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Bioplastic (PHBV) addition to soil alters microbial community structure and negatively affects plant-microbial metabolic functioning in maize

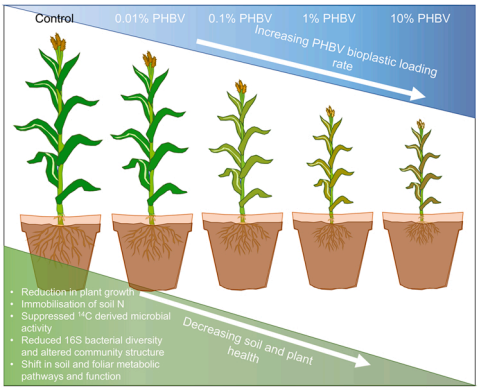
Robert W. Brown [a,*], David R. Chadwick [a], Huadong Zang [b], Martine Graf [a], Xuejun Liu [c], Kai Wang [c], Lucy M. Greenfield [a], Davey L. Jones [a,d,e]

[a] School of Natural Sciences, Bangor University, Bangor, Gwynedd LL57 2UW, UK
[b] College of Agronomy and Biotechnology, China Agricultural University, 100193 Beijing, China
[c] College of Resources and Environmental Sciences, National Academy of Agriculture Green Development, China Agricultural University, Beijing 100193, China
[d] Centre for Environmental Biotechnology, School of Natural Sciences, Bangor University, Bangor, Gwynedd LL57 2UW, UK
[e] Centre for Sustainable Farming Systems, Food Futures Institute, Murdoch, WA 6150, Australia

HIGHLIGHTS

- Effect of biopolymer PHBV microplastic on soil and plant health was investigated.
- PHBV addition caused a dose-dependent negative effect on soil and plant health.
- PHBV addition altered foliar metabolism and reduced plant growth and foliar N.
- PHBV reduced soil microbial activity and changed the bacterial community structure.
- Bioplastics may not be environmentally benign.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic contamination poses a significant threat to agroecosystem functioning, provoking a move away from the use of conventional oil-based plastics in agriculture, to biodegradable alternatives that may be degraded over a shorter timescale. The impact of these bioplastics on plant and soil health, however, has received relatively little attention. Here, we investigated the effect of soil loading (0.01%, 0.1%, 1% and 10%) of biobased microplastic poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) on soil and plant (*Zea mays* L.) health and function. We showed that PHBV caused a dose-dependent reduction in plant growth and foliar nitrogen (N) content while untargeted metabolite analysis revealed significant shifts in foliar metabolic function. These results were also reflected in soil, where PHBV led to reduced plant availability of both ammonium and nitrate. Soil ^{14}C-isotope tracing and 16S metabarcoding revealed that PHBV suppressed microbial activity, reduced bacterial diversity and shifted microbial community structure, inducing a major shift in soil metabolic pathways, and thus functioning. Overall, our data suggests that the bioplastic PHBV is not environmentally benign and that

* Corresponding author.
E-mail address: rob.brown@bangor.ac.uk (R.W. Brown).

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contamination levels as low as 0.01% (0.01 mg kg^{-1}) can induce significant short-term changes in both plant and soil microbial functioning, with potential implications for long term agroecosystem health.

1. Introduction

Plastic pollution is now a ubiquitous phenomenon in all terrestrial biomes (Rillig and Lehmann, 2020; Wang et al., 2021; Wang et al., 2022a). Although much of this plastic is present as macroplastics (> 5 mm in diameter), it is the presence of micro- (< 5 mm) and nano- (< 0.1 μm) plastic particles in soil that has drawn most concern (Qi et al., 2020a; Allouzi et al., 2021; Wang et al., 2022b; Zhang et al., 2022). One of the largest sources of plastic contamination in soil is from the use of plastic mulch films which are notoriously difficult to completely remove from the field at the end of the growing season (Chae and An, 2018). This leads to large amounts of mulch film becoming incorporated into the soil in subsequent tillage operations. Microplastics can be produced from the progressive breakdown of macroplastics due to photo-degradation and physical degradation, however, they can also be introduced into soil following atmospheric deposition (Xu et al., 2020) or the input of organic wastes (e.g., biosolids, composts; Zhang et al., 2020c). It is evident that a more sustainable alternative is required (Zhao et al., 2022). As such, it has been suggested that the use of conventional oil-based plastics be progressively phased out and replaced with more bio-based plastics made from renewable sources (Riggi et al., 2012; Yan et al., 2016; Zhao et al., 2021; Chong et al., 2022; Koul et al., 2022).

Adequate soil function and quality are key to ecosystem service provision, particularly in agroecosystems (i.e., provision of food, fuel and fibre) (Bünemann et al., 2018). It is now well established that conventional microplastics composed of polyethylene or polypropylene can exert a wide range of negative effects on the soil leading to a decline in soil quality (Qi et al., 2020a; Zang et al., 2020; Zhou et al., 2022). For example, these microplastics can interfere with earthworm growth and reproduction, alter microbial activity and function and change the physical properties of the soil (de Souza Machado et al., 2019; Rodríguez-Seijo et al., 2019; Lin et al., 2020; Azeem et al., 2021; Wang et al., 2021; Wang et al., 2022a). In contrast, however, the impact of bio-based biodegradable macro- and micro-plastics on soil functioning remains largely unknown. A range of studies have shown that biodegradable plastic films readily fragment in soil leading to the formation of smaller particles (Griffin-LaHue et al., 2022; Yang et al., 2022). This breakdown is catalysed by both photooxidation and biodegradation processes depending on the chemical nature of the material (Liwarska-Bizukojc, 2021). Further, evidence suggests that biodegradable plastics can alter soil microbial communities by changing the microclimate of the soil (Lian et al., 2022; Wang et al., 2022c) and by providing a substrate to support microbial growth, particularly fungal taxa (Bandopadhyay et al., 2018). It is also likely that this will have both direct and indirect effects on the biogeochemical cycling of key nutrients associated with soil organic matter (e.g. N, P, S). As most plastic biopolymers contain low amounts of N and P, relative to C, this may induce immobilization of plant nutrients within the microbial biomass, particularly with fast degrading films. However, it has also been suggested that biopolymer addition to soil may accelerate nutrient cycling by stimulating microbial activity (Bandopadhyay et al., 2018). One approach to holistically assess soil biogeochemical cycling is to investigate the soil metabolome, the collection of all low molecular weight metabolites that are produced by organisms during metabolism (Lankadurai et al., 2013). The metabolome is the product of the genome, transcriptome and proteome (Biswas and Sarkar, 2018) and has been shown to be sensitive to soil type (Withers et al., 2020), environmental stress (Brown et al., 2021) and microplastic addition (Sun et al., 2022b).

In view of the current uncertainty surrounding the sustainability of bioplastic use in agriculture, further studies are clearly needed to assess their environmental impact. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is one of the most widely used bioplastics in agriculture and can be synthesied from waste products (Chong et al., 2021a, 2021b). For risk assessment purposes, it would also be useful to determine critical thresholds of plastic contamination in soil where negative or positive effects on ecosystem service delivery occur. This would provide key information to growers and regulators on the safety and sustainability of biodegradable macro- and micro-plastics on agroecosystems.

This study, therefore, aimed to determine whether biobased microplastics pose a risk to plant and soil health. This was assessed using a combination of untargeted metabolomics to assess functional changes between treatments, and ^{14}C-labled glucose tracing as a proxy for microbial growth potential. Pure micro-bioplastic was chosen, as much of the current literature does not disentangle the effect of pure bioplastic from the plastic additives, e.g. plasticisers (Aguilar et al., 2020) and UV stabilisers (Sun et al., 2022a). Specifically, we hypothesized that; (i) the biodegradable nature of the bioplastic would promote soil microbial activity in a dose-dependent manner, (ii) the high C:N:P ratio of the bioplastic would induce nutrient immobilization (e.g. N and P), and (iii) increased root-microbial competition for nutrients would suppress plant growth.

2. Materials and methods

2.1. Soil sampling

Soil was collected from the Ahp horizon (0–10 cm depth) of a recently ploughed field at the Henfaes Research Centre, Abergwyngregyn, North Wales (53°14'29"N, 4°01'15"W). The soil is classified as a freely draining Eutric Cambisol (IUSS Working Group WRB, 2015) or Typic Hapludalf (US Soil Taxonomy) with crumb structure and sandy clay loam texture. The site has a temperate oceanic climate regime with long term (> 10 y) mean annual air temperature of 10.8 °C and annual rainfall of 1066 mm y^{-1}. The site has no previous history of plastic mulching or organic waste inputs likely to contain plastics (e.g. composts, biosolids). Prior to collecting the soil, the site had a land use history of cereal production (e.g. wheat, barley, maize) and grassland (*Lolium perenne* L.) in rotation. After collection, the soil was transported to the laboratory and sieved to pass 5 mm to remove stones and plant litter. This sieve size was used to minimise changes in soil structure and microbial activity (Jones and Willett, 2006). Field soil physicochemical properties are summarised in Table 1.

2.2. Experimental design

The experiment used the biobased microplastic PHBV (1–15 μm diameter; $[COCH_2CH(CH_3)O]_m[COCH_2CH(C_2H_5)O]_n$; TianAn Biologic Materials Ltd, Ningbo City, China). PHBV is a co-polymer of hydroxybutyrate and hydroxyvalerate and is used for biodegradable plastic manufacture and undergoes photodegradation and biodegradation in the environment to produce microplastics (Imam et al., 1999; Wei and McDonald, 2016). PHBV was mixed with individual batches of soil (n = 10) at rates of 0.01, 0.1, 1 or 10% on a gravimetric basis. Loading rates were chosen to represent 'homogenous mixing in soil' 'heterogenous mixing in soil', 'plastic contamination hotspots in soil', and 'field plastic waste dump sites'. These rates were chosen based on field observations and reports on the abundance of microplastics in typical soils under plastic mulch films (Huang et al., 2020; Qi et al., 2020a). The soil hotspots reflect the highly heterogenous distribution of plastic waste in a field after tillage, while the field waste hotspot loading rate reflects

plastic waste disposal sites at field margins. For context, for a typical 15 μm thick PHBV film with a density of 1.25 g cm^{-3}, the annual loading rate in the top 10 cm of soil would be 0.188 t ha^{-1} equivalent to 0.02% on a gravimetric basis, assuming a soil bulk density of 1.05 g cm^{-3} and homogenous mixing.

The batches of plastic-contaminated soil (0.5 kg) were then placed in individual opaque polyethylene plastic pots (1 litre). A control set of pots were also established containing no plastic. There were 10 replicates for each treatment. A pre-germinated maize seedling (*Zea mays* L. cv. Debalto; KWS Seeds Inc., Bloomington, MN, USA; roots 2 cm long) was then sown in each pot and the plants were placed in a greenhouse with automated ventilation and mean air temperature of 20 °C, reflecting conditions at the field site described in Section 2.1. All pots were weighed at the start of the experiment. The pots were reweighed five times a week and the amount of water lost by evaporation and transpiration was replaced with deionised water accordingly (Dumroese et al., 2015). The height of the shoots was measured weekly.

A Rhizon® MOM sampler (5 cm long, 0.15 μm pore size; Rhizosphere Research Products B.V., Wageningen, The Netherlands) was inserted in the centre of the pot at a 45° angle to recover soil solution. On a twice weekly basis, samples of soil solution were recovered 30 min after watering by attaching a sterile pre-evacuated vacutainer (VWR International, Wayne, PA) to the Rhizon® and leaving it in place overnight. The average recovery of soil solution was 5.3 ± 0.4 ml.

