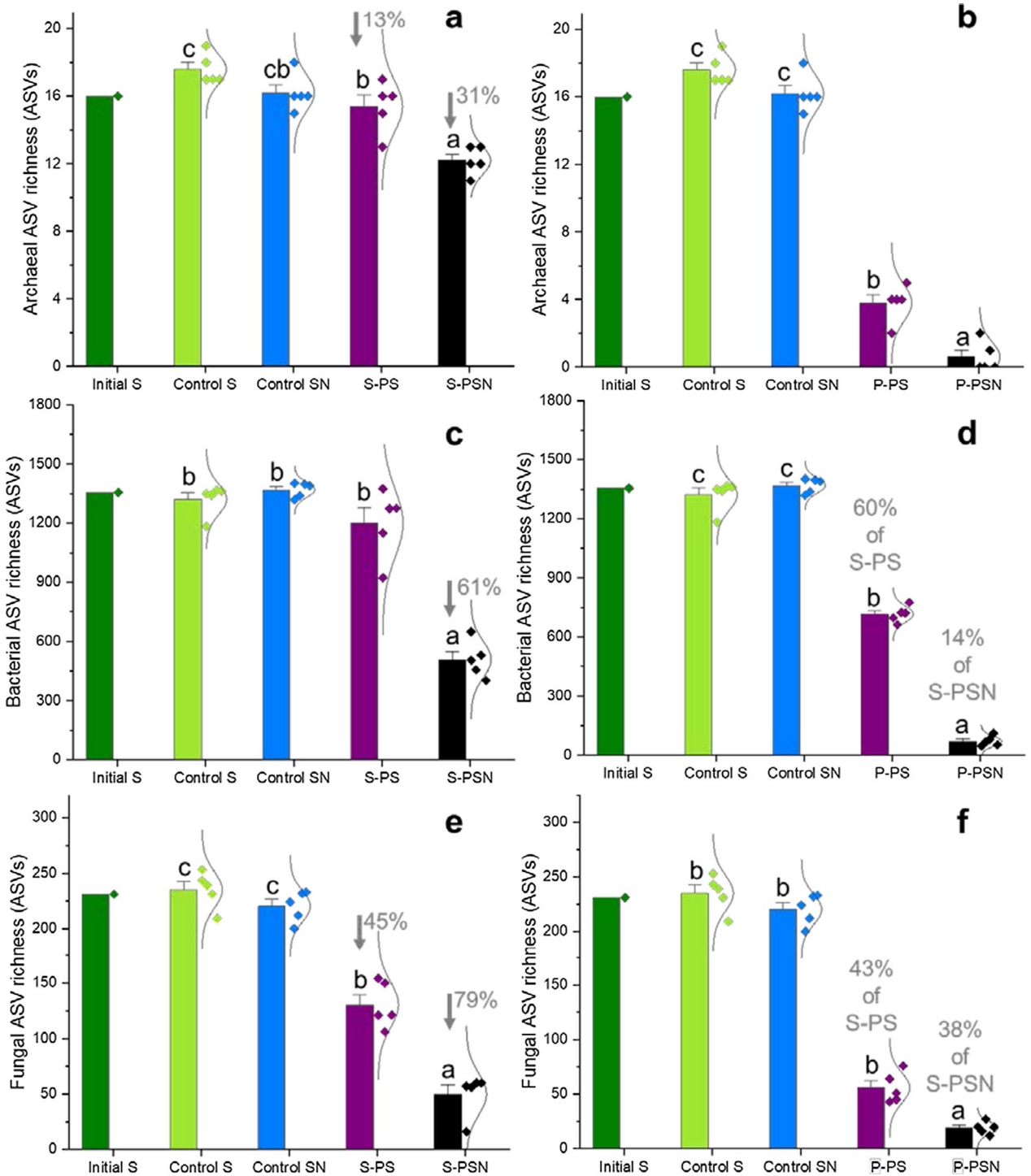

Fig. 2 ASV richness (number of ASVs) for archaea (**a**, **b**), bacteria (**c**, **d**) and fungi (**e**, **f**) in soil (left) and on PBSA (right) samples. Details of the treatment abbreviations can be found in the legend of Fig. 1

and control soils. Eurotiomycetes (24.0%, 20.6% contributed by *Exophiala equina* ASV 02) and Leotiomycetes (28.6%, 23.1% contributed by *Tetracladium furcatum* ASV 03) were co-dominant with Sordariomycetes (31.4%, 9.5% contributed by *Ilyonectria macrodidyma* ASV 07 and 4.7% contributed by *Chaetomium globosum* ASV 09),

whereas Dothideomycetes almost disappeared from the PS-treated soil (Fig. 1c).

Adding $(NH_4)_2SO_4$ together with PBSA strongly shifted the microbial community composition of soil under PSN treatment from that of the initial, control soils and control soils with $(NH_4)_2SO_4$ addition. Archaeal, bacterial and fungal community compositions in soil were more sensitive to the combination of the two amendment additions (PBSA and $(NH_4)_2SO_4$ addition) (Figs. 1, 3). After adding $(NH_4)_2SO_4$ and PBSA, the abundance of *Candidatus Nitrocosmicus* ASV 59 in the soil of the PSN treatment increased compared with its abundance in initial and control soils, whereas the abundance of *Nitrososphaeraceae* ASV 13 slightly decreased, and some other archaeal ASVs disappeared. Strong hyper-dominant patterns were observed for both bacterial and fungal communities in PSN-treated soil. We found that only Proteobacteria (66.6%, 20% contributed by *Achromobacter insolitus* ASV 02) was dominant in the bacterial community and that Sordariomycetes (80.3%, 43.0% contributed by *Fusarium solani* ASV 01 and 10.2% contributed by *Clonostachys rosea* ASV 11) were dominant in the fungal community in PSN-treated soil (Fig. 1b, c). Interestingly, in two of five soil samples under PSN treatment, PBSA decomposition rates were lower than those of the other three replicates, whereas Bacteroidota (contributed by *Chitinophaga* ASV 41) and Eurotiomycetes (contributed by *E. equina* ASV 02) almost completely disappeared (Fig. 1b, c).

