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Microplastics in mixed waste organic outputs

Soil chemical fate and ecotoxicology assessment

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List of abbreviations

16S rRNA	16S ribosomal ribonucleic acid
Al	Aluminium
***amo*A**	Ammonia monooxgenase gene
ANOSIM	Analysis of similarities
ANOVA	Analysis of variance
As	Arsenic
BPA	Bisphenol A
Cd	Cadmium
CEC	Cation exchange capacity
Co	Cobalt
Cu	Copper
DEHP	Bis(2-ethylhexyl)phthalate
DNA	Deoxyribonucleic acid
EC	Electrical conductivity
Fe	Iron
HDPE	High density polyethylene
K_d	Partition coefficient
K_{ow}	Octanol-water partition coefficient
MCPA	2-methyl-4-chlorophenoxyacetic acid
Mn	Manganese
Mo	Molybdenum
MWHC	Maximum water holding capacity
MWOO	Mixed waste organic outputs
Ni	Nickel
***nif*H**	Nitrogenase gene
***nir*K**	Nitrite reductase gene
%OC	Percent organic carbon
OECD	Organisation for Economic Cooperation and Development
PCA	Principal component analysis
PCR	Polymerase chain reaction
PET	Polyethylene terephthalate
pK_a	acid dissociation constant

PVC	Polyvinyl chloride
qPCR	Quantitative polymerase chain reaction
SIN	Substrate induced nitrification
SIR	Substrate induced respiration
TRFLP	Terminal restriction fragment length polymorphism
TRF	Terminal restriction fragment
w/w	Proportion weight per weight
Zn	Zinc

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1 Executive Summary

Sustainable alternatives to landfill disposal for municipal mixed wastes in Australia represents a major challenge to governments and waste management industries. In NSW, alternative waste treatments or mechanical biological treatments are one option being used by industry to produce mixed waste organic outputs (MWOO) for land application. The presence of chemical compounds, including organic and metal contaminants, and physical contaminants, including microplastics, in MWOO has raised concerns about potential negative effects on soil health and agriculture following land application.

The aim of this project was to determine whether microplastics and associated chemical compounds in MWOO can affect the health of soil biota and important functions, such as carbon mineralisation and nitrification. The objectives were:

- To determine the fate of selected inorganic and organic compounds associated with MWOO and MWOO amended with microplastics in three agricultural soils following incubation of the soils for up to 9 months; and
- To examine the effects of MWOO and MWOO amended with microplastics in agricultural soils to a range of soil biota and important soil functions following incubation of soils for up to 9 months.

The three types of microplastics used for the assessment were high density polyethylene (HDPE) sourced from shopping bags, polyethylene terephthalate (PET) sourced from drink bottles and polyvinyl chloride (PVC) sourced from a tablecloth were selected for addition to MWOO and soils. The microplastics were selected for their relevance to waste streams, based on waste surveys and consumer use, and were shredded and sieved to microplastic size of <2mm. Three NSW agricultural soils with a broad range of physicochemical and structural properties to ensure representativeness were selected for mixing with MWOO and microplastics. Microplastics were added to soil and MWOO-amended soils at conservatively high rates of up to 1 % w/w of microplastic, equivalent to 100 t/ha MWOO application. The soil, MWOO and microplastic mixtures were then incubated for up to 9 months, after which they were subjected to a number of soil chemical fate and ecotoxicological assessments.

Ecotoxicological assessments included acute, chronic and early life-stage exposures of earthworms (*Eisenia fetida*), nematodes (*Caenorhabditis elegans*) and wheat (*Triticum aestivum*), while effects on microbial communities were assessed by measuring soil respiration rates, nitrogen cycling and microbial community structure.