The plants were fertiliesed at 4 weeks, to ensure nutrients did not limit plant growth or function, fertiliser was added at a rate equivalent to 100 kg N ha^{-1}, 20 kg P ha^{-1} and 50 kg K ha^{-1} (as NH_4NO_3, KH_2PO_4 and KCl) to all the pots. The experimental growth period lasted 8 weeks in total, at this point the pots were destructively sampled. The shoots were cut at the soil surface and weighed. Subsequently, 1.5 g of shoot material was immediately placed in a glass vial, frozen at − 80 °C (72 h) to quench metabolic activity and then freeze-dried (48 h) for subsequent metabolomic analysis. The remaining shoot material was weighed, placed in paper bags and oven dried (80 °C, 24 h). A subsample of soil (50 g) was also immediately placed at − 80 °C to quench metabolic activity and freeze-dried for metabolomic analysis and microbial community assessment.

2.3. Soil and plant analysis

At plant harvest, soil N availability was evaluated by performing a 1:5 (w/v) soil-to-0.5 M K_2SO_4 extract (200 rev min^{-1}, 1 h), centrifuging the extracts (18,000 *g*, 5 min) and analysing the NH_4^+ and NO_3^- concentration colorimetrically according to the salicylate procedure of Mulvaney (1996) and vanadate method of Miranda et al. (2001), respectively. Plant-available P was evaluated by performing a 1:5 (w/v) soil-to-0.5 M acetic acid extract (200 rev min^{-1}, 1 h), centrifuging the extracts (18,000 *g*, 5 min) and analysing the P concentration colorimetrically according to the molybdate blue method of Murphy and Riley (1962). Available Na, K and Ca in the same 0.5 M acetic acid extracts was determined with a Sherwood model 410 flame photometer (Sherwood Scientific Ltd, Cambridge, UK). Soil pH and electrical conductivity (EC) were measured in a 1:2.5 (w/v) soil:distilled H_2O extract after shaking (200 rev min^{-1}, 10 min) using standard electrodes. Total C and N of the soil and shoots at harvest were determined on oven-dried soil (105 °C, 24 h) and plants (80 °C, 24 h) using a TruSpec® CN analyser (Leco Corp., St Joseph, MI). Soil organic matter was quantified by loss-on-ignition in a muffle furnace (450 °C, 16 h) (Ball, 1964), although it must also be noted that this technique will oxidise the bioplastic in the sample as well as the native SOM. The pH, EC, NO_3^- and NH_4^+ concentrations in soil solution recovered using the Rhizon samplers were determined as described above for the soil extracts.

2.4. Untargeted plant and soil metabolomic sample preparation, extraction, and analysis

Plant shoot and soil samples ($n = 5$) were randomly selected from the 10 replicate pots. Post-lyophilisation, the samples were ground to a fine powder using a MM200 stainless-steel ball mill (Retsch GmbH, Haan, Germany) before being transferred to sterile polypropylene 1.5 ml microfuge tubes. Samples were then shipped on dry ice (−78.5 °C) to the West Coast Metabolomics Center (UC Davis Genome Center, Davis, CA, USA) for untargeted primary metabolism analysis.

Untargeted primary metabolism extraction was performed as described by Brown et al. (2021). Briefly, extraction consisted of vortexing a 1:0.025 (w/v) soil/plant-to-3:3:2 (v/v/v) MeCN/IPA/H_2O solution, before shaking for 5 min at 4 °C, centrifuging and an aliquot of the supernatant recovered for analysis. Metabolite analysis was performed on a 6890 GC (Agilent Technologies Inc., Santa Clara, CA) coupled to a Pegasus III TOF MS (Leco Corp.), injected via a Gerstel CIS4 with dual MPS Injector (Gerstel, Muehlheim, Germany) using the chromatographic parameters described in Fiehn et al. (2008). ChromaTOF vs. 2.32 was used for data pre-processing before being further processed in the metabolomics BinBase database as described in Fiehn

Table 1
Influence of PHBV microbioplastic addition rate on key soil and plant properties at the end of the experimental trial. Results are expressed on mean dry weight basis ± SEM ($n = 10$) unless otherwise stated. Letters denote significant differences between treatments using either an ANOVA with a Tukey Post-hoc test for data that conformed to parametric parameters or a Kruskal-Wallis test with a Bonferroni corrected Dunn Post-hoc test for non-parametric data ($p < 0.05$). * represents $p < 0.05$, * * represents $p < 0.01$, * ** represents $p < 0.001$.

	Soil PHBV application rate (% by weight)					[a]ANOVA/[b]Kruskall
	0%	0.01%	0.1%	1%	10%	
Soil						
pH	5.74 ± 0.05^{b}	5.74 ± 0.04^{b}	5.89 ± 0.05^{ab}	5.99 ± 0.02^{a}	5.81 ± 0.04^{b}	* **^{b}
EC (μS cm^{-1})	41.3 ± 3.7^{c}	54.2 ± 9.3^{c}	67.4 ± 4.4^{bc}	97.3 ± 5.5^{b}	144.4 ± 13.1^{a}	* **^{a}
Organic matter (%)	5.41 ± 0.45^{bc}	4.80 ± 0.59^{c}	5.77 ± 1.01^{bc}	6.42 ± 0.26^{b}	16.18 ± 0.61^{a}	* **^{b}
Moisture (%)	25.7 ± 0.7^{cb}	16.4 ± 2.0^{d}	21.0 ± 1.4 ^{cd}	29.0 ± 2.3^{ab}	33.4 ± 0.5^{a}	* **^{a}
NO_3^- (mg N kg^{-1})	0.60 ± 0.16^{ab}	2.23 ± 0.99^{ab}	1.37 ± 0.28^{a}	0.55 ± 0.09^{b}	0.84 ± 0.21^{ab}	*^{b}
NH_4^+ (mg N kg^{-1})	4.53 ± 1.38^{a}	2.32 ± 0.99^{ab}	1.64 ± 0.41^{bc}	1.10 ± 0.14 ^{cd}	0.77 ± 0.08^{d}	* **^{b}
PO_4^{3-} (mg P kg^{-1})	20.0 ± 0.6^{a}	17.3 ± 0.7^{ab}	16.1 ± 1.1^{b}	14.2 ± 1.4^{b}	16.1 ± 0.6^{b}	* *^{a}
C:N ratio	9.72 ± 0.75^{c}	9.68 ± 0.43^{cb}	9.19 ± 0.29^{c}	10.8 ± 0.21^{b}	37.4 ± 1.84^{a}	* **^{b}
Na (mg kg^{-1})	39.6 ± 2.1	34.5 ± 1.4	40.2 ± 2.7	40.4 ± 2.3	45.0 ± 3.5	
K (mg kg^{-1})	244 ± 31^{a}	110 ± 15^{c}	158 ± 15^{bc}	234 ± 11^{a}	190 ± 7^{ab}	* **^{a}
Ca (mg kg^{-1})	1230 ± 32^{a}	1221 ± 26^{a}	1380 ± 123^{a}	1260 ± 40^{a}	1035 ± 21^{b}	* **^{b}
Microbial CUE	0.65 ± 0.02^{b}	0.69 ± 0.01^{ab}	0.73 ± 0.01^{a}	0.67 ± 0.02^{ab}	0.71 ± 0.02^{ab}	* **^{a}
Plant						
Height (cm)	70.3 ± 1.2^{a}	62.9 ± 2.0^{b}	49.1 ± 1.5^{c}	24.0 ± 0.9^{d}	24.3 ± 1.0^{d}	* **^{b}
Shoot biomass (g $plant^{-1}$)	3.55 ± 0.11^{a}	2.51 ± 0.20^{b}	1.78 ± 0.19^{c}	0.70 ± 0.12^{d}	0.68 ± 0.15^{d}	* **^{a}
Foliar C:N ratio	42.1 ± 2.1^{c}	51.6 ± 3.3^{bc}	54.0 ± 2.2^{bc}	61.9 ± 8.0^{ab}	73.8 ± 3.1^{a}	* **^{b}

EC, electrical conductivity; Microbial CUE, microbial carbon use efficiency.

et al. (2008).

2.5. Soil microbial community analysis

Fresh soil was placed into a MoBio PowerMag Soil DNA Isolation Bead Plate (MoBio Laboratories Inc., Carlsbad, CA). DNA was extracted following MoBio's instructions on a KingFisher Flex robot (Thermo Fisher Scientific Corp, Waltham, MA). Bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the V4 region (515F 5'-GTGCCAGCMGCCGCGGTAA-3', and 806R 5'-GGACTACHVGGGTWTCTAAT-3'), as per the protocol of Kozich et al. (2013). Amplicons were sequenced with an Illumina MiSeq using the 300-bp paired-end kit (v.3) (Illumina Inc., San Diego, CA). Sequences were denoised, taxonomically classified using Silva (v. 138) as the reference database, and clustered into 97%-similarity operational taxonomic units (OTUs) with the '*mothur*' software package (v. 1.44.1) (Schloss et al., 2009).

The potential for contamination was addressed by co-sequencing DNA amplified from samples and from template-free controls (negative control) and extraction kit reagents processed the same way as the samples. A positive control consisting of cloned sulfur-oxidizing bacteria (SUP05) DNA, was also included. OTUs were considered putative contaminants (and were removed) if their mean abundance in controls reached or exceeded 25% of their mean abundance in the samples. OTUs were filtered if they had fewer than 3 counts and occurred in fewer than 10% of the samples. Sequencing read files analysed in this study can be accessed from the National Center for Biotechnology Information (project PRJNA856841).

2.6. Soil microbial activity

At the end of the experiment, soil microbial activity was measured by adding ^{14}C-[U]-glucose (Lot 3632,475; PerkinElmer Inc., Waltham, MA; 0.5 ml, 16 kBq ml^{-1}, 10 mM) to 5 g of soil contained in a sterile 50 cm^3 polypropylene centrifuge tube. Experimentation was performed with 5 replicates. After addition of the ^{14}C-gluose, a 1 M NaOH trap (1 ml) was suspended above the soil to catch any respired $^{14}CO_2$. The tubes were then hermetically sealed and incubated at room temperature (20 ± 1 ℃) in the dark. The NaOH traps were replaced after 1, 2, 4, 7, 22, 48, 72 and 168 h, post glucose application. The efficiency of the NaOH traps was > 98% (as determined by collecting $^{14}CO_2$ generated from adding excess 0.1 M HCl to 0.001 M Na$H^{14}CO_3$). The amount of ^{14}C in the NaOH traps was measured by mixing with Optiphase HiSafe 3 liquid scintillation cocktail (PerkinElmer Inc., Waltham, MA, USA) and placing on a Wallac 1404 scintillation counter (Wallac EG&G, Milton Keynes, UK) with automated quench correction.

2.7. Data processing and statistical analysis

All statistical and graphical analysis was performed in the R environment (v 4.1.1; R Core Team, 2021), and graphical analysis was constructed using the '*ggplot2*' package (Wickham, 2016), unless otherwise stated. Analysis was deemed significant if $p < 0.05$.

Only metabolites that were present in > 10% of either soil or plant samples were taken forward for analysis, to reduce analysis bias. All metabolomic statistical analysis was performed using generalized logarithm transformed (glog) and Pareto scaled data. A principal component analysis (PCA) was constructed for the plant and soil metabolite data using the '*vegan*' package (Oksanen et al., 2020), to reduce the dimensionality of the dataset and give a visual representation of data variance. The PCA was quantitively evaluated with Permutational multivariate analysis of variance (PERMANOVA) using '*adonis2*' in '*vegan*' and pairwise comparisons were performed in '*RVAideMemoire*' (Hervé, 2021), with false discovery rate ('*fdr*') correction (Benjamini and Yekutieli, 2001). Treatment comparisons were further illustrated using a heatmap constructed using Metaboanalyst 5.0 (Pang et al., 2020, 2021). One-way ANOVA (analysis of variance) was also performed to identify significant differences in compound concentrations between treatments.