Different colonisers of PBSA under PS and PSN treatments

The compositions of archaeal, bacterial, and fungal communities in PBSA receiving PS and PSN treatments were significantly different from those of their soil counterparts (Additional file 1: Table S4). Among the total 27 archaeal ASVs, only seven were present on PBSA under PS treatment. Among these, *Nitrososphaeraceae* ASV 13, *Candidatus Nitrocosmicus* ASV 59, *and Nitrososphaeraceae* ASV 72 were dominant (Fig. 1a and Additional file 2: Table S1). The bacterial community in the PBSA samples under PS treatment was dominated by Proteobacteria (56.1%, 7.5% contributed by *Caulobacter rhizosphaerae* ASV 08, 3.5% contributed by *Azotobacter chroococcum* ASV 18) with a small contribution from Actinobacteriota (10.3%, 1.1% contributed by *Agromyces ramosus* ASV 30) and Bacteroidota (8.7%, 1.1% contributed by *Ohtaekwangia* ASV 97) (Figs. 1b, 4 and Additional file 2: Table S2). In the fungal community, Leotiomycetes (28.3%, 22.8% contributed by *Tetracladium furcatum* ASV 03), Eurotiomycetes (27.2%, 22.6% contributed by *E. equina* ASV 02) and Sordariomycetes (27.6%, 18.1% contributed by *I. macrodidyma* ASV 07) were co-dominant in the PBSA samples under PS treatment (Figs. 1c, 5 and Additional file 2: Table S3). For PBSA under PSN treatment, only *Nitrososphaeraceae* ASV 13 was detected in two of five independent PBSA replicates and *Candidatus Nitrocosmicus* ASV 59 in one replicate, whereas in the other three replicates, archaea were completely absent (Fig. 1a). In the PSN treatment, only a few bacterial phyla and fungal classes could colonise the PBSA (Fig. 1b, c). Proteobacteria (94.6%, 19.4% contributed by *Stenotrophomonas maltophilia* ASV 03, 18.9% contributed by *A. insolitus* ASV 02 and 15.3% contributed by *Achromobacter denitrificans* ASV 04) and Sordariomycetes (78.9%, 49.1% contributed by *F. solani* ASV 01 and 14% contributed by *C. rosea* ASV 11) dominated the bacterial and fungal communities, respectively, of PBSA samples under PSN treatment (Figs. 1b, c, 4, 5, Additional file 2: Tables S2 and S3).

Soil pH response to PBSA addition and links to microbial community composition and richness

The initial soil pH of 7.5 slightly decreased when $(NH_4)_2SO_4$ was added (pH 7.3) (Additional file 1: Fig. S2a). After 90 days of incubation, pH values in the control soil and PS-treated soil were similar to the initial soil pH. Contrarily, soil pH after 90 days of incubation significantly decreased under control SN and PSN treatment (pH value 6.7 and 6.3, respectively). pH values were significantly positively correlated with archaeal, and bacterial richness ($R = 0.54–0.55$, $P = 0.011–0.014$, Additional file 1: Fig. S2b, c). We found no correlation between pH values and fungal richness (Additional file 1: Fig. S2d). Overall, treatments explained 13%, 23%, and 28% of variations in archaeal, bacterial, and fungal community composition, respectively (Additional file 1: Fig. S3a–c). PBSA explained 54%, 35%, and 64% of the total explainable variation in archaeal, bacterial, and fungal community composition, respectively. The addition of $(NH_4)_2SO_4$ did not explain archaeal community composition, but explain 13%, and 11% of the total explainable variation in bacterial, and fungal community composition. pH alone

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Fig. 3 Non-metric multidimensional scaling (NMDS) ordinations of archaeal (**a**, **b**), bacterial (**c**, **d**) and fungal (**e**, **f**) community compositions in soil based on presence/absence data and the Jaccard dissimilarity measure. Data are presented for initial soil (0), control soil (1), control SN (2), and PBSA and soils from the PS (3), and PSN (4) treatments. Details of the treatment abbreviations can be found in the legend of Fig. 1. PERMANOVA and ANOSIM results are provided in Additional file 1: Table S4

