Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supporting Information

Nitrogen fixing bacteria facilitate microbial biodegradation of a bio-based and biodegradable plastic in soils under ambient and future climatic conditions

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Methodology: decomposability and microbiome of PBSA over 328 days under ambient and future climatic conditions.

We reanalysed and integrated existing (N fixing bacteria, fungi and fungal biomass) as well as the newly analysed (nifH gene abundances, represent the activity of N fixing bacteria) datasets on the PBSA microbiome from our previous PBSA decomposition study. Furthermore, we set-up laboratory experiment to demonstrate that N fixation is the rate-limiting step for PBSA decomposition. All information related to study sites, experimental set-up and PBSA microbiome characterization are presented in supplementary information. Briefly, The PBSA degradation experiment was carried out for 328 days in soils at the conventional farming treatment (crop rotation: winter rape, winter wheat and winter barley) plots of the Global Change Experimental Facility (Schädler et al., 2019) (GCEF), Bad Lauchstädt, Central Germany (51°22060 N, 11°50060 E, 118 m a.s.l.). The study area is characterized by an oceanic climate (Cfb) climate with average annual precipitations at 489 – 525 mm and mean temperature at 8.9 – 9.7 °C. The climate treatment simulates the future climate for Central Germany in the time period 2070 – 2100, in particular reduced summer precipitation by approximately 20%, increased precipitation in spring and autumn by approximately 10% and increased mean temperature by 0.55 °C is predicted. We placed the PBSA film (BioPBS FD92, 21 cm x 30 cm, thickness 50 µm) on the top of the soil in conventional farming plots (both ambient and future climate treatments) in August 2018 (*ca*. 4 years after climate treatments were effectively manipulated) for 20 days to allow for PBSA to be colonized by air and soil microbes and exposed to the surrounding environment. Later, the PBSA films were buried under the soil at 5 cm depth.

We determined the decomposability and microbiome of PBSA over 328 days on four sampling times (0, 30, 180 and 328 days) in 5 true replicates (each replicate comes from each individual distinct conventional farming plot (16 × 24 m) of the GCEF, in total 5 ambient and 5 future climate treatment plots were used). Fungal biomass were detected at all sampling times except the 0 day while bacterial biomass was very low at 30 day consistently detected from 180 days, thus in this study we mainly focus on the interaction between N fixing bacteria and fungi at 180 (accounting for 12 – 13% gravimetric and 3 – 5% molar mass losses) and 328 (accounting for 28 – 30% gravimetric and molar mass losses) days. Gravimetric weight and molar mass was determined on 12.5 cm^2 oven-dried PBSA samples (60 °C, 72 hr or until constant weight) using 5 digits balance (Mewes Wägetechnik, Haldensleben, Germany) and gel permeation chromatography (GPC)[1,2].

Changes of PBSA surface and associated microrganisms analyzed with Scanning Electron Microscopy (SEM).

The PBSA microbiome was characterized using rRNA operon amplicon sequencing by Illumina MiSeq and quantitative real-time polymerase chain reaction (qPCR) assays. Sample processing for DNA extraction of PBSA buried in soil were modified from a protocol published elsewhere[3]. Briefly, we randomly cut 12 cm^2 PBSA samples and removed loosely adherent soil particles by vortexing in sterile phosphate-buffered saline (0.01M) for 5 min. PBSA samples were then submerged and shaken vigorously in 45 mL sterile Tween (0.1%) and this step was repeated 3 times. The samples were then washed 7 times using sterile water. Microbial biomass attached firmly with PBSA sample was used for DNA extraction using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany). Fungal biomass and nifH gene abundances were quantified using SYBR Green-based qPCR assay. For bacterial amplicon library, the 16S rRNA gene V4 region was amplified using the universal prokaryote primer pair 515F (5' -GTGCCAGCMGCCGCGGTAA- 3') and 806R (5' -GGACTACHVGGGTWTCTAAT- 3')[4]. For the fungal amplicon libraries, the fungal internal transcribed spacer 2 (ITS2) gene was amplified using the fungal primer pair fITS7, and ITS4 primer[5,6]. We performed paired-end sequencing of 2 x 300 bp 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 of Environmental Research. The raw 16S and ITS rDNA sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under study accession number PRJNA595487 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA595487).