The chemical and ecotoxicological soil assessments revealed:

- Soil microbial community diversity was not affected by the addition of microplastics to MWOO-amended soil after up to 9 months of incubation. The genes related to nitrogen cycling (*amoA, nirK* and *nifH*) and soil microbial biomass, were also not significantly reduced following the addition of microplastics to MWOO-amended soils.
- Soil diversity and microbial numbers were found to increase in soil treatments and controls over the 9 month incubation period, suggesting microbial steady-state may not have been achieved in the soil treatments and they may have required incubation periods greater than 9 months.
- Soil functioning, based on the response to the addition of carbon (SIR) and nitrogen (SIN) substrates, showed no significant negative trends related to the addition of microplastics. In some cases, the addition of microplastics did have an effect on SIR or SIN; for example, addition of PVC had a negative impact on both SIN and SIR after 9 months of incubation but this was not a microplastic concentration-dependent response and did not consistently occur in the presence or absence of MWOO.
- Similarly, there were no trends showing significant negative effects for the earthworm avoidance, chronic toxicity and reproduction assays, nematode survival and reproduction assays and wheat seedling emergence rates and biomass assays following addition of microplastics to MWOO-amended soils.
- The addition of MWOO to soils increased the concentration of a number of trace metals in soil solution. Trace metal concentrations generally decreased in soil solutions over the incubation period, although concentrations of zinc consistently increased with incubation time across the majority of treatments. The availability to soil organisms of metals released from microplastics and MWOO will depend on the physical and chemical properties of soils, such as pH, Fe/Mn oxide and organic carbon contents and cation and anion exchange capacities. There was high variability observed of metal concentrations in soil solutions from incubated soils following addition of increasing amounts of individual plastics to treatments. In general, metal concentrations in soil solutions from all treatments were similar or

significantly lower following addition of increasing amounts of individual plastics compared to the lowest addition rate for the different soil incubation periods. This suggests the added microplastics were able to remove some metals into fractions, possibly through adsorption or precipitation reactions, which could not be readily released into soil solutions.

- Targeted analysis of 39 organic contaminants, including phenols, pesticides and phthalates, was unable to measure quantifiable concentrations in both soil solutions and solvent extracts of soils after 9 months incubation and following addition of microplastics. Batch sorption of three potential organic contaminants (BPA, thiabendazole and MCPA) were variably affected by the addition of MWOO and plastics, although there was no clear trend that could be ascertained.
- Under the experimental conditions within three distinct NSW agricultural soils incubated for up to 9 months in the presence and absence of MWOO (10 t/ha application rate equivalent) and three commonly used microplastics at a number of microplastic addition rates (up to 100 t/ha MWOO addition rate equivalent), there were few and inconsistent negative effects apparent in the soil ecotoxicological assessments.

Based on these findings a number of recommendations can be made:

1. **Extending soil incubation period to account for stability of microplastics and microbial community.**

   The nature of the microplastics used in this study, being highly resistant to environmental degradation, would suggest that a longer incubation period may be necessary to cover a greater extent of their chemical or physical degradation and subsequent interaction with organic and inorganic chemicals present in the soil mixtures. Longer incubation periods may necessitate larger mesocosms or field-based studies to account for additional weathering processes, such as solar radiation and rainfall.

2. **Focussing on soil microbial community structure and functions for future ecotoxicity assessments.**

Of the ecotoxicological assays, soil microbial functions and community structure were found to be the most sensitive to the microplastic treatments, which would indicate that focussing on soil microbial endpoints in future assessments may allow effects to be determined before they manifest themselves in higher level organisms, such as invertebrate and plant species. This does not preclude the use of other species in future assessments that were not included in this study, such as arthropods.

3. **Consider using higher rates of microplastics to account for future changes in regulations.**

The dosing rates of plastics in this study were selected to cover currently regulated addition rates of MWOO and associated microplastic content. Were regulation to change in the future, particularly where multiple applications or substantially higher rates of MWOO addition to terrestrial environments is anticipated, then additional higher microplastic addition rates should be further considered in terms of soil chemical fate and ecotoxicology.

4. **Include other microplastics, such as biodegradable polymers, that reflect future consumer use patterns of plastics.**

The type and nature of microplastics considered in this study are related to commonly used plastics in the community at present, which are present in produced MWOO. If any trends in consumer use of plastics becomes evident, such as the substantial uptake in the use of other polymer types through regulatory or market-driven preferences, then the inclusion of these plastic types would enable a more comprehensive and realistic analysis relating to potential negative impacts of microplastics in the terrestrial environment.