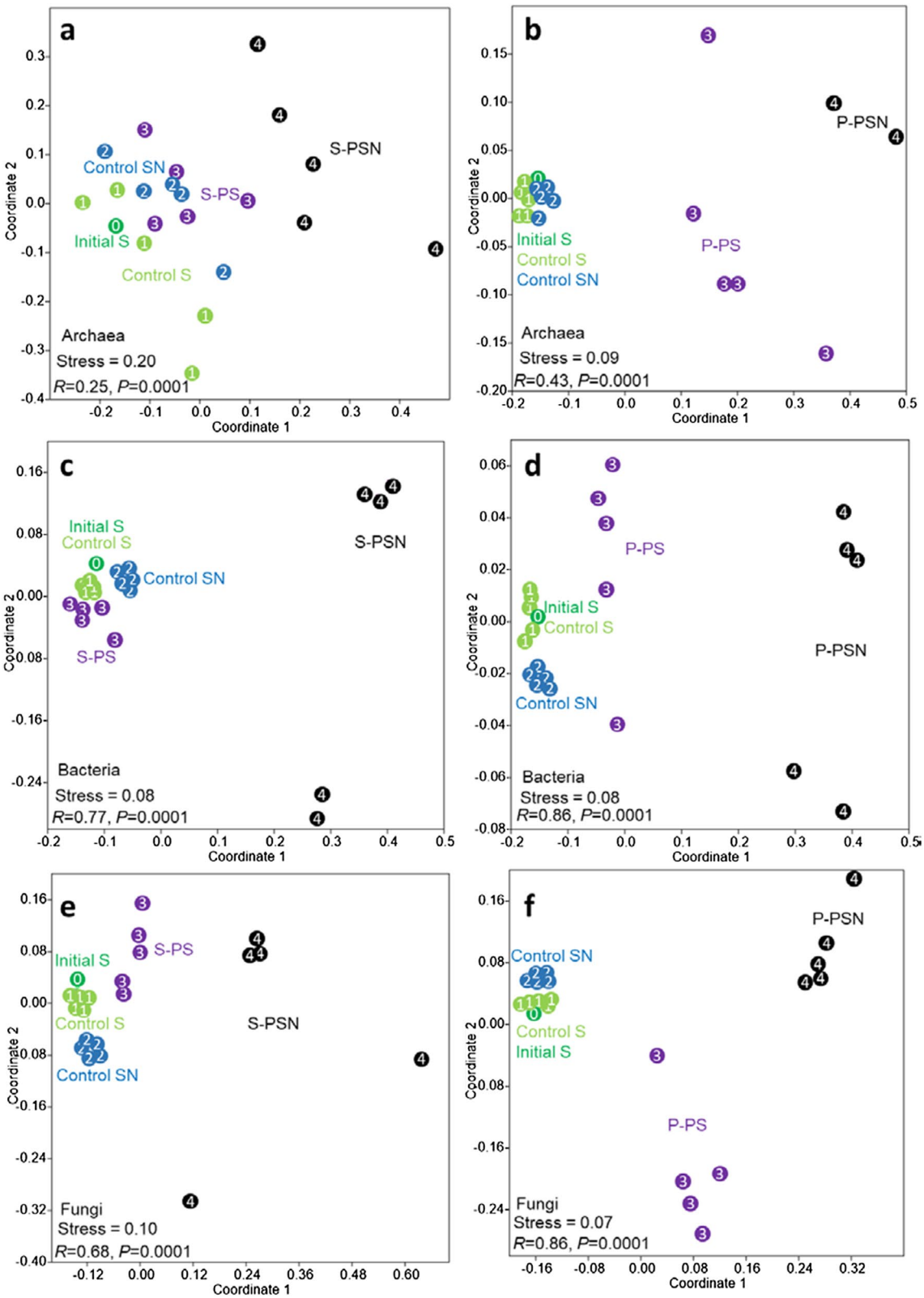

Fig. 3 (See legend on previous page.)