From the 16 S data, alpha diversity was calculated using the Shannon index on raw OTU abundance tables after filtering out contaminants, as described in Section 2.4. The significance of diversity differences between treatments was tested using an ANOVA model followed by a post hoc Tukey HSD test using the '*agricolae*' package (de Mendiburi, 2021). To obtain the overall variance in microbial composition, the similarities in microbial beta diversity across samples and location treatments were visualized by principal coordinates analysis (PCoA) multidimensional scaling ordinations based on Bray-Curtis dissimilarity in '*vegan*', PERMANOVA and pairwise comparisons performed, as described above.

Soil chemical and physical characteristics and plant health were visually assessed for normality and homogeneity. For data that did not conform to parametric assumptions even after using $\log_{10}$ transformation (pH, NO_3-N, NH_4-N, soil C:N, SOM, Ca, plant height, plant C:N) a Kruskal-Wallis test ('*stats*' package; R Core Team, 2021) was used to assess the similarities between MP treatments, otherwise a one-way ANOVA was used (EC, PO_4-P, Na, K). ^{14}C microbial activity (i.e., total $^{14}CO_2$ production as a percentage of ^{14}C labelled glucose applied after 168 h) was also tested by ANOVA. Microbial C use efficiency was calculated from the $^{14}CO_2$ mineralization data using the kinetic modelling approach of Jones et al. (2018).

3. Results

3.1. Soil physicochemical properties and plant health

Addition of PHBV to soil significantly affected some key aspects of soil chemistry and plant health, as summarised in Table 1. These changes were strongly evidenced by a significant reduction in plant height ($H_{(4)} = 40.3$, $p < 0.001$) and shoot biomass ($F_{(4,32)} = 53.6$, $p < 0.001$) with increasing PHBV loading rate. At high PHBV loadings the plants also showed the presence of anthocyanins in the leaves and had a lower foliar C:N ratio ($H(4) = 25.11$, $p < 0.001$) (Table 1). Prior to fertilization, the amount of available NO_3^- and NH_4^+ in soil solution decreased in a dose-dependent manner with increasing PHBV addition with effects seen at the lowest PHBV addition rate (Table S1). After fertilisation, while there were some significant differences between treatments ($H_{(4)} = 12.28$, $p < 0.05$), soil NO_3^- concentrations showed variability with no consistent trend evident with bioplastic application, whereas soil NH_4^+ concentrations decreased with increasing PHBV loading ($H_{(4)} = 28.31$, $p < 0.001$) (Table 1). A significant increase in EC ($F_{(4,42)} = 27.97$, $p < 0.001$) with increasing PHBV loading was also observed during the experiment (Table S1) and at plant harvest (Table 1). As expected, the amount of soil organic matter was significantly higher under the 10% loading rate due to the presence of C in the PHBV ($H_{(4)} = 27.66$, $p < 0.001$) (Table 1). Correspondingly, the C:N ratio of the soil also increased significantly under the 1% and 10% loading rate compared to the control ($H_{(4)} = 30.20$, $p < 0.001$).

3.2. Soil primary metabolites

Untargeted primary metabolomic analysis tentatively identified 85 compounds that were present in > 10% of soil samples. Of these compounds, 31 showed statistically significant differences between treatments ($p < 0.05$) by ANOVA analysis. In terms of the overall soil metabolome, PCA revealed a gradient of metabolome change with increasing PHBV loading, with divergence from the control treatment occurring increasingly at higher loading rates (Fig. 1). PERMANOVA revealed significant differences between PHBV loading rates ($F = 5.37$, $p < 0.001$). Further pairwise comparisons showed significant differences between centroids of all treatments ($p < 0.05$), except 1% and 10% loading ($p = 0.09$) (Fig. 1). Heatmap analysis showed similar grouping trends with 1% and 10% loading rates clustering closely. In terms of

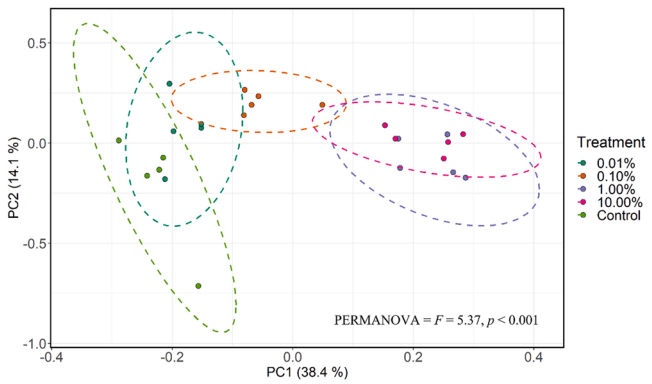

Fig. 1. PCA ordination analysis of the whole soil metabolite profile under different PHBV microbioplastic addition rates. Analysis was based on Euclidean distance with ellipses representing 95% confidence intervals for each treatment.

individual metabolites, there were two groupings (Fig. 2), Response A contained a number of compounds ($n = 25$) which were present in relatively lower concentrations under the higher PHBV loading rates compared to the Control treatment. Of these, 21 compounds were significantly lower ($p < 0.05$). In contrast, Response B had a much more variable response to PHBV loading. A heatmap of all metabolites detected in the samples, can be found in supplementary information (Fig. S1).

3.3. Plant primary metabolites

Untargeted primary metabolomic analysis tentatively identified 200 compounds that were present in > 10% of plant leaf samples. Of these compounds, 114 showed statistically significant differences between treatments ($p < 0.05$) by ANOVA analysis. In terms of the overall plant metabolome, PCA revealed a similar trend to soil, demonstrating a gradient of metabolome change with increasing PHBV loading, with strong divergence from the control treatment occurring at higher loading rates (Fig. 3). PERMANOVA revealed significant differences

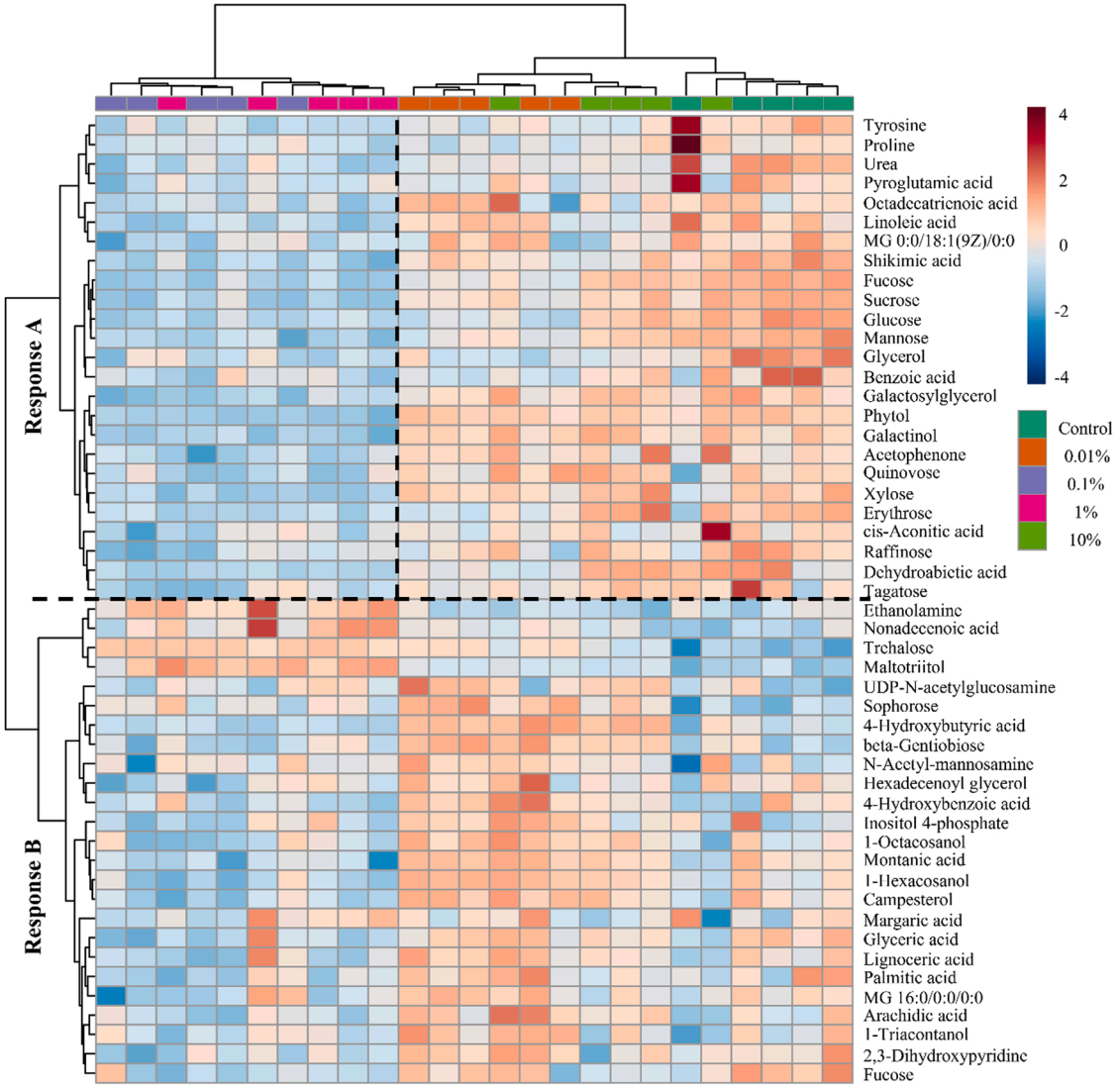

Fig. 2. Influence of PHBV microbioplastic addition rate on the metabolome of soil cultivated with maize (*Z. mays*). Heatmap showing expression profiles of soil treatment groups based on the 50 most significant tentatively identified metabolites as classified by ANOVA *p*-value ($p < 0.03$). Metabolites shown were present in > 10% of samples and were subsequently clustered using Euclidean distance and Ward linkage. Data were normalised using a log transformation and Pareto scaling. The colour of samples ranges from red (relatively more abundant) to blue (relatively less abundant), indicating metabolite concentration z-score. Response A compounds ($n = 25$) were present in relatively lower concentrations under the higher PHBV loading rates compared to the Control treatment. In contrast, Response B had a much more variable response to PHBV loading.