2 Introduction

Sustainable alternatives to landfill disposal for municipal mixed wastes in Australia represents a major challenge to governments and waste management industries. In NSW, alternative waste treatments or mechanical biological treatments are one option being used by industry to produce mixed waste organic outputs (MWOO) for land application. These MWOO are produced through a process of sequential technologies to remove unwanted physical contaminants, composting to reduce pathogen loads and hammer milling to reduce particle size to less than 2 mm. There are four facilities currently producing MWOO in NSW and more are either commencing construction soon or in the planning process.

The production of MWOO commenced prior to the NSW EPA having a specification for appropriate land application. The regulatory mechanism in NSW that allows for the beneficial land application of MWOO falls under a resource recovery order and resources recovery exemption (Resource Recovery Order and Exemption under Part 9, Clauses 91, 92, and 93 of the Protection of the Environment Operations (Waste) Regulation 2014, NSW EPA). The presence of chemical compounds, including organic and metal contaminants, and physical contaminants, including plastics and glass, in MWOO has raised concerns about potential negative effects on soil health and agriculture following land application.

Microplastics are increasingly being recognised as a potential threat to ecosystems. In aquatic environments, there has been research interest in determining the fate and effects, especially trophic transfer, of plastics in fresh and marine waters and sediments (Barnes et al. 2009 , Eerkes-Medrano et al. 2015, Duis and Coors 2016). So far, there has been little scientific research undertaken to examine the effects of microplastics in the terrestrial environment (Rillig, 2012). The microplastics (primary and secondary fragments) and chemical compounds associated with or released may have adverse behavioural, morphological and reproductive effects on terrestrial biota.

A basic conceptual model of the fate of chemical compounds associated with or released from microplastics in MWOO and soils can be found in Figure 1. The conceptual model takes into consideration the physical, chemical and biological interactions that MWOO are likely to undergo in the environment and the toxicants that may be released, transformed or degraded in the process. The model also considers the impact of the waste and associated toxicants on soil biota and associated functions.

Once applied and mixed into the surface of agricultural soils, microplastics in MWOO can be degraded by physical, chemical and biological weathering processes with time (material ageing). These processes can cause the microplastics to disintegrate resulting in fragmented secondary particles of various sizes. The secondary particles may have different chemical functionality, including functional groups or surface charge, compared to the parent particles depending on the type of microplastics in the MWOO. Furthermore, the type of polymer used in plastics is known to be important for the rate of degradation in environments. Polymer-based materials that contain ester linkages such as polyester polyurethanes are known to be readily biodegraded by the action of esterases (Albertsson and Karlsson 1993). Biotic degradability of polymers has been found to decrease with increased ethylene content, as reported by Kumar et al. (2006) from a 6-month study on the degradability of ethylene–propylene copolymers. Composition can also affect how sensitive a polymer is to photo-degradation. Kaczmarek et al. (2007) used blends of poly(ethylene oxide) and pectin and found that after twenty hours of exposure the blends most sensitive to UV degradation were those with an equal weight-ratio of each polymer.

Abiotic and biotic degradation of microplastics in MWOO can result in the release of chemical compounds including plasticizers, transformed polymers, organic compounds, and metals. These chemical compounds may be further degraded, transformed or sorbed to the MWOO matrix and/or leached from the MWOO matrix into the soil environment. Microplastics may also act as sites of interaction through, for example, complexation and sorption for other chemical substances present in MWOO, including organic and metal contaminants. The leachability of chemical substances from MWOO into soils will depend on the aqueous solubility of the substance, as well as the strength of their interactions with solid phases, including microplastics.

In the soil environment, the fate and ecotoxicity of leached chemical compounds from either MWOO or microplastics may depend on their transformations and interactions with the solid phase. The physical and chemical properties of soils, including pH, organic matter content (% OC), texture and clay mineralogy, can all have a significant effect on the partitioning and bioavailability of chemical compounds released from the MWOO. The soluble fraction in soils is considered to be the most bioavailable and mobile fraction in soils and soil pore water concentrations are particularly useful indicators of assessing bioavailable fractions of contaminants in soils.