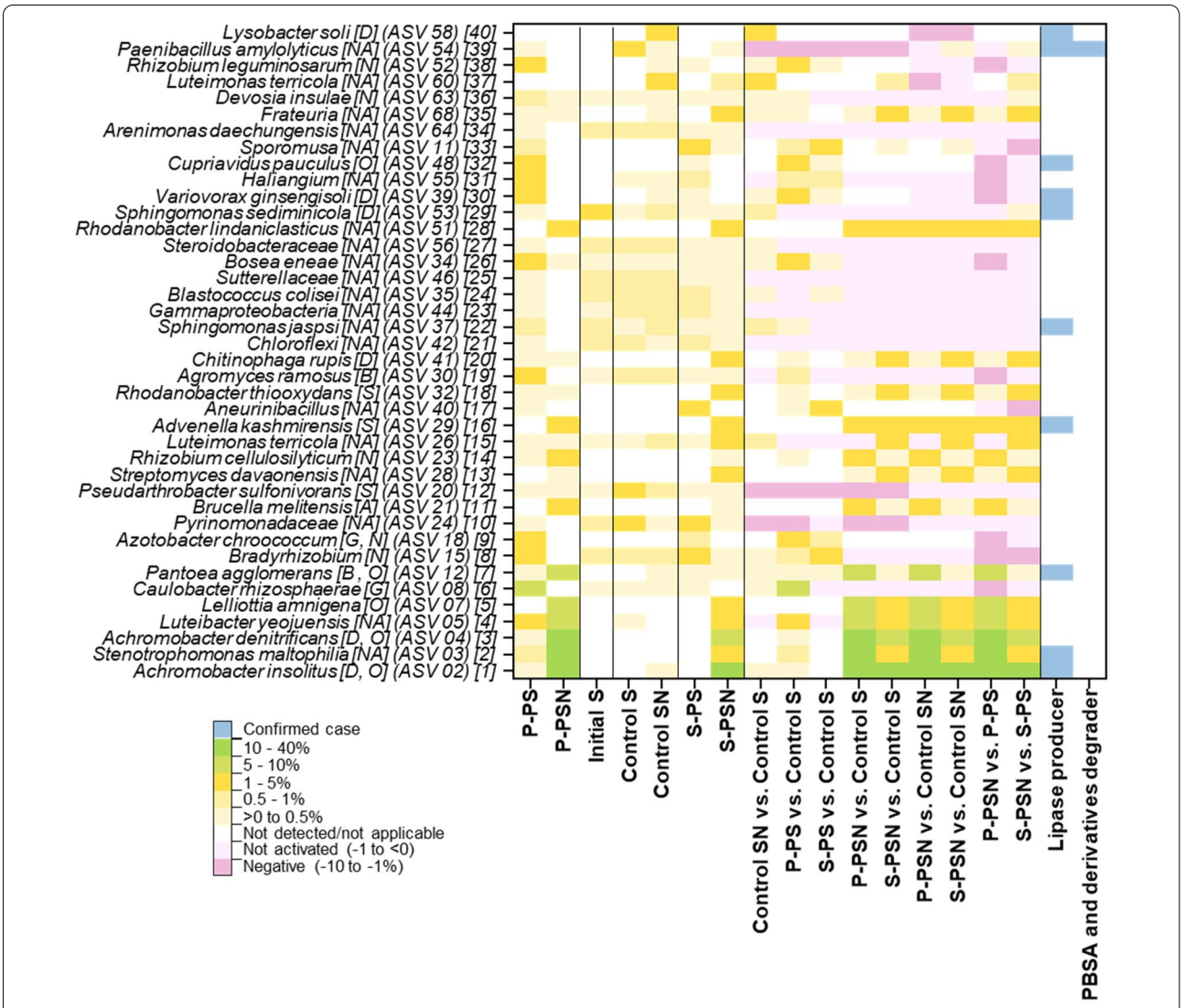

Fig. 4 Top-40 bacterial ASVs that had the highest relative abundances in all treatments (initial soil, control soil, control SN, and PBSA and soils in PS, and PSN treatments). The enrichment or the decline of bacterial ASVs in PBSA and soil samples from the PS and PSN treatment were compared with those in the control soil and control soil with $(NH_4)_2SO_4$ addition. The last two columns indicate bacterial ASVs that have been reported to produce lipase enzyme and to degrade PBS and its derivatives in previous studies. The letters in the square parentheses indicate the functions of each ASV: A: animal and human pathogen, B: biological control, D: denitrifying bacteria, G: plant growth promotion, N: N fixing, NA: not applicable, O: opportunistic human pathogen, P: plant pathogen, and S: sulphur cycling. The number in the square parentheses indicates the ranking of each ASV among the top-40 ASVs. Details of the treatment abbreviations can be found in the legend of Fig. 1

did not explain the microbial community compositions. However, the combinations of pH and $(NH_4)_2SO_4$ addition explained 31%, 43%, and 21% of the total explainable variation in archaeal, bacterial, and fungal community composition, respectively.

Potential lipase-producing and PBS/PBSA-degrading microbes

Considering the 40 most abundant bacteria, potential lipase-producing bacteria were almost absent or were detected in low relative abundance in the initial, control soils and control soils with $(NH_4)_2SO_4$ addition, whereas potential PBS-degrading bacteria were not detected in the initial soil (Fig. 4). In control soils and control soils with $(NH_4)_2SO_4$ addition, only *Paenibacillus amylolyticus* ASV 54 (a potential lipase producer and potential PBS degrader) [1, 46] was detected with 1.8% and 0.006% relative abundance, respectively. In PS- and PSN-treated soils, *P. amylolyticus* was not detected or detected at very low relative abundance ($\leq 0.01\%$). Potential