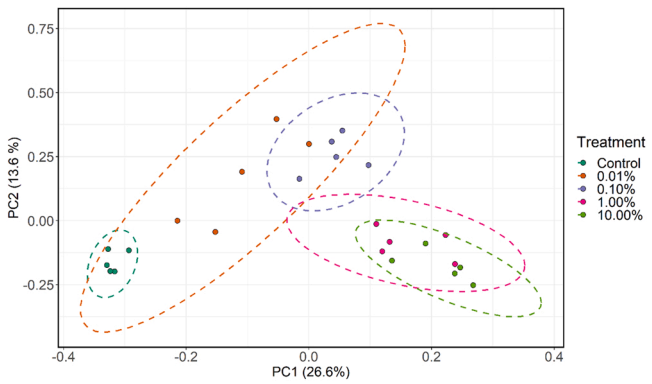

Fig. 3. PCA ordination analysis of maize (*Z. mays*) aboveground biomass metabolite profile under different PHBV microbioplastic addition rates. Analysis was based on Euclidean distance with ellipses representing 95% confidence intervals for each treatment.

between PHBV loading rates ($F = 5.30$, $p < 0.001$). Further pairwise comparisons showed significant differences between centroids of all treatments ($p < 0.05$), except 1% and 10% loading ($p = 0.30$) (Fig. 3). Heatmap analysis showed similar grouping trends with 1% and 10% loading rates clustering closely. In terms of individual metabolites, there were four distinct responses to PHBV loading. Response A compounds ($n = 21$) were found in significantly ($p < 0.05$) elevated concentrations at higher PHBV loading rates (1% and 10%) compared to the other treatment (Fig. 4, response A). Response B compounds ($n = 9$) were more variable and were generally found in significantly lower concentrations in the Control treatment compared to other treatments ($p < 0.05$). In Response C ($n = 20$), compounds were found in significantly lower relative concentrations in the 1% and 10% loading rate treatments compared to the Control and 0.01% and 0.1% loading rates ($p < 0.05$). A heatmap of all metabolites detected in samples, can be found in supplementary information (Fig. S2).

3.4. Soil 16S bacterial community

On average 22137 quality-filtered reads were generated per sample. From these, 6457 OTUs (not including those occurring total count < 3) were clustered across all 16S rRNA gene reads. Proportional abundance of 16S bacterial phyla varied significantly across, with the proportions of proteobacteria increasing with PHBV loading rates. At loading rates of 1% and 10% it was the dominant 16S bacterial phyla. Conversely, proportional abundances of firmicutes and gemmatimonadetes decreased with PHBV loading. Equally several phyla, verucomicrobia and acidobacteria, increased in abundance at lower (0.01% and 0.1%) PHBV loading rates compared to the Control and higher (1% and 10%) PHBV loading (Fig. S3). This was reflected in a significant decline in alpha diversity (Shannon index, accounting for richness and evenness) in the 10% loading rate compared all other treatments ($p < 0.001$) (Fig. 5B). In terms of bacterial beta diversity PCoA (Fig. 5A) we observed a large separation between treatments which was further confirmed by PERMANOVA ($R^2 = 0.74$, $p < 0.001$) and pairwise comparisons showing significant separation between all centroids ($R^2 > 0.31$, $p < 0.05$).

3.5. Soil microbial activity

The microbial community demonstrated a rapid use of ^{14}C-labelled glucose in all treatments consistent with previous studies in this soil (Hill et al., 2008). Within the first 7 h there was a distinct separation in ^{14}C mineralisation rate between higher PHBV loading rates (1% and 10%) and the Control and lower loading rate treatments (0.01% and 0.1%) (Fig. 6). However, beyond 24 h further differences in the overall trends in ^{14}C mineralisation became apparent, with PHBV application at all rates above 0.01% causing a significant reduction in microbial activity ($F_{(4,21)} = 9.88$, $p < 0.001$) relative to the Control treatment (Fig. 6). Small but differences were apparent in microbial C use efficiency between the treatments ($F_{(4,21)} = 3.36$, $p = 0.028$), however, there was no clear dose-dependent pattern.

4. Discussion

4.1. Functional changes in the soil-plant metabolome

While terrestrial plastic pollution has been identified as a major threat to the soil ecosystem (de Souza Machado et al., 2018), little research has focused on how bioplastics affect the biochemical profile of the soil and associated vegetation. Here, we observed significant changes in both the soil (Fig. 1) and maize plant metabolome (Fig. 3), with evidence that the response was strongly dose-dependent. In soil, the most significant response was related to a relative reduction in several compounds under higher (1% and 10%) PHBV loading rates (Response A, Fig. 2). While in maize plants, the most significant response was related to a relative increase in a number of compounds under higher (1% and 10%) PHBV loading rates (Response A, Fig. 4).

4.1.1. Plant leaf metabolome

Plant compounds in response A were varied, however, a number of compounds related to the TCA cycle (citric acid, α-ketoglutarate and isocitrate) had accumulated relative to other treatments. Usually, the enzymes that underpin the interconversion of the TCA cycle metabolic intermediaries are tightly regulated (Zhang and Fernie, 2018), and accumulation of compounds suggests inefficient energy production and/or nutrient imbalance (e.g. P deficiency; Fernie and Martinoia, 2009). A number of saccharide compounds and derivatives (maltotriose (osmotic regulator; Kaplan et al., 2007), 6-deoxyglucose (potential glycolytic inhibitor; Pajak et al., 2020), ribonic acid (cellular growth; Aguiar et al., 2018), ribose (coding, decoding and regulation; Mahoney et al., 2018) and xylulose (glucose signalling compound; Uyeda, 2021)) were also present in Response A. This suggests altered shoot carbohydrate metabolism under higher plastic loading. Compounds identified in Response A lend themselves to be candidate biomarker compounds for stress attributed to bioplastic exposure. It should be noted, however, that, it is unlikely that bioplastics would be found in the highest loading concentrations (10%) in the field, apart from in waste plastic burial hotspots. Also, while proline has previously been suggested as a potential (bio)marker for ecotoxicity related to bioplastics in plants (Martin-Closas et al., 2014), in this study proline was not detected in the plant samples.

Lactic acid was also found to be significantly higher in the Response B compounds, even at low PHBV levels (0.01%). This provides evidence that the plants were exhibiting stress symptoms, potentially in response to PHBV-induced hypoxia in the soil, leading to the generation of lactic acid though anaerobic respiration, and subsequent transport to the shoots (Eprintsev et al., 2018). Response C (Fig. 4) comprised of compounds present in higher concentrations in the leaves of either the Control or low PHBV treatments (0.01% and 0.1% loading) relative to the higher loading rate (1% and 10%). This suggests a suppression of anabolic and catabolic processes related to these compounds under plastic treatments, potentially due to increased root-microbial competition for resources (de Souza Machado et al., 2019; Yi et al., 2021).

Many of the compounds identified (e.g. fructose, mannose and phytol; full list in Fig. S1) may also be precursor compounds to further metabolite creation particularly for secondary metabolites. Secondary metabolism in plants is integral to their fitness, making them competitive in their environment (through sensing, signalling and defence; Clemensen et al., 2020). Further exploration of potential biomarker compounds (through untargeted metabolomics) is therefore recommended, to determine whether these biomarkers are conserved on

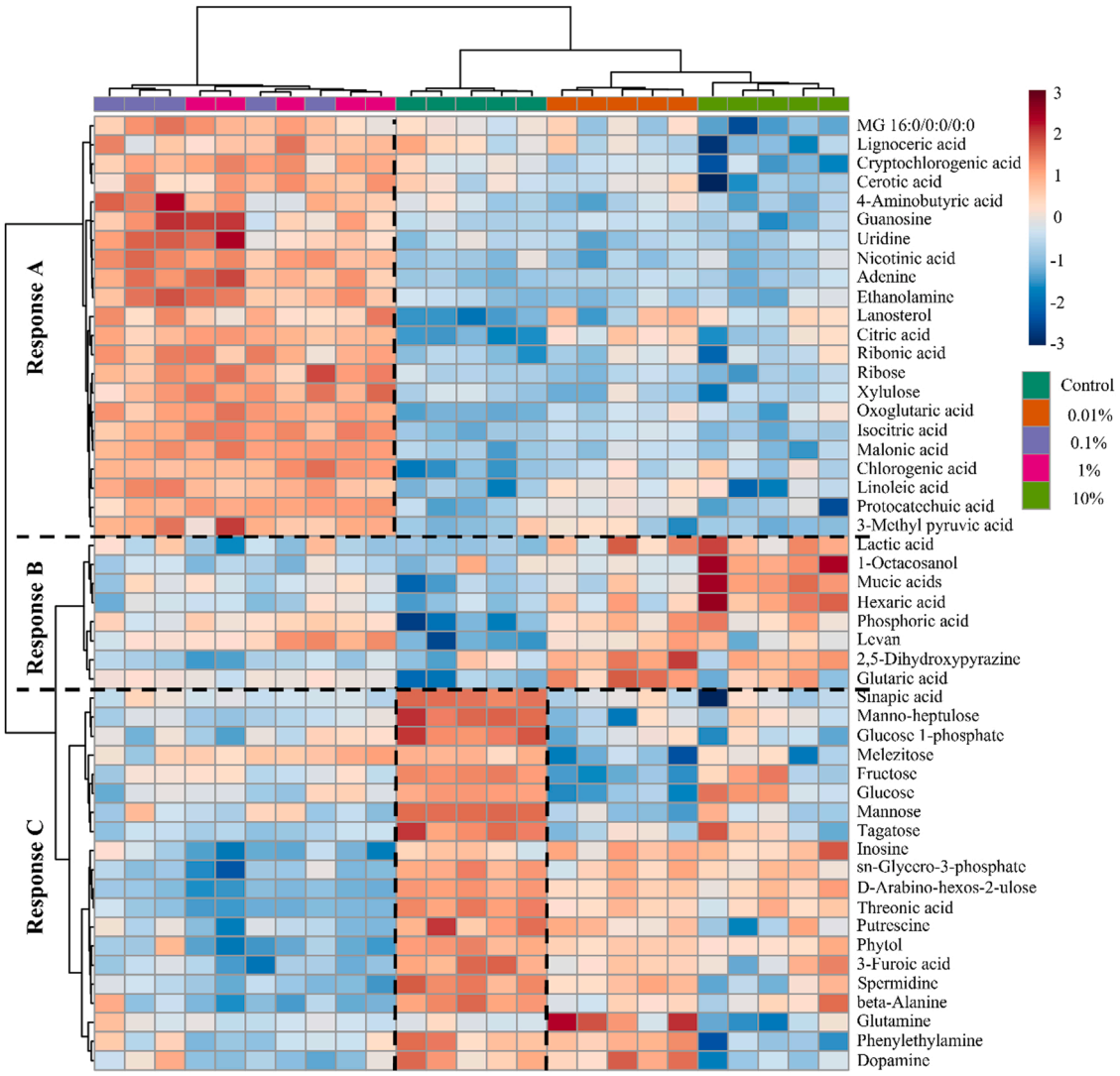

Fig. 4. Influence of PHBV microbioplastic addition on the metabolome of maize (*Z. mays*) aboveground biomass. Heatmap showing expression profiles for each treatment based on the 50 most significant tentatively identified metabolites as classified by ANOVA *p*-value. Metabolites shown were present in > 10% of samples and were subsequently clustered using Euclidean distance and Ward linkage. Data were normalised using a log transformation and Pareto scaling. The colour of samples ranges from red (relatively more abundant) to blue (relatively less abundant), indicating metabolite concentration z-score. Response A illustrates compounds ($n = 21$) that were found in significantly ($p < 0.05$) elevated concentrations at higher PHBV loading rates (1% and 10%) compared to the other treatment. Response B compounds ($n = 9$) were more variable and were generally found in significantly lower concentrations in the Control treatment compared to other treatments ($p < 0.05$). Response C compounds ($n = 20$) were found in significantly lower relative concentrations in the 1% and 10% loading rate treatment compared to the Control and 0.01% and 0.1% loading rates ($p < 0.05$).

exposure to different types of plastic. Additionally, plant growth and biomass quality was reduced even at the lowest PHBV loading rate, suggesting that the effect threshold lies below a loading rate of 0.01%. We therefore accept hypothesis (iii) and conclude that phytotoxicity and agroecosystem service provision is likely to be negatively affected in most field situations (Zhang et al., 2020b).