Environmental factors are expected to influence the transformations of microplastics and fate of chemicals associated with these. If chemical compounds are leached from MWOO into soils, there will likely be an initial fast reaction for sorption sites onto solid phases as observed for other

contaminants (Ma et al. 2006, Oorts et al. 2007, Delgado-Moreno and Gan 2013). This initial fast reaction is followed by slower reactions that can remove chemical compounds from labile pools into a pool or pools from which desorption is slow, a process referred to as 'chemical-ageing'. The immobilised chemical compound can often still be measured using conventional techniques for bulk chemical analysis, including strong acid digestion or solvent extractions of soils, but has essentially become unavailable for movement into soil solutions, rendering it inaccessible to soil biota. Consequently, toxicity may decrease with time as the amount of actual contaminant exposure is reduced, assuming soil physicochemical properties remain relatively constant over time.

It is important to examine the fate and toxicity of chemical compounds leached from MWOO to soils following scenarios of realistic exposure pathways and concentrations to determine their potential risk to soil environments. The soil environment is expected to have a significant effect on the fate and ecotoxicity of chemical compounds leached from surface applied MWOO treatments, containing microplastics.

2.1 Aims and objectives

The NSW EPA commenced a program of scientific research in 2011 to investigate the potential benefits and risks associated with land application of MWOO. The research program consists of four projects:

Project 1: Assessing the impacts of physical contaminants in MWOO on the soil environment;

Project 2: Field trial assessing the impacts of MWOO using field based crop/soil responses (project leader: NSW Department of Primary Industries);

Project 3: Assessing the toxicity of MWOO leachate (project leader: NSW Office of Environment and Heritage; and

Project 4: Assessing the behaviour of MWOO in different NSW soils (project leader: University of New England).

This project specifically relates to project 1e. The key objective was to determine whether the chemical compounds in/on microplastics present in MWOO poses a risk to the terrestrial environment and if so what risk this presents.

The aim of this project was to determine whether microplastics and associated chemical compounds in MWOO affect the health of soil biota and important soil functions. The objectives were:

- To determine the fate of selected inorganic and organic compounds associated with MWOO and MWOO amended with microplastics in agricultural soils following incubation of the soil treatments for up to 9 months; and
- To examine the effects of MWOO and MWOO amended with microplastics in three agricultural soils to a range of soil biota and important soil functions following incubation for up to 9 months