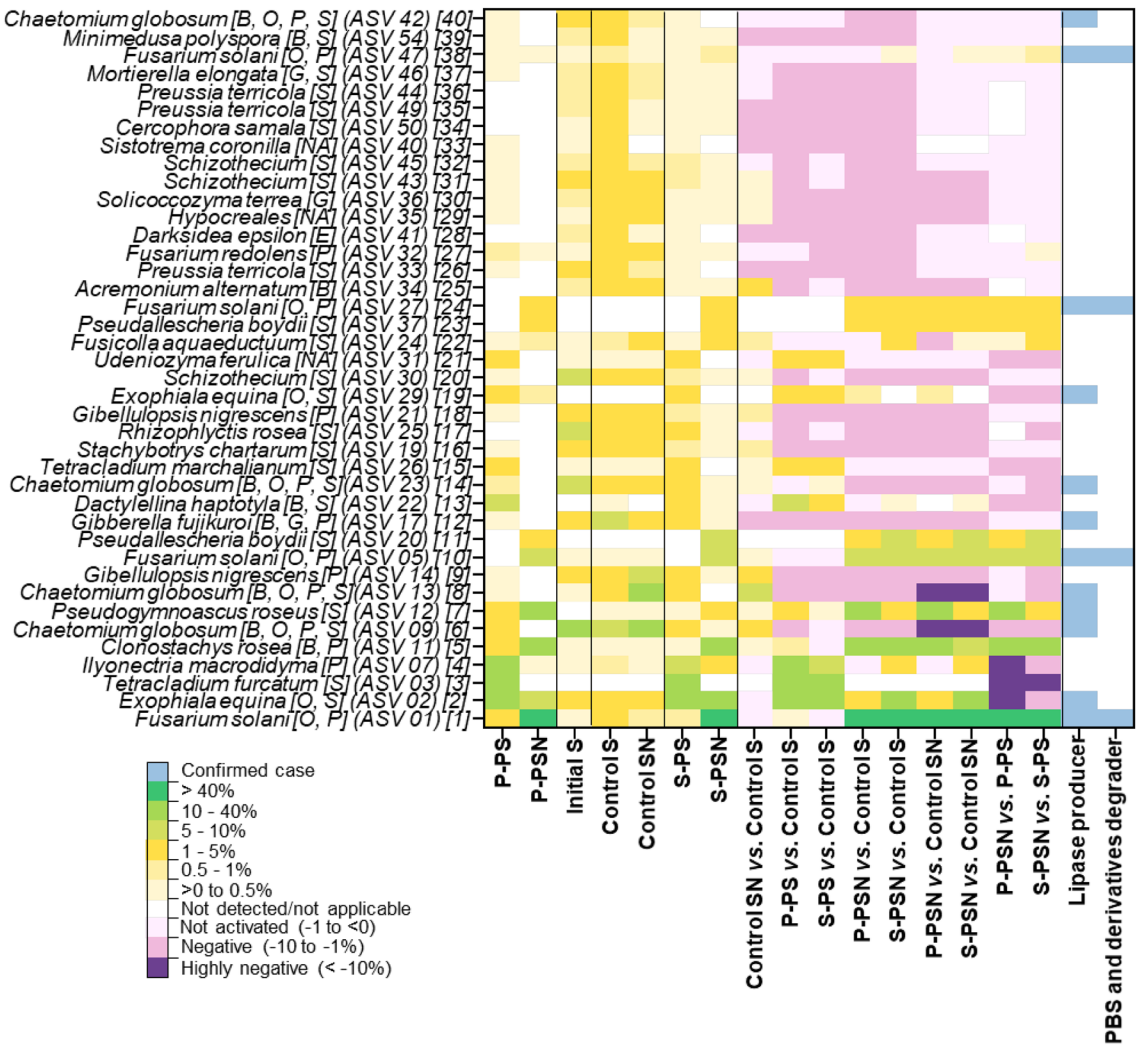

Fig. 5 Top-40 fungal ASVs that had the highest relative abundances in all treatments (initial soil, control soil, control SN, and PBSA and soils in the PS, and PSN treatments). The enrichment or the decline of fungal ASVs in PBSA and soil samples from the PS and PSN treatment were compared with those in the control soil and control soil with $(NH_4)_2SO_4$ addition. The last two columns indicate fungal ASVs that have been reported to produce lipase enzyme and to degrade PBS and its derivatives in previous studies. The letters in the square parentheses indicate the functions of each ASV: B: biological control, E: endophyte, G: plant growth promotion, NA: not applicable, O: opportunistic human pathogen, P: plant pathogen, and S: saprotroph. The number in the square parentheses indicates the ranking of each ASV among the top-40 ASVs. Details of the treatment abbreviations can be found in the legend of Fig. 1

lipase-producing bacteria were found to be associated with both soil and PBSA samples across all other treatments (PS, and PSN treatments). Most of them were more enriched on PBSA than in soil from the same treatment. However, the community of these potential lipase-producing and PBS-degrading bacteria differed among treatments. After incorporating PBSA into soil, five out of 10 potential lipase-producing bacteria were detected in PS-treated soil at very low relative abundances ($\leq$0.3%). In PBSA samples under PS treatment, eight out of 10 potential lipase-producing bacteria were detected, and *Cupriavidus pauculus* ASV 48, *Variovorax ginsengisoli* ASV 39 were enriched. In the PSN treatment, *A. insolitus* ASV 02 was highly enriched in both soil and PBSA samples. *Pantoea agglomerans* ASV 12, and *Stenotrophomonas maltophilia* ASV 03 were detected in soil and highly enriched in PBSA samples under PSN treatment.

Among the 40 most abundant fungi, all potential PBS-degrading fungi were *F. solani* ASV 01, ASV 05, ASV 27, and ASV 47 [47] (Fig. 5). *F. solani* was also reported to potentially produce lipase [48]. In the initial, control soils and control soils with $(NH_4)_2SO_4$ addition, three

out of four potential PBS-degrading *F. solani* ASVs were detected with 0.04–1.4% relative abundance. Under PS treatment, two out of four *F. solani* ASVs were found to be associated with both soil and PBSA samples (0.08–1.7% relative abundance). Under PSN treatment, *F. solani* ASV 01 was highly enriched in both soil and PBSA samples with relative abundances of 43–49%. The community of other potential lipase-producing fungi also differed among the different treatments. *Chaetomium globosum* were the most abundant in the initial (11.9%), control soils (5.7%) and control soils with $(NH_4)_2SO_4$ addition (10.5%). Under PS treatment, *E. equina* ASV 02 was enriched in both soil and PBSA samples (21–23%). Under the PSN treatment, *E. equina* ASV 02 and *Pseudogymnoascus roseus* ASV 12 were enriched in both soil and PBSA samples (7–14% and 4–12%, respectively).

Beneficial and pathogenic microorganisms associated with PBSA: top 40 bacteria and fungi

We found that both beneficial and pathogenic microbes colonised PBSA samples, and they were listed among the top-40 most abundant bacteria and fungi (Figs. 4, 5). Three main groups of potentially beneficial bacteria associated with PBSA were as follows: (i) biological control agents (*A. ramosus*, and *P. agglomerans*), (ii) plant growth-promoting microorganisms (*A. chroococcum* and *C. rhizosphaerae*), and (iii) N fixers (*A. chroococcum*, *Bradyrhizobium*, *Devosia insulae*, *Rhizobium cellulosilyticum*, and *R. leguminosarum*). Among the beneficial bacteria, *C. rhizosphaerae* (7.5%), *A. ramosus*, *A. chroococcum*, *Bradyrhizobium*, and *R. leguminosarum* were enriched (1.1–7.5%) on PBSA under PS treatment. Most beneficial bacteria, especially N-fixing bacteria (three of five ASVs), were suppressed in PBSA under PSN treatment (Fig. 4). Only a biological control agent (*P. agglomerans*) was enriched in PBSA under PSN treatment. In fungi, we detected only two potential beneficial groups associated with PBSA: (i) biological control agents (*Chaetomium globosum, C. rosea*, *Dactylellina haptotyla*, *Gibberella fujikuroi,* and *Minimedusa polyspora*) and (ii) plant growth promoters (*G. fujikuroi, Mortierella elongata* and *Solicoccozyma terrea*). Among them, *D. haptotyla* was suppressed in PBSA under PSN treatment (Fig. 5). Only one biological control agent (*C. rosea*) was enriched in PBSA under PSN treatment.