In summary, the plant leaf metabolome showed a significant, dose-dependant response to PHBV loading in the soil, suggesting changed leaf function. This was supported by the reduced plant growth and biomass quality, suggesting that PHBV-associated stress was substantial from loading rates as low as 0.01%. Extrapolated to a wider scale, this may have significant impacts on crop yields over long temporal periods. Additionally, we also identify a novel group of potential metabolic biomarkers for bioplastic associated stress.

4.1.2. Soil-plant metabolome

With regard to the soil, it was impossible to separate the microbial metabolic response from the plant root exudate response as all soil within the pots was likely to be influenced by the maize roots. The most evident change was the reduction in the relative abundance of compounds associated with high levels of PHBV loading, as well as some separation between the control samples and lower PHBV loading rates (Fig. 1). This grouping contained a mix of compound classes, including saccharides, alcohols and amino acids, many of which are associated with root-rhizosphere plant-microbe signalling (Fig. 2 Response A; Chaparro et al., 2013; Moe, 2013; Hennion et al., 2019). A relative reduction in these compounds compared to the control and lower loading rate (0.01% and 0.1%) treatments may suggest altered root exudation and rhizosphere function, consistent with previous research on bioplastics in soil (Qi et al., 2020b; Zhou et al., 2021b). However, further work is clearly needed to critically assess how bioplastics affect the quantity and quality of rhizodeposits and the effect on soil biological quality.

Typically, in a healthy soil, microbial (particularly bacterial) metabolism and growth, and therefore the associated biochemical profile, is primarily limited by water availability, then the quality and quantity of

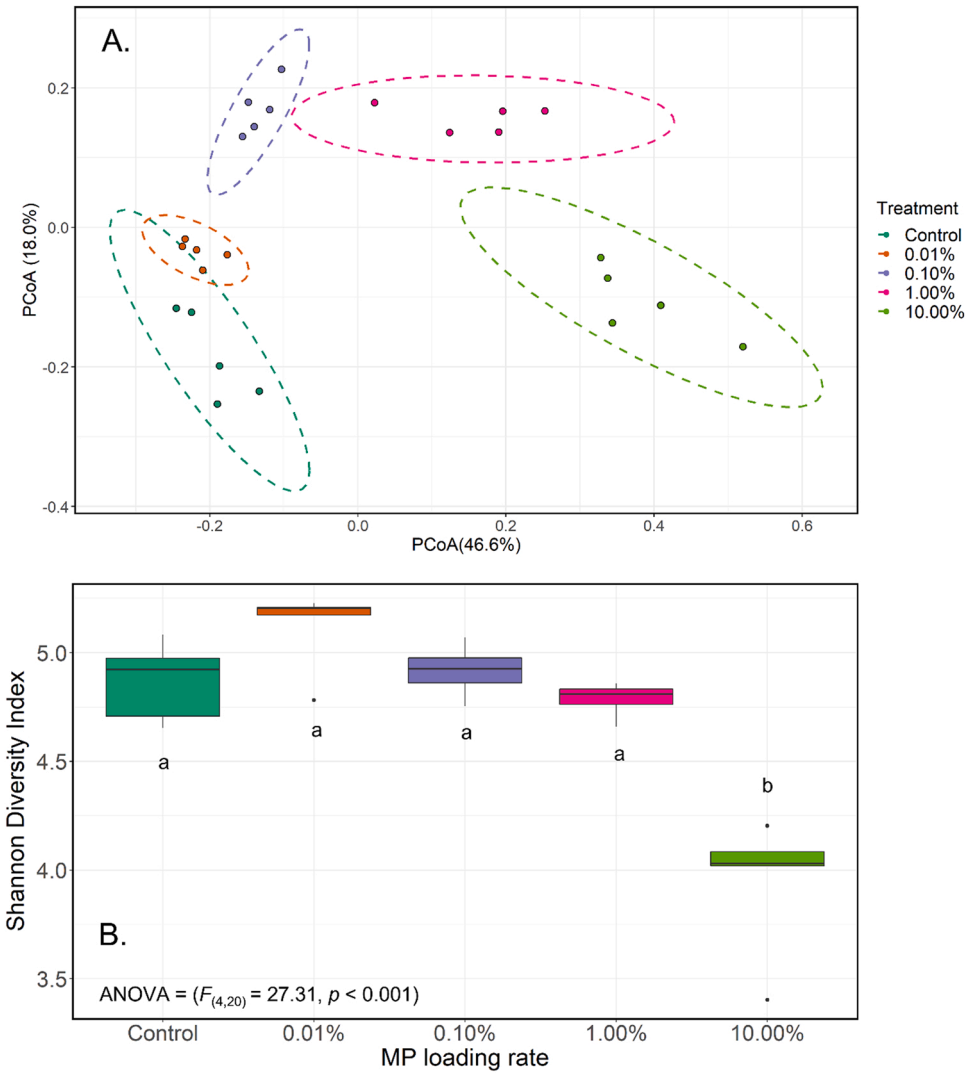

Fig. 5. A) PCoA ordination of microbiome composition similarity among samples. Soil 16S OTU abundances microbiome under different PHBV microbioplastic addition rates. Analysis was based on Bray-Curtis dissimilarities with ellipses representing 95% confidence intervals for each treatment. B) Boxplot of 16S microbiome alpha diversity in response to PHBV microbioplastic addition.

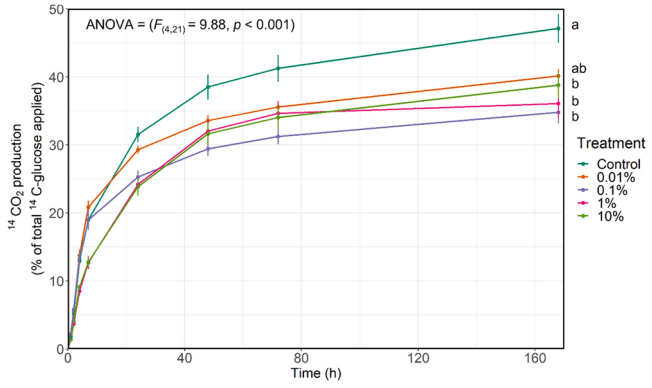

Fig. 6. Microbial mineralisation of ^{14}C-labelled glucose to $^{14}CO_2$ in soil over 1 week (168 h) under different PHBV microbioplastics loading rates. Treatments were replicated in quintuplicate ($n = 5$), and error bars indicate the SEM.

C substrates, and finally macro- and micro- nuturient concentrations (Aanderud et al., 2018). Often, an influx of C into the soil can lead to changes in the C source preference of the soil microbial community with more labile, accessible substrates being used over more recalcitrant, energetically demanding substrates, potentially explaining why microbial activity was suppressed (Fig. 6). Bioplastic (biodegradable plastics, not biosynthesised plastic) is designed to break down relatively rapidly (months to year) once disposed of in the enevironment (Liwarska-Bizukojc, 2021). For example, biodegradation of PHBV has been estimated to occur over 70 days to 22 months (Arcos-Hernández et al., 2013; Salomez et al., 2019), adding substantial amounts of C substrates to soil in a short period, particularly at high bioplastic loading rates (+ 1%), and altering the soil stoichiometry (reducing microbial C limitation) (Qi et al., 2020b). This is likely to lead to immobilisation of soil inorganic, available N (Brown et al., 2022a) as the microbial community continues to grow (Table 1). Over time this may lead to oligotrophic soil niches favouring specific microoganisms (as illustrated here with dominance of bacteria in the families Betaproteobacteria and Caulobacteraceae under high PHBV loading (Fig. S2), disscussed in Section 4.2).

Here, the bioplastics were applied directly as microplastic particles, so it is likely that the high surface to volume ratio increased the susceptibility to, and therefore, the speed of degradation. It is likely that in some cases some of these bioplastic hydrolysis products are bioavailable and used in preference to native soil C sources, leading to a shift in microbial metabolome (and associated transcriptome and enzymatic

proteome) (Qi et al., 2020b; Sun et al., 2022b). We therefore reject hypothesis (i) as microbial activity was in fact suppressed in the presence of bioplastic in comparison to the control treatment.

To summarise, this study is one of the first to explore changes in the soil metabolome associated with bioplastics addition. Overall, the plant-soil metabolome response was similar to that of the maize leaves, demonstrating a dose-dependant response to PHBV loading in the soil suggesting a change in plant-microbial functioning, likely induced by the increase in bioplastic-associated-C leading to changes in soil stoichiometry. Overall, more work is required to understand the long-term implications to these change in metabolic function, and soil-microbe-plant interactions.

4.2. Soil bacterial community and microbial activity response

Changing soil nutrient stoichiometry and the availability of labile C will not only shift microbial metabolism but also affect the structure of the soil microbial community (Aanderud et al., 2018). Here, we saw significant divergence in the community composition from the un-amended control at all microplastic loading rates (Fig. 5A), particularly at bioplastic loading rates above 0.01%. Further, a significant reduction in alpha diversity was observed at 10% PHBV loading (Fig. 5B). A number of studies on bioplastics in soil have reported alteration of the soil microbial community (Li et al., 2014; Zhang et al., 2019; Tanunchai et al., 2021), while others reported no significant change (Bandopadhyay et al., 2020). Generally, while a large amount of functional redundancy exists within the soil (Louca et al., 2018; Jia and Whalen, 2020), a significant reduction in alpha diversity is likely to impact on the ability of the soil to function, particularly in an agroecosystem context (i.e., production of food; Wagg et al., 2014; Zhou et al., 2021a).

Further, as the soil within the pots used in this experiment is likely to be dominated by rhizosphere interaction, it is highly likely that the change in the soil bacterial community be indicative of changing plant-microbe interaction. Plant health plays an important role in shaping the rhizosphere microbiome, influencing the quality and quantity of rhizodeposits (Sasse et al., 2018; Walters et al., 2018). The effects of abiotic stress on plant root exudates and associated rhizosphere community are well documented (Olanrewaju et al., 2018). To date, however, little research has focused on how plastics may alter root exudation and the impact of this on plant-microbiome interaction and (agro)ecosystem function.

In terms of the microbial community activity, as measured by ^{14}C-labelled glucose mineralisation, all bioplastics treatments had reduced mineralisation rates compared to the untreated control (Fig. 6). Glucose is often seen as a model substrate as it is used as a labile C source utilised ubiquitously across soil microorganisms (Blagodatskaya and Kuzyakov, 2013; Gunina and Kuzyakov, 2015). However, here, addition of ^{14}C-labelled glucose at relatively low concentration (10 mM) did not seem to be used in preference to bioplastic derived C substrates, potentially due to the microbial community being metabolically primed to use bioplastic substrate, as the enzymatic proteome has previously been shown to be sensitive to bioplastic addition (Zhou et al., 2021b). However, while PHBV is a bioplastic and is capable of being fully degraded to CO_2, H_2O and microbial biomass C (Salomez et al., 2019), the specific metabolic decomposition pathways and potential metabolic end-/by-products have yet to be fully elucidated; these will be key in fully understanding the impact on soil biology and biochemistry.

4.3. Soil chemistry

Microbioplastic incorporation had a major effect on the chemistry of the soil, even at lowest PHBV addition rate. Our results gave clear evidence that PHBV induced the microbial immobilization of available N in a dose-dependent manner (Table 1; Table S1) (Reid et al., 2022; Shi et al., 2022). This also correlated well with the decline in plant growth and reduced N content in the maize shoots. Conversely, PHBV led to an increase in soil EC suggesting that other ions were accumulating in the soil due to reduced plant demand caused by the lack of available N (Heiniger et al., 2003). There was also some evidence that available P was reduced under higher bioplastic loading treatments compared to the control (Table 1; Aanderud et al., 2018).