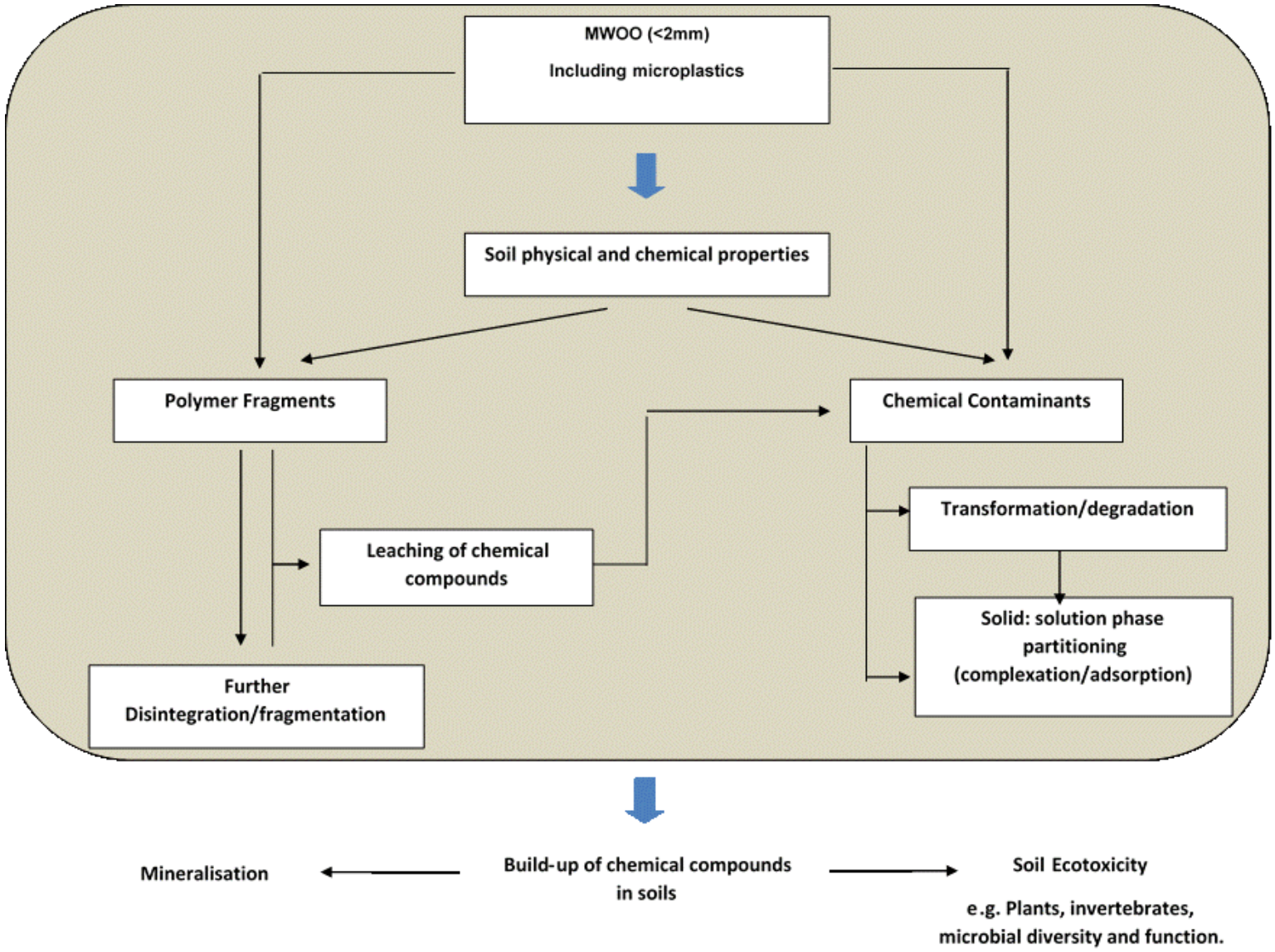


Figure 1. Conceptual model of the fate of chemical compounds associated with or released from microplastics in MWOO and soils.

3 Methods and Materials

3.1 Collection and preparation of agricultural soils and MWOO

The mixed waste organic outputs (MWOO) was sourced from the *[name redacted]* facility in June 2016 and used as received. MWOO was immediately refrigerated at <4^{o}C upon receipt.

The selection of soils for this study was based on the diversity of the soil properties, as well as sourcing them from an agricultural region from NSW. The three agricultural soils selected for this study, Kirby Sand, Kirby Clay and Warialda Loam, were collected from the New England agricultural region. The soils selected covered a range of pH, organic carbon content (%OC) and textures known to be important in the fate of inorganic and organic chemicals. An additional advantage of including these soils was that they were also used for a previous assessment of MWOO toxicity (Wilson et al. 2015).

Kirby Sand (371479E, 6632389N), Kirby Clay (368782E, 6632154N) and Warialda Loam (247122E, 6728341N) were collected from near Armidale, NSW on the 23-26th May 2016 (Figure 2). The vegetative cover was removed from the surface of soils at each site. The soils were collected from the top 20 cm of the profile using steel shovels into 20 kg containers for shipment to CSIRO. At CSIRO, the soils were air-dried in a glass house to a constant mass, homogenised and sieved to < 2mm. MWOO was used as received for soil dosing.

Figure 2. Map showing the relative location of Armidale region, where experimental soils were collected, to Sydney.