Opportunistic human pathogenic bacteria and plant pathogenic fungi were the frequently detected pathogenic microorganisms in PS-treated PBSA samples. Opportunistic human pathogenic bacteria dominated the 40 most abundant bacterial ASVs, and these included *A. denitrificans, A. insolitus, C. pauculus*, *Lelliottia amnigena* and *P. agglomerans*. Among them, only *C. pauculus* was enriched in PS-treated PBSA samples, although it was not detected in either the initial or both control soils (control S and SN). Furthermore, *A. denitrificans and A. insolitus* (not detected in initial and control soils and almost absent in control soil with $(NH_4)_2SO_4$ addition) was highly enriched in PSN-treated PBSA samples (15% and 19%, respectively). Apart from opportunistic pathogenic bacteria, there was an animal and human pathogen (*Brucella melitensis*) that was only detected in PSN-treated PBSA sample, where it was abundant. The 40 most abundant fungi were dominated by plant pathogens (Fig. 5). Among them, *I. macrodidyma* was enriched after PBSA was incorporated into the soil (PS treatment). PSN treatment markedly enriched the relative abundances of *C. rosea* and *F. solani*, whereas it significantly suppressed those of *I. macrodidyma*. Some plant pathogens, such as *C. globosum* and *F. solani* were also classified as opportunistic human pathogens. In this study, we found that individual bacterial and fungal ASVs may represent more than one function, even the opposite one. For example, *P. agglomerans* is an opportunistic pathogen that also helps in biological control, and *C. rosea* is classified as either a biological control or a plant pathogen depending on the plant species being considered.

Effects of PBSA on beneficial and pathogenic microbes in soil

PBSA not only offers a habitat for beneficial and pathogenic microbes, but also alters the relative abundances of such microbes in soils. The relative abundances of these microbes detected on PBSA and in soil did not always correspond with each other. We detected three patterns: (i) enriched both in soil and in PBSA [bacteria: *Bradyrhizobium* (N fixation), fungi: *Dactylellina haptotyla* (biological control, and saprotroph), *E. equina* (opportunistic human pathogen and saprotroph), and *I. macrodidyma* (plant pathogen)]; (ii) enriched only on plastic but not in soil [bacteria: *A. ramosus* (biological control), *C. rhizosphaerae* (plant growth-promoting bacteria), *C. pauculus* (opportunistic human pathogen), *R. leguminosarum* (N fixation), fungi: *C. rosea* (biological control and plant pathogen)] and (iii) enriched only in soil but not on plastic [fungi: *A. alternatum* (biological control), *G. fujikuroi* (biological control, plant growth promoter and plant pathogen), *Gibellulopsis nigrescens* (plant pathogen)]. Addition of $(NH_4)_2SO_4$ together with PBSA (PSN treatment) highly enriched both bacteria [*A. denitrificans* and *A. insolitus* (denitrification and opportunistic human pathogens)] and fungi [*C. rosea* (biological control and plant pathogen) and *F. solani* (plant pathogen and opportunistic human pathogen)] both in soil and on PBSA. *E. equina* (opportunistic human pathogen and saprotroph) was relatively resistant to the combined effect of $(NH_4)_2SO_4$ and PBSA addition

as its relative abundance was not significantly different between PS- and PSN-treated soils.

Discussion

How soil and soil microorganisms respond to PBSA addition?

We are able to maintain richness and community compositions of soil prokaryotes (archaeal and bacterial) and fungi in control treatment under laboratory system for 90 days to be resemble to those of the initial field soil, thus changes in microbial communities observed in our experiment are highly representative by our treatment settings. The decline in the soil microbial richness under PBSA addition varied among microbial kingdoms in the following order: fungi > archaea > bacteria. Fungi were the most sensitive, as their diversity largely declined (45%) and their community composition was significantly changed by PBSA addition. Bacterial richness was resistant to PBSA, but bacterial community composition significantly changed by gaining N-fixing bacteria, such as *A. chroococcum*, *R. leguminosarum*, and *Bradyrhizobium* sp., under PBSA addition. Contrastingly to bacterial and fungal communities, the archaeal community composition was resistant to PBSA but the archaeal richness significantly reduced. pH did not explain the changes in soil archaeal and fungal diversity and bacterial and fungal community compositions in PS-treated soil compared with those in control soil as the pH values in these two treatments were similar. We suggest that the presence of PBSA promotes specific fungi (especially, *E. equina*, and *I. macrodidyma*) that may outcompete other fungi and eventually affect fungal–bacterial interactions. Strong inter-kingdom relationships between bacteria and fungi, especially between N-fixing bacteria and fungi, in PBSA were demonstrated in soil under field conditions [49]. Indeed, we found that *Bradyrhizobium* sp. was the most enriched taxa in soil after PBSA was incorporated for 90 days, which occurred concurrently with an increase in the relative abundance of *Exophiala* spp., known as efficient lipase-producing fungi [50].