4.4. Bioplastics in the soil system – implications and outlook

Bioplastics have been rapidly replacing conventional plastic mulch films in agriculture, to prevent plastic (and micro- and nano- plastic) accumulation in agricultural soils. This shift in management, however, may have significant implications for soil and plant health and agro-ecosystem service provision (Rillig et al., 2019; Zhang et al., 2020a; Sridharan et al., 2021). Due to their higher biotic and abiotic breakdown rates, bioplastics are less recalcitrant, leading to significant amounts of C substrate becoming available to the soil biological community. As such this may not eliminate the threat of plastics to soil system, merely change the dynamics of microplastic formation and their persistence. Arguably in the short- to medium-term, conventional plastics provide little problem in the soil system (Brown et al., 2022b), although nano-plastic transport and uptake may become a persistent issue in the future (Mateos-Cárdenas et al., 2021; Wahl et al., 2021). However, the rapid breakdown of bioplastics is likely to increase the availability and chronic effect of additives which may contribute up to 70% of bioplastic mass (Nizzetto et al., 2016; Steinmetz et al., 2016), increase the speed of micro-bioplastic formation with unknown consequences to soil and plant health (Fojt et al., 2020) and availability of C may lead to native soil organic matter priming. These issues should therefore be explored in a range of soil and crop types as a matter of urgency to better understand the legacy of bioplastics in soil.

Bioplastics is a catch-all term and includes both biodegradable plastics and plastics from renewable biomass sources (Coppola et al., 2021). This may be misleading (Dilkes-Hoffman et al., 2019; Nandakumar et al., 2021), as bioplastics from renewable biomass may still be persistent in the environment for long time periods. Ultimately, bioplastic degradation will vary significantly between plastic type due to the inherent chemical composition as well as the prevailing climate conditions (Satti et al., 2018). Long-term field scale studies are therefore recommended to better understand changes in soil physiochemistry and function as mesocosm studies may overestimate soil responses to plastic loading (Brown et al., 2022b; Greenfield et al., 2022).

5. Conclusions

While research on the impact of bioplastics on soil and plant function is a nascent field, there is growing evidence that bioplastic may provide just as much, if not more, of a threat to soil, plant and ecosystem health due to its labile, unstable nature. This study begins to assess the biochemical impact of pure bioplastic addition, however, much more must be done to understand the full extent of the effect, particularly on long-term agroecosystem productivity. PHBV bioplastic loading rates at 1% and above (representing hotspots, or bioplastic accumulation) caused significant changes in the soil metabolome and microbial community likely associated with changing function. We observed strong negative effects on plant health and metabolic function at loading rates of 1% and above. We attribute this effect to the rapid influx of labile C substrates into the soil, leading to an alleviation in metabolic C limitations. This increased C turnover is likely to have significant implications for soil C cycling and ecosystem service provision, the extent of which should be the subject of future field and laboratory study. Overall, this study suggests that the bioplastic PHBV is not environmentally benign and that contamination levels as low as 100 mg kg^{-1} can induce significant short-term changes in plant-soil-microbial functioning.

CRediT authorship contribution statement

Robert W. Brown: Investigation, Data curation, Formal analysis, Visualisation, Writing – Original Draft, Writing – Review and Editing. David R. Chadwick: Writing – Review and Editing, Supervision, Funding acquisition. Huadong Zang: Writing – Review and Editing. Martine Graf: Writing – Review and Editing. Xuejun Liu: Writing – Review and Editing. Kai Wang: Writing – Review and Editing. Lucy M. Greenfield: Writing – Review and Editing. Davey L. Jones: Conceptualization, Formal analysis, Writing – Original draft, Writing – Review and Editing, Supervision, Funding acquisition.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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Statement of environmental implication

This manuscript is of high environmental relevance; biodegradable plastics have been widely adopted in the agricultural sector as a 'green' alternative to conventional plastic mulch films, which are used globally to improve yields. While bioplastic mineralise more readily than conventional plastic, the impact of this accessible carbon source on soil and plant health has not been widely explored. Our results suggest that bioplastics may negatively affect soil and plant health at relatively low loading rates, which is likely to reduce crop yields and threaten food security in the long term, as such, they should be considered a hazardous material.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.129959.

References

Aanderud, Z.T., Saurey, S., Ball, B.A., Wall, D.H., Barrett, J.E., Muscarella, M.E., Griffin, N.A., Virginia, R.A., Adams, B.J., 2018. Stoichiometric shifts in soil C:N:P promote bacterial taxa dominance, maintain biodiversity, and deconstruct community assemblages. Front. Microbiol. 9, 1401. https://doi.org/10.3389/FMICB.2018.01401/BIBTEX.

Aguiar, N.O., Olivares, F.L., Novotny, E.H., Canellas, L.P., 2018. Changes in metabolic profiling of sugarcane leaves induced by endophytic diazotrophic bacteria and humic acids. PeerJ 6, e5445. https://doi.org/10.7717/PEERJ.5445/SUPP-1.

Aguilar, J.M., Bengoechea, C., Pérez, E., Guerrero, A., 2020. Effect of different polyols as plasticizers in soy based bioplastics. Ind. Crops Prod. 153, 112522 https://doi.org/10.1016/J.INDCROP.2020.112522.

Allouzi, M.M.A., Tang, D.Y.Y., Chew, K.W., Rinklebe, J., Bolan, N., Allouzi, S.M.A., Show, P.L., 2021. Micro (nano) plastic pollution: the ecological influence on soil-plant system and human health. Sci. Total Environ. 788, 147815 https://doi.org/10.1016/J.SCITOTENV.2021.147815.

Arcos-Hernández, M. v, Laycock, B., Donose, B.C., Pratt, S., Halley, P., Al-Luaibi, S., Werker, A., Lant, P.A., 2013. Physicochemical and mechanical properties of mixed culture polyhydroxyalkanoate (PHBV. Eur. Polym. J. 49, 904–913. https://doi.org/10.1016/J.EURPOLYMJ.2012.10.025.

Azeem, I., Adeel, M., Ahmad, M.A., Shakoor, N., Jiangcuo, G.D., Azeem, K., Ishfaq, M., Shakoor, A., Ayaz, M., Xu, M., Rui, Y., 2021. Uptake and accumulation of nano/microplastics in plants: a critical review. Nanomaterials 11, 2935. https://doi.org/10.3390/NANO11112935.

Bandopadhyay, S., Martin-Closas, L., Pelacho, A.M., DeBruyn, J.M., 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, H.Y., DeBruyn, J.M., 2020. Effects of biodegradable plastic film mulching on soil microbial communities in two agroecosystems. PeerJ 8, e9015. https://doi.org/10.7717/PEERJ.9015.

Benjamini, Y., Yekutieli, D., 2001. The control of the false discovery rate in multiple testing under dependency. Ann. Stat. 29, 1165–1188. https://doi.org/10.1214/AOS/1013699998.

Biswas, R., Sarkar, A., 2018. 'Omics' Tools in Soil Microbiology: The State of the Art. In: Adhya, T., Lal, B., Mohapatra, B., Paul, D., Das, S. (Eds.), Advances in Soil Microbiology: Recent Trends and Future Prospects. Microorganisms for Sustainability, volume 3. Springer, Singapore, pp. 35–64. https://doi.org/10.1007/978-981-10-6178-3_3.

Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol. Biochem. 67, 192–211. https://doi.org/10.1016/J.SOILBIO.2013.08.024.

Brown, R.W., Chadwick, D.R., Zang, H., Jones, D.L., 2021. Use of metabolomics to quantify changes in soil microbial function in response to fertiliser nitrogen supply and extreme drought. Soil Biol. Biochem. 160, 108351 https://doi.org/10.1016/J.SOILBIO.2021.108351.

Brown, R.W., Chadwick, D.R., Bending, G.D., Collins, C.D., Whelton, H.L., Daulton, E., Covington, J.A., Bull, I.D., Jones, D.L., 2022a. Nutrient (C, N and P) enrichment induces significant changes in the soil metabolite profile and microbial carbon partitioning. Soil Biol. Biochem. 172, 108779 https://doi.org/10.1016/J.SOILBIO.2022.108779.

Brown, R.W., Chadwick, D.R., Thornton, H., Marshall, M.R., Bei, S., Distaso, M.A., Bargiela, R., Marsden, K.A., Clode, P.L., Murphy, D.V., Pagella, S., Jones, D.L., 2022b. Field application of pure polyethylene microplastic has no significant short-term effect on soil biological quality and function. Soil Biol. Biochem. 165, 108496 https://doi.org/10.1016/J.SOILBIO.2021.108496.

Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., de Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J.W., Brussaard, L., 2018. Soil quality – A critical review. Soil Biol. Biochem. 120, 105–125. https://doi.org/10.1016/J.SOILBIO.2018.01.030.

Chae, Y., An, Y.J., 2018. Current research trends on plastic pollution and ecological impacts on the soil ecosystem: a review. Environ. Pollut. 240, 387–395. https://doi.org/10.1016/J.ENVPOL.2018.05.008.

Chaparro, J.M., Badri, D. v, Vivanco, J.M., 2013. Rhizosphere microbiome assemblage is affected by plant development. ISME J. 8, 790–803. https://doi.org/10.1038/ISMEJ.2013.196.

Chong, J.W.R., Khoo, K.S., Yew, G.Y., Leong, W.H., Lim, J.W., Lam, M.K., Ho, Y.C., Ng, H.S., Munawaroh, H.S.H., Show, P.L., 2021a. Advances in production of bioplastics by microalgae using food waste hydrolysate and wastewater: a review. Bioresour. Technol. 342, 125947 https://doi.org/10.1016/J.BIORTECH.2021.125947.

Chong, J.W.R., Yew, G.Y., Khoo, K.S., Ho, S.H., Show, P.L., 2021b. Recent advances on food waste pretreatment technology via microalgae for source of polyhydroxyalkanoates. J. Environ. Manag. 293, 112782 https://doi.org/10.1016/J.JENVMAN.2021.112782.

Chong, J.W.R., Tan, X., Khoo, K.S., Ng, H.S., Jonglertjunya, W., Yew, G.Y., Show, P.L., 2022. Microalgae-based bioplastics: future solution towards mitigation of plastic wastes. Environ. Res. 206, 112620 https://doi.org/10.1016/J.ENVRES.2021.112620.

Clemensen, A.K., Provenza, F.D., Hendrickson, J.R., Grusak, M.A., 2020. Ecological implications of plant secondary metabolites - Phytochemical diversity can enhance agricultural sustainability. Front. Sustain. Food Syst. 4, 233. https://doi.org/10.3389/FSUFS.2020.547826.

Coppola, G., Gaudio, M.T., Lopresto, C.G., Calabro, V., Curcio, S., Chakraborty, S., 2021. Bioplastic from renewable biomass: a facile solution for a greener environment. Earth Syst. Environ. 5, 231–251. https://doi.org/10.1007/S41748-021-00208-7.

Dilkes-Hoffman, L., Ashworth, P., Laycock, B., Pratt, S., Lant, P., 2019. Public attitudes towards bioplastics – knowledge, perception and end-of-life management. Resour., Conserv. Recycl. 151, 104479 https://doi.org/10.1016/J.RESCONREC.2019.104479.

Dumroese, R.K., Pinto, J.R., Montville, M.E., 2015. Using container weights to determine irrigation needs: a simple method. Nativ. Plants J. 16, 67–71. ⟨https://www.muse.jhu.edu/article/578515⟩.