We screened for good-quality sequences of the 16S and ITS rDNA raw sequence reads (a minimum length of 200 nt (bacteria) or 120 nt (fungi); a minimum average quality of 25 Phred score for bacteria and fungi; containing homopolymers with a maximum length of 20 nt; without ambiguous nucleotides) using MOTHUR[7] and OBI Tools[8] software suits. We detected chimeric sequences using the UCHIME algorithm[9] as implemented in MOTHUR and removed them from the datasets. The obtained reads were then clustered into operational taxonomic units (OTUs) using the CD-HIT-EST algorithm[10] at a threshold of 97% sequence similarity. The OTU representative sequences (defined as the most abundant sequence in each OTU) were taxonomically assigned against the reference sequences from the SILVA database[11] v132 for prokaryote 16S and from the Unite database[12] (version unite.v7) for fungal ITS using the naive Bayesian classifier[13] as implemented in MOTHUR using the default parameters. Rare OTUs (singletons to tripletons), which potentially might represent artificial sequences were removed. The read counts were rarefied to the smallest read number per sample (31,141 and 26,056 reads for bacteria and fungi, respectively). Rarefaction curves of all the samples indicated sufficient sampling effort, thus we obtain reliable information on both richness and community composition of microbiome associated with PBSA degradation. Potential N fixing bacteria assignments were carried out using relevant literature[14], including both N fixing bacteria associated with cereals (dominant plant in the study plots)[15] and legume plants (common plants in this study area)[16]. All identified N fixing bacteria are presented in Table S1 supplementary

information. We separated N fixing bacteria in to different functional groups: non-symbiotic N fixing aerobic bacteria (NSNB), non-symbiotic N fixing facultative anaerobic bacteria (NSNFB) and symbiotic N fixing bacteria (SNB). Various indicators from N fixing bacteria (richness and relative abundances of specific N fixing bacterial Operational Taxonomic Unit (OTU) and functional group as well as *nifH* gene abundances) and fungi (fungal biomass, specific relative abundances of dominant fungal OTUs (relative abundances >0.5%) and the entire fungal community) were used for testing our hypotheses on the role N fixing bacteria to facilitate microbial biodegradation of PBSA.

To demonstrate that N fixation is the rate limiting step of PBSA decomposition in soil, we set-up PBSA decomposition experiment in laboratory using the soil from the GCEF experimental plots. PBSA films were cut into pieces (2mm – 5mm) and buried in sterile glass jars containing soils from three treatment (nonsterile soil, non-sterile soil with N addition and sterile soil, five replicates). In N addition treatments, we added N (in form of $(NH4)_2SO_4$) directly soil to replace N fixation step and make N immediately available for soil microbes. We observed the microbial colonization and mass loss at 30 and 90 days. PBSA associated microrganisms were analyzed with Scanning Electron Microscopy (SEM) at 90 days. Microbial respiration were measured using Replicon from 0 – 90 days. More detailed experimental description and results are present in our previous publication [17].

Scanning Electron Microscopy (SEM)

Scanning electron microscope images were taken on a Philips XL30 FEG device (Philips, Amsterdam, NL) using an acceleration voltage of 5 kV. In general, the polymer samples were placed on carbon conductive tapes and coated by sputtering with 10 nm of a platinum/palladium 80/20 (w/w) alloy. A sputter coater "208 HR" with the associated thickness measuring unit "mtm 20" from Cressington (Cressington Scientific Instruments Ltd., Watford, England) was used for this purpose. Selected samples were cleaned and lyophilized before this type of preparation.

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Figure S1 *nifH* gene abundances quantified with quantitative real-time PCR.

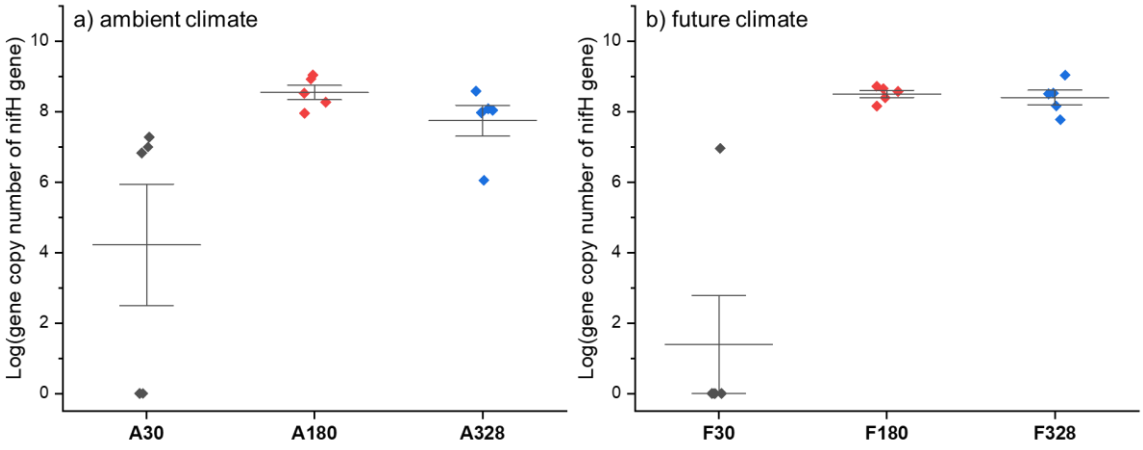

Figure S2. FTIR results of PBSA in different treatments.