The combination of the two amendment additions, PBSA and $(NH_4)_2SO_4$ fertiliser, caused a significant decline in diversity and changed the community composition of all three kingdoms. This can be partly explained by the alteration of soil pH caused by the addition of $(NH_4)_2SO_4$. Ammonium sulphate has a pH of 5.5 and immediately changed soil pH (by 0.2 unit) under $(NH_4)_2SO_4$ treatment. In soil, ammonium (NH_4^+) ions are oxidised to nitrate (NO_3^-) by nitrification, causing the release of H^+ ions, leading to a reduction in soil pH [51]. It has been previously reported that $(NH_4)_2SO_4$ application at a rate of 210 kg/ha (which is similar to the application rate in our study) causes a reduction in pH from 5.8 to 5.2 in rice field soil (Inceptisols) [51]. In our system, there were no plants to neutralise the H^+ from nitrification process, which made the pH decrease approximately 1.2 units from 7.5 to 6.3. Soil acidification is known to negatively affect a broad range of soil organisms, especially bacteria [52]. In our experiment, however, the soil pH (except two replicates from PSN treatment, pH = 5.8) remained close to physiological optimum (6–6.5), therefore microorganisms were not subjected to the acidic stress. The microbial community composition was also shifted towards nitrophilic microorganisms, which can outcompete other microbes under high $(NH_4)_2SO_4$ conditions in soil. For example, we frequently detected *Nitrocosmicus* and *Nitrososphaeraceae* (archaea) [53] and *A. insolitus* and *A. denitrificans*, (bacteria) [54], which are known to be nitrophilic organisms.

Positive effects of bio-based and biodegradable plastic on soil systems

Bio-based and biodegradable plastics may introduce some beneficial microbes to the soil system and facilitate their enrichment [24]. In this study, beneficial microbes were enriched in PBSA and/or soil. These beneficial microbes, which included N-fixing bacteria, plant growth-promoting microbes, and biological control agents, can potentially improve plant growth and production [55]. To increase the richness of these beneficial soil microbes, plant species need to be considered. For example, *C. globosum* can function as either a biological control or a plant pathogenic fungus depending on the plant species with which it is interacting. A previous report showed that *C. globosum* causes leaf spot disease on potato [56] and pomegranate [57, 58]. Conversely, *C. globosum* has exhibited biocontrol potential against *Colletotrichum*, *Fusarium*, *Phytophthora*, *Pyricularia*, *Rhizoctonia*, and *Sclerotium* pathogens [59] by suppressing the growth of these fungi through competition for substrates and nutrients, mycoparasitism, antibiosis, or a combination of various mechanisms [60, 61]. The efficacy of *C. globosum* as a biocontrol agent has also been demonstrated for the control root rot disease in citrus, black pepper, strawberry, damping-off disease in sugar beet as well as late blight disease in potatoes [62, 63].

Negative effects of bio-based and biodegradable plastics on soil systems

Our study is the first to list the microbes associated with PBSA using in-depth analyses of the fungal, bacterial, and archaeal community compositions at a high resolution. In this study, we detected opportunistic human pathogens in PBSA–soil system. However, these opportunistic human pathogens have also been identified across different environmental samples. Adding PBSA together

with fertilisation by $(NH_4)_2SO_4$ can increase the abundances of a very important animal and human pathogen, *B. melitensis* [64]. Some opportunistic human pathogens were enriched in PS-treated PBSA samples, although it was not detected in either the initial or control (Control S and Control SN) soils. These pathogens may also present in the initial and control soils but occurred at extremely low abundances, so that they cannot be detected by using Illumina Miseq sequencing as this technique has a certain detection limit (~ 10 cells/ml) [65].

Some plant pathogens were detected or enriched on PBSA and in soil such as *I. macrodidyma*. *F. solani*, a plant pathogen with a broad host range, was highly enriched on PBSA under PSN treatment [66], indicating that this fungus was proliferating and metabolically active. Compared with decomposing wheat straw (frequently used as natural mulch [67]) in the field where our initial soil was collected, PBSA (under both PS and PSN treatments) harboured lower number of fungal plant pathogens [68]. Although some crop production studies [69, 70] reported stable yield and no disease increases after multiple years of biodegradable mulch application, our results revealed a potential interaction between PBSA and nitrogen fertilisation promoting some important plant pathogens, especially *F. solani*. However, the possibility of disease outbreak may highly depend on plant species, level of N fertilisation, mulching film waste management, and any other ecosystem properties related to plant–pathogen interaction. To be at the safe side, we suggest to remove PBSA mulching film after use to avoid the accumulation of their residues in soil. Future research should focus on the effects of different forms (manure and synthetic N fertilisers) and application rates of N fertilisers on animal, human and plant pathogens, interaction of microbes in the PBSA–soil system and the introduction of beneficial microbes to control plant pathogenic fungi.