Eprintsev, A.T., Komarova, N.R., Falaleeva, M.I., Larchenkov, V.M., 2018. Distinctive features of the functioning of lactic acid and the alcohol fermentation enzyme in sorghum and pea leaves under the conditions of oxygen deficiency. Biol. Bull. 45, 426–431. https://doi.org/10.1134/S1062359018050059.

Fernie, A.R., Martinoia, E., 2009. Malate. Jack of all trades or master of a few? Phytochemistry 70, 828–832. https://doi.org/10.1016/J.PHYTOCHEM.2009.04.023.

Fiehn, O., Wohlgemuth, G., Scholz, M., Kind, T., Lee, D.Y., Lu, Y., Moon, S., Nikolau, B., 2008. Quality control for plant metabolomics: reporting MSI-compliant studies. Plant J. 53, 691–704. https://doi.org/10.1111/j.1365-313X.2007.03387.x.

Fojt, J., David, J., Přikryl, R., Řezáčová, V., Kučerík, J., 2020. A critical review of the overlooked challenge of determining micro-bioplastics in soil. Sci. Total Environ. 745, 140975 https://doi.org/10.1016/J.SCITOTENV.2020.140975.

Greenfield, L.M., Graf, M., Rengaraj, S., Bargiela, R., Williams, G., Golyshin, P.N., Chadwick, D.R., Jones, D.L., 2022. Field response of N_2O emissions, microbial communities, soil biochemical processes and winter barley growth to the addition of conventional and biodegradable microplastics. Agric., Ecosyst. Environ. 336, 108023 https://doi.org/10.1016/J.AGEE.2022.108023.

Griffin-LaHue, D., Ghimire, S., Yu, Y., Scheenstra, E.J., Miles, C.A., Flury, M., 2022. In-field degradation of soil-biodegradable plastic mulch films in a Mediterranean climate. Sci. Total Environ. 806, 150238 https://doi.org/10.1016/J.SCITOTENV.2021.150238.

Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. Soil Biol. Biochem. 90, 87–100. https://doi.org/10.1016/J.SOILBIO.2015.07.021.

Heiniger, R.W., McBride, R.G., Clay, D.E., 2003. Using soil electrical conductivity to improve nutrient management. Agron. J. 95, 508–519. https://doi.org/10.2134/AGRONJ2003.5080.

Hennion, N., Durand, M., Vriet, C., Doidy, J., Maurousset, L., Lemoine, R., Pourtau, N., 2019. Sugars en route to the roots. Transport, metabolism and storage within plant roots and towards microorganisms of the rhizosphere. Physiol. Plant. 165, 44–57. https://doi.org/10.1111/PPL.12751.

Hervé, M., 2021. RVAideMemoire: testing and plotting procedures for biostatistics. R. Package Version 0, 9–80.

Hill, P.W., Farrar, J.F., Jones, D.L., 2008. Decoupling of microbial glucose uptake and mineralization in soil. Soil Biol. Biochem. 40, 616–624. https://doi.org/10.1016/J.SOILBIO.2007.09.008.

Huang, Y., Liu, Q., Jia, W., Yan, C., Wang, J., 2020. Agricultural plastic mulching as a source of microplastics in the terrestrial environment. Environ. Pollut. 260, 114096 https://doi.org/10.1016/J.ENVPOL.2020.114096.

Imam, S.H., Gordon, S.H., Shogren, R.L., Tosteson, T.R., Govind, N.S., Greene, R., 1999. Degradation of starch–poly(β-Hydroxybutyrate-Co-β-Hydroxyvalerate) bioplastic in tropical coastal waters. Appl. Environ. Microbiol. v. (65), 431. https://doi.org/10.1128/AEM.65.2.431-437.1999.

Jia, Y., Whalen, J.K., 2020. A new perspective on functional redundancy and phylogenetic niche conservatism in soil microbial communities. Pedosphere 30, 18–24. https://doi.org/10.1016/S1002-0160(19)60826-X.

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38, 991–999. https://doi.org/10.1016/J.SOILBIO.2005.08.012.

Jones, D.L., Olivera-Ardid, S., Klumpp, E., Knief, C., Hill, P.W., Lehndorff, E., Bol, R., 2018. Moisture activation and carbon use efficiency of soil microbial communities along an aridity gradient in the Atacama Desert. Soil Biol. Biochem. 117, 68–71. https://doi.org/10.1016/J.SOILBIO.2017.10.026.

Kaplan, F., Kopka, J., Sung, D.Y., Zhao, W., Popp, M., Porat, R., Guy, C.L., 2007. Transcript and metabolite profiling during cold acclimation of Arabidopsis reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. Plant J. 50, 967–981. https://doi.org/10.1111/J.1365-313X.2007.03100.X.

Koul, B., Yakoob, M., Shah, M.P., 2022. Agricultural waste management strategies for environmental sustainability. Environ. Res. 206, 112285 https://doi.org/10.1016/J.ENVRES.2021.112285.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112–5120. https://doi.org/10.1128/AEM.01043-13.

Lankadurai, B.P., Nagato, E.G., Simpson, M.J., 2013. Environmental metabolomics: an emerging approach to study organism responses to environmental stressors. Environ. Rev. 21, 180–205. https://doi.org/10.1139/er-2013-0011.

Li, C., Moore-Kucera, J., Miles, C., Leonas, K., Lee, J., Corbin, A., Inglis, D., 2014. Degradation of Potentially Biodegradable Plastic Mulch Films at Three Diverse U.S. Locations. Agroecol. Sustain. Food Syst. 38, 861–889. https://doi.org/10.1080/21683565.2014.884515.

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. In press.

Lin, D., Yang, G., Dou, P., Qian, S., Zhao, L., Yang, Y., Fanin, N., 2020. Microplastics negatively affect soil fauna but stimulate microbial activity: insights from a field-based microplastic addition experiment. Proc. R. Soc. B 287, 20201268. https://doi.org/10.1098/RSPB.2020.1268.

Liwarska-Bizukojc, E., 2021. Effect of (bio)plastics on soil environment: A review. Sci. Total Environ. 795, 148889 https://doi.org/10.1016/J.SCITOTENV.2021.148889.

Louca, S., Polz, M.F., Mazel, F., Albright, M.B.N., Huber, J.A., O'Connor, M.I., Ackermann, M., Hahn, A.S., Srivastava, D.S., Crowe, S.A., Doebeli, M., Parfrey, L.W., 2018. Function and functional redundancy in microbial systems. Nat. Ecol. Evol. 2, 936–943. https://doi.org/10.1038/s41559-018-0519-1.

Mahoney, D.E., Hiebert, J.B., Thimmesch, A., Pierce, J.T., Vacek, J.L., Clancy, R.L., Sauer, A.J., Pierce, J.D., 2018. Understanding D-ribose and mitochondrial function. Adv. Biosci. Clin. Med. 6, 1–5. https://doi.org/10.7575/AIAC.ABCMED.V.6N.1P.1.

Martin-Closas, L., Botet, R., Pelacho, A.M., 2014. An in vitro crop plant ecotoxicity test for agricultural bioplastic constituents. Polym. Degrad. Stab. 108, 250–256. https://doi.org/10.1016/J.POLYMDEGRADSTAB.2014.03.037.

Mateos-Cárdenas, A., van Pelt, F.N.A.M., O'Halloran, J., Jansen, M.A.K., 2021. Adsorption, uptake and toxicity of micro- and nanoplastics: Effects on terrestrial plants and aquatic macrophytes. Environ. Pollut. 284, 117183 https://doi.org/10.1016/J.ENVPOL.2021.117183.

de Mendiburi, F., 2021. agricolae: Statistical Procedures for Agricultural Research v 1.3.5.

Miranda, K.M., Espey, M.G., Wink, D.A., 2001. Spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5, 62–71. https://doi.org/10.1006/NIOX.2000.0319.

Moe, L.A., 2013. Amino acids in the rhizosphere: From plants to microbes. Am. J. Bot. 100, 1692–1705. https://doi.org/10.3732/AJB.1300033.

Mulvaney, R.L., 1996. Nitrogen - Inorganic forms. In: Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3. Soil Science Society of America, Madison, WI, USA, pp. 1123–1184.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.

Nandakumar, A., Chuah, J.A., Sudesh, K., 2021. Bioplastics: A boon or bane. Renew. Sustain. Energy Rev. 147, 111237 https://doi.org/10.1016/J.RSER.2021.111237.

Nizzetto, L., Futter, M., Langaas, S., 2016. Are agricultural soils dumps for microplastics of urban origin. Environ. Sci. Technol. 50, 10777–10779. https://doi.org/10.1021/ACS.EST.6B04140.

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGilnn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. vegan: Community Ecology Package. R. Package Version 2, 5–7.

Olanrewaju, O.S., Ayangbenro, A.S., Glick, B.R., Babalola, O.O., 2018. Plant health: feedback effect of root exudates-rhizobiome interactions. Appl. Microbiol. Biotechnol. 103:3 103, 1155–1166. https://doi.org/10.1007/S00253-018-9556-6.

Pajak, B., Siwiak, E., Sołtyka, M., Priebe, A., Zieliński, R., Fokt, I., Ziemniak, M., Jaśkiewicz, A., Borowski, R., Domoradzki, T., Priebe, W., 2020. 2-deoxy-d-glucose and its analogs: from diagnostic to therapeutic agents. Int. J. Mol. Sci. 21, 234. https://doi.org/10.3390/IJMS21010234.

Pang, Z., Chong, J., Li, S., Xia, J., 2020. MetaboAnalystR 3.0: Toward an Optimized Workflow for Global Metabolomics. Metabolites 10, 186. https://doi.org/10.3390/metabo10050186.

Pang, Z., Chong, J., Zhou, G., de Lima Morais, D.A., Chang, L., Barrette, M., Gauthier, C., Jacques, P.É., Li, S., Xia, J., 2021. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucleic Acids Res. 49, W388–W396. https://doi.org/10.1093/NAR/GKAB382.

Qi, R., Jones, D.L., Li, Z., Liu, Q., Yan, C., 2020. Behavior of microplastics and plastic film residues in the soil environment: A critical review. Sci. Total Environ. 703, 134722 https://doi.org/10.1016/J.SCITOTENV.2019.134722.

Qi, Y., Ossowicki, A., Yang, X., Huerta Lwanga, E., Dini-Andreote, F., Geissen, V., Garbeva, P., 2020a. Effects of plastic mulch film residues on wheat rhizosphere and soil properties. J. Hazard. Mater. 387, 121711 https://doi.org/10.1016/J.JHAZMAT.2019.121711.

R Core Team, 2021. R: A language and environment for statistical computing.

Reid, E. v, Samuelson, M.B., Blanco-Canqui, H., Drijber, R., Kadoma, I., Wortman, S.E., 2022. Biodegradable and biobased mulch residues had limited impacts on soil properties but reduced yield of the following crop in a low fertility soil. Renew. Agric. Food Syst. 1–14. https://doi.org/10.1017/S1742170522000217.

Riggi, E., Santagata, G., Malinconico, M., 2012. Bio-Based and biodegradable plastics for use in crop production. Recent Pat. Food, Nutr. Agric. 3, 49–63. https://doi.org/10.2174/2212798411103010049.

Rillig, M.C., Lehmann, A., 2020. Microplastic in terrestrial ecosystems. Science 368, 1430–1431. https://doi.org/10.1126/SCIENCE.ABB5979.

Rillig, M.C., Lehmann, A., de Souza Machado, A.A., Yang, G., 2019. Microplastic effects on plants. New Phytol. 223, 1066–1070. https://doi.org/10.1111/NPH.15794.