Limitations and implications

Our study clearly illustrates the changes of soil microbial community composition and reduction of their richness due to the high load of PBSA addition and its interaction with $(NH_4)_2SO_4$ fertilisation. Such responses occurred over a relatively short period of 90 days under controlled temperature and moisture conditions in the laboratory. Under real field conditions, soil microbial communities change in response to amendment addition and management (e.g. cover crop, tillage, manure addition, etc.), dominant plant species, and by seasonal effects (e.g. temperature and precipitation fluctuations in winter, spring, summer, and fall) [71, 72], which were beyond the scope of our study. As we used the simplified soil systems addressing the two main factors (PBSA addition and inorganic nitrogen fertilisation), our results may not cover other complex interactions, especially those from plants, seasonal changes, and land use occurring in real ecosystems. Soil moisture, temperature and agricultural practices alter significantly during different seasons [71, 72]. While the disturbances to soil systems as well as the extreme weather conditions (drought, freezing, flooding) can negatively impact soil microbial communities, such negative effects can be strongly reduced with increasing plant diversity [73]. These mentioned factors should be considered in future studies, although mechanistic interpretations are difficult in real ecosystems. Another limitation of our study is the high dose of PBSA addition (6% PBSA–soil (w/w)). This is higher than the dose that normally used for evaluating the effect of plastics in soils (1%), and the typical dose of the biodegradable plastics mulching film in a normal single use (~ 0.0063%) [74, 75]. Although, there is a possibility that the bio-based and biodegradable mulching films can be highly accumulated in agricultural soils after many years of applications, especially in dry and cold areas where the degradation rate under field conditions is slow [23], the amount may not reach 6%. Such high load of plastic film can be found in land-fill where the percentage reaches 6.5% (w/w) [76]. Overall, our results should be interpreted with caution. Our study rather provides information on diverse soil microbial communities associated with PBSA degradation and how they respond to $(NH_4)_2SO_4$ fertilisation. We clearly demonstrated that $(NH_4)_2SO_4$ fertilisation significantly increased the degradation rate of PBSA film which may have an implication for its waste management.

Conclusions

For the first time, the effects of a bio-based and biodegradable plastic, PBSA, on the soil microbiome have been investigated using high-resolution molecular technique. In contrast to previous reports, we conclude that PBSA significantly affects the soil microbiome; however, different microbial kingdoms have different responses to the presence of PBSA. We show that adding PBSA to soil facilitated the growth of a fungal minority, thereby reducing the fungal richness and strongly changing both bacterial and fungal community composition. The combined amendment additions of PBSA and N fertilisation [in the form of $(NH_4)_2SO_4$] on the soil microbiome are much greater than the effects of PBSA alone owing to a strong decrease in soil pH and a shift in the soil microbiome towards nitrophilic microbes. We only detected some opportunistic human pathogens, except when $(NH_4)_2SO_4$ fertilisation was used in combination with PBSA, which increased the sequence read abundances of the animal and human pathogen *B. melitensis*. Plant pathogenic fungi were also detected and enriched in PBSA films as well as in the surrounding soils. Adding PBSA

and N fertilisation can either reduce the sequence read abundances of some plant pathogens to almost zero or increase the abundance of specific pathogens, especially the economically important pathogen *F. solani*. Apart from plant pathogens, we also detected many potential microbial control agents and plant growth-promoting microorganisms owing to PBSA addition. Future studies should focus on evaluating the effects of bio-based and biodegradable plastics on the soil microbiome, plant–microbe interactions, and soil feedbacks under various field conditions.

Abbreviations

ASVs: Amplicon sequence variants; C:N ratio: Carbon:nitrogen ratio; PBAT: Polybutylene adipate terephthalate; PBS: Polybutylene succinate; PBSA: Polybutylene succinate-co-adipate; PHA: Polyhydroxyalkanoate; PLA: Polylactic acid.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-021-00528-5.

Additional file 1: Appendix 1. PBSA chemical and physical properties. **Figure S1.** Rarefaction curves of archaea (a), bacteria (b), and fungi (c). **Figure S2**. Links between soil pH and microbial richness: a) Soil pH values of initial soil, initial-N soil, control S, control SN, and soils in PS, and PSN treatment on 90 days. Correlations between soil pH and richness of archaea (b), bacteria (c) and fungi (d). **Figure S3**. Factors explain variations in microbial community composition: percent explainable variations of archaeal (a), bacterial (b), and fungal (c) communities explained by PBSA addition, N fertilization, soil pH, and combinations of these factors. Total variations of archaeal, bacterial, and fungal communities explained by PBSA addition, N fertilization, soil pH, and combinations of these factors are 13, 23 and 28%, respectively. **Table S4.** Comparisons between microbial community compositions of (i) PBSA and soil counterparts and (ii) soils of different treatments and (iii) control soils (Control S and Control SN) and PBSA (PS and PSN treatments) using analysis of similarities (ANOSIM) and non-parametric multivariate analysis of variance (NPMANOVA).

Additional file 2: Table S1. Information on relative abundance (%) of archaeal ASVs detected in poly(butylene succinate-co-adipate) (PBSA) and soils of all treatments. Please see in another excel file. **Table S2.** Information on relative abundance (%) of bacterial ASVs detected in poly(butylene succinate-co-adipate) (PBSA) and soils of all treatments. Please see in another excel file. **Table S3.** Information on relative abundance (%) of fungal ASVs detected in poly(butylene succinate-co-adipate) (PBSA) and soils of all treatments. Please see in another excel file.

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Authors' contributions

WP, EB and BT conceived and designed the study. BT, WP, VG, MU and SW led the laboratory experimental setup. BT, WP, EB, MU and VG collected the samples and metadata. WP, EB and FB contributed reagents and laboratory equipment. BT, WP, and SW led the DNA analysis. SW led bioinformatics. BT, WP and KJ led the microbial taxonomy and data analyses. BT, KJ and WP wrote the manuscript. BG and WP supervised BT. MN, BG, ASL, EGA, EB and FB reviewed and gave comments and suggestions for manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The Illumina sequencing of prokaryotic and fungal datasets are deposited in The National Center for Biotechnology Information (NCBI) database under BioProject ID: PRJNA702448 (http://www.ncbi.nlm.nih.gov/bioproject/702448). Microbial taxonomic and relative abundance information is provided in Additional file 2 Table S1, S2 and S3.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

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

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