Rodríguez-Seijo, A., Santos, B., Ferreira da Silva, E., Cachada, A., Pereira, R., 2019. Low-density polyethylene microplastics as a source and carriers of agrochemicals to soil and earthworms. Environ. Chem. 16, 8–17. https://doi.org/10.1071/EN18162.

Salomez, M., George, M., Fabre, P., Touchaleaume, F., Cesar, G., Lajarrige, A., Gastaldi, E., 2019. A comparative study of degradation mechanisms of PHBV and PBSA under laboratory-scale composting conditions. Polym. Degrad. Stab. 167, 102–113. https://doi.org/10.1016/J.POLYMDEGRADSTAB.2019.06.025.

Sasse, J., Martinoia, E., Northen, T., 2018. Feed your friends: do plant exudates shape the root microbiome. Trends Plant Sci. 23, 25–41. https://doi.org/10.1016/J.TPLANTS.2017.09.003.

Satti, S.M., Shah, A.A., Marsh, T.L., Auras, R., 2018. Biodegradation of poly(lactic acid) in soil microcosms at ambient temperature: Evaluation of natural attenuation, bio-augmentation and bio-stimulation. J. Polym. Environ. 26, 3848–3857. https://doi.org/10.1007/s10924-018-1264-x.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.

Shi, J., Wang, J., Lv, J., Wang, Z., Peng, Y., Wang, X., 2022. Microplastic presence significantly alters soil nitrogen transformation and decreases nitrogen bioavailability under contrasting temperatures. J. Environ. Manag. 317, 115473 https://doi.org/10.1016/J.JENVMAN.2022.115473.

de Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S., Rillig, M.C., 2019. Microplastics can change soil properties and affect plant performance. Environ. Sci. Technol. 53, 6044–6052. https://doi.org/10.1021/ACS.EST.9B01339.

Sridharan, S., Kumar, M., Bolan, N.S., Singh, L., Kumar, S., Kumar, R., You, S., 2021. Are microplastics destabilizing the global network of terrestrial and aquatic ecosystem services. Environ. Res. 198, 111243 https://doi.org/10.1016/J.ENVRES.2021.111243.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O., Schaumann, G.E., 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, L., Qian, X., An, X., 2022. Transparent and highly efficient full-band UV-shielding bioplastic designed by in-situ embedded metal-organic frameworks (MIL-68(In)-NH_2) in cellulose matrix. Cellulose 29, 1017–1034. https://doi.org/10.1007/s10570-021-04322-7.

Sun, Y., Duan, C., Cao, N., Ding, C., Huang, Y., Wang, J., 2022. Biodegradable and conventional microplastics exhibit distinct microbiome, functionality, and metabolome changes in soil. J. Hazard. Mater. 424, 127282 https://doi.org/10.1016/J.JHAZMAT.2021.127282.

Tanunchai, B., Juncheed, K., Wahdan, S.F.M., Guliyev, V., Udovenko, M., Lehnert, A.S., Alves, E.G., Glaser, B., Noll, M., Buscot, F., Blagodatskaya, E., Purahong, W., 2021. Analysis of microbial populations in plastic–soil systems after exposure to high poly (butylene succinate-co-adipate) load using high-resolution molecular technique. Environ. Sci. Eur. 33, 1–17. https://doi.org/10.1186/s12302-021-00528-5.

Uyeda, K., 2021. Energy Metabolism - Pyruvate Kinase. Encyclopedia of Biological Chemistry: Third Edition 1, 166–169. doi:10.1016/B978–0-12–809633-8.21272–7.

Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc. Natl. Acad. Sci. USA 111, 5266–5270. https://doi.org/10.1073/PNAS.1320054111.

Wahl, A., le Juge, C., Davranche, M., el Hadri, H., Grassl, B., Reynaud, S., Gigault, J., 2021. Nanoplastic occurrence in a soil amended with plastic debris. Chemosphere 262, 127784. https://doi.org/10.1016/J.CHEMOSPHERE.2020.127784.

Walters, W.A., Jin, Z., Youngblut, N., Wallace, J.G., Sutter, J., Zhang, W., González-Peña, A., Peiffer, J., Koren, O., Shi, Q., Knight, R., del Rio, T.G., Tringe, S.G., Buckler, E.S., Dangl, J.L., Ley, R.E., 2018. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proc. Natl. Acad. Sci. USA 115, 7368–7373. https://doi.org/10.1073/PNAS.1800918115.

Wang, F., Feng, X., Liu, Y., Adams, C.A., Sun, Y., Zhang, S., 2022a. Micro(nano)plastics and terrestrial plants: Up-to-date knowledge on uptake, translocation, and phytotoxicity. Resour., Conserv. Recycl. 185, 106503 https://doi.org/10.1016/J.RESCONREC.2022.106503.

Wang, F., Wang, Q., Adams, C.A., Sun, Y., Zhang, S., 2022b. Effects of microplastics on soil properties: current knowledge and future perspectives. J. Hazard. Mater. 424, 127531 https://doi.org/10.1016/J.JHAZMAT.2021.127531.

Wang, Q., Adams, C.A., Wang, F., Sun, Y., Zhang, S., 2021. Interactions between microplastics and soil fauna: A critical review. Critical Rev. Environ. Sci. Technol. 52, 3211–3243. https://doi.org/10.1080/10643389.2021.1915035.

Wang, Q., Adams, C.A., Wang, F., Sun, Y., Zhang, S., 2022. Interactions between microplastics and soil fauna: a critical review. Crit. Rev. Environ. Sci. Technol. 52, 3211–3243. https://doi.org/10.1080/10643389.2021.1915035.

Wei, L., McDonald, A.G., 2016. Accelerated weathering studies on the bioplastic, poly(3-hydroxybutyrate-co-3-hydroxyvalerate. Polym. Degrad. Stab. 126, 93–100. https://doi.org/10.1016/J.POLYMDEGRADSTAB.2016.01.023.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Withers, E., Hill, P.W., Chadwick, D.R., Jones, D.L., 2020. Use of untargeted metabolomics for assessing soil quality and microbial function. Soil Biol. Biochem. 143, 107758 https://doi.org/10.1016/j.soilbio.2020.107758.

Xu, C., Zhang, B., Gu, C., Shen, C., Yin, S., Aamir, M., Li, F., 2020. Are we underestimating the sources of microplastic pollution in terrestrial environment. J. Hazard. Mater. 400, 123228 https://doi.org/10.1016/J.JHAZMAT.2020.123228.

Yan, C., He, W., Xue, Y., Liu, E., Liu, Q., 2016. Application of biodegradable plastic film to reduce plastic film residual pollution in Chinese agriculture. Chin. J. Biotechnol. 32, 748–760. https://doi.org/10.13345/J.CJB.160008.

Yang, Y., Li, Z., Yan, C., Chadwick, D., Jones, D.L., Liu, E., Liu, Q., Bai, R., He, W., 2022. Kinetics of microplastic generation from different types of mulch films in agricultural soil. Sci. Total Environ. 814, 152572 https://doi.org/10.1016/J.SCITOTENV.2021.152572.

Yi, M., Zhou, S., Zhang, L., Ding, S., 2021. The effects of three different microplastics on enzyme activities and microbial communities in soil. Water Environ. Res. 93, 24–32. https://doi.org/10.1002/WER.1327.

Zang, H., Zhou, J., Marshall, M.R., Chadwick, D.R., Wen, Y., Jones, D.L., 2020. Microplastics in the agroecosystem: are they an emerging threat to the plant-soil system. Soil Biol. Biochem. 148, 107926 https://doi.org/10.1016/j.soilbio.2020.107926.

Zhang, D., Ng, E.L., Hu, W., Wang, H., Galaviz, P., Yang, H., Sun, W., Li, C., Ma, X., Fu, B., Zhao, P., Zhang, F., Jin, S., Zhou, M., Du, L., Peng, C., Zhang, X., Xu, Z., Xi, B., Liu, X., Sun, S., Cheng, Z., Jiang, L., Wang, Y., Gong, L., Kou, C., Li, Y., Ma, Y., Huang, D., Zhu, J., Yao, J., Lin, C., Qin, S., Zhou, L., He, B., Chen, D., Li, H., Zhai, L., Lei, Q., Wu, S., Zhang, Y., Pan, J., Gu, B., Liu, H., 2020. Plastic pollution in croplands threatens long-term food security. Glob. Change Biol. 26, 3356–3367, 10.1111/GCB.15043.

Zhang, H., Flury, M., Miles, C., Liu, H., Devetter, L., 2020. Soil-biodegradable plastic mulches undergo minimal in-soil degradation in a perennial raspberry system after 18 months. Horticulturae 2020 (6), 47. https://doi.org/10.3390/HORTICULTURAE6030047.

Zhang, J., Ren, S., Xu, W., Liang, C., Li, J., Zhang, H., Li, Y., Liu, X., Jones, D.L., Chadwick, D.R., Zhang, F., Wang, K., 2022. Effects of plastic residues and microplastics on soil ecosystems: a global meta-analysis. J. Hazard. Mater. 435, 129065 https://doi.org/10.1016/J.JHAZMAT.2022.129065.

Zhang, L., Xie, Y., Liu, J., Zhong, S., Qian, Y., Gao, P., 2020. An overlooked entry pathway of microplastics into agricultural soils from application of sludge-based fertilizers. Environ. Sci. Technol. 54, 4248–4255. https://doi.org/10.1021/ACS.EST.9B07905.

Zhang, M., Jia, H., Weng, Y., Li, C., 2019. Biodegradable PLA/PBAT mulch on microbial community structure in different soils. Int. Biodeterior. Biodegrad. 145, 104817 https://doi.org/10.1016/J.IBIOD.2019.104817.

Zhang, Y., Fernie, A.R., 2018. On the role of the tricarboxylic acid cycle in plant productivity. J. Integr. Plant Biol. 60, 1199–1216. https://doi.org/10.1111/JIPB.12690.

Zhao, Z.Y., Wang, P.Y., Wang, Y.B., Zhou, R., Koskei, K., Munyasya, A.N., Liu, S.T., Wang, W., Su, Y.Z., Xiong, Y.C., 2021. Fate of plastic film residues in agro-ecosystem and its effects on aggregate-associated soil carbon and nitrogen stocks. J. Hazard. Mater. 416, 125954 https://doi.org/10.1016/J.JHAZMAT.2021.125954.

Zhao, Z.Y., Wang, P.Y., Xiong, X., bin, Wang, Y.B., Zhou, R., Tao, H.Y., Grace, U.A., Wang, N., Xiong, Y.C., 2022. Environmental risk of multi-year polythene film mulching and its green solution in arid irrigation region. J. Hazard. Mater. 435, 128981 https://doi.org/10.1016/J.JHAZMAT.2022.128981.

Zhou, J., Wen, Y., Marshall, M., Zhao, J., Gui, H., Yang, Y., Zeng, Z., Jones, D., Zang, H., 2021a. Microplastics as an emerging threat to plant and soil health in agroecosystems. Sci. Total Environ. 787, 147444 https://doi.org/10.1016/J.SCITOTENV.2021.147444.

Zhou, J., Gui, H., Banfield, C.C., Wen, Y., Zang, H., Dippold, M.A., Charlton, A., Jones, D.L., 2021b. The microplastisphere: biodegradable microplastics addition alters soil microbial community structure and function. Soil Biol. Biochem. 156, 108211 https://doi.org/10.1016/J.SOILBIO.2021.108211.

Zhou, J., Wen, Y., Cheng, H., Zang, H., Jones, D., 2022. Simazine degradation in agroecosystems: will it be affected by the type and amount of microplastic pollution? Land Degrad. Dev. 33, 1128–1136. https://doi.org/10.1002/ldr.4243.