3.2 Microplastics selection and preparation

A NSW EPA audit of municipal wastes found plastics can constitute around 10% of the total mass of disposed waste material (NSW EPA 2016; personal communication). It was therefore conservatively assumed in this study that this amount would be present in the final MWOO material. Of the identified plastics in this audit, the most common plastics were polyethylene (PE) and polyethylene terephthalate (PET). The lowest mass plastic was identified as polyvinyl chloride (PVC). Plastic shopping bags, produced from high density PE (HDPE) and PET plastic bottles were commonly found in waste material. A previous study assessing the ability of plastics to sorb chemicals found PE to have a high capacity to absorb organic contaminants, compared with PET and PVC (Teuten et al. 2007, Rochman et al. 2013). PVC, despite being a significantly smaller component of overall plastics in waste material, contains a high level of plasticisers and especially the phthalate ester plasticisers and has been found to have a comparatively greater toxicity than other plastics, such as HDPE (Lithner et al. 2012). The presence of phthalates in the PVC selected for assessment in this project (from tablecloth) was confirmed by gas chromatography-mass spectrometry (GC-MS) (Figure B3).

Based on the abundance of plastics in municipal wastes, potential absorptive capacity of the plastics (and potential effect on contaminant fate) and their potential ecotoxicity the following plastics were selected for this study:

- High density polyethylene (HDPE)
- Polyethylene terephthalate (PET)
- Polyvinyl chloride (PVC)

HDPE was sourced from consumer shopping bags (QIS Packaging, Australia), PET was sourced from drink bottles (Synergy Packaging, Australia) and PVC was sourced from a table cloth (Spotlight, Australia). The selected plastics were either colourless (PET, PVC) or white (HDPE) to minimise the presence of colouring agents. The plastics were shredded using a mill rotating blade cutter (Security Engineered Machinery, Model 1012 disintegrator, Westboro, MA, USA) at a document destruction facility located in Adelaide, South Australia. Shredder blades were thoroughly cleaned with compressed air, while each batch of different polymers were first run through the blade and discarded with an additional cleaning of the blades with compressed air. This ensured cross-contamination of plastics was minimised during preparation The shredded plastics were

subsequently sieved to < 2mm using stainless steel sieves. Micrographs and particle size analysis of individual microplastics can be found in Table B1 and Figure B1.

3.3 Soil dosing and incubation

The soil samples were amended with MWOO at a rate equivalent to 10 t MWOO/ha soil or 1 % w/w, which is equivalent to the maximum permissible rate for broadacre agriculture in NSW (Protection of the Environment Operations (Waste) Regulation 2005 – General Exemption under Part 6, Clause 51 and 51A; The organic outputs derived from mixed waste exemption 2014). This 1 % w/w assumes that the MWOO would be incorporated in the field to a depth of 100 mm. Treatments included the addition of HDPE, PET and PVC microplastics to the soil/MWOO mixture at varying rates. The rates of microplastic addition to the soils were 0.1, 0.25, 0.5 and 1 % w/w for all three microplastics, while an additional 0.01 % w/w treatment was included for PVC due to its lower proportion of contamination in MWOO waste streams (Table 1). These rates of microplastics addition were based on a previous audit of wastes destined for MWOO production containing approximately 10 % w/w plastics (NSW EPA; personal communication), so that the microplastics were added to represent a scenario where 10, 25, 50 and 100 t/ha of MWOO was added to soil and the 10 % w/w of plastic in the MWOO was composed of each individual microplastic. The rate of 50 t/ha MWOO represents the highest allowable rate of application for non-contact or plantation forestry agriculture in NSW. The microplastics were homogenously added into soil and MWOO-amended soil treatments by mixing the soils (or soils and MWOO) and microplastics in stainless steel bowls prior to addition to large glass containers, in which they were regularly mixed throughout the incubation period. It should be noted that concentrations given in this study relate to nominal, rather than measured, concentrations of microplastics due to the emerging nature of microplastics analysis in solid environmental matrices, such that using nominal concentrations currently represents an acceptable approach for microplastic toxicity testing (Lwanga et al. 2016). Additional treatments included using soils without added MWOO or microplastics, while microplastics were added to soils without MWOO as a treatment in the batch sorption and soil microbial toxicity experiments to cover the scenario where the largely organic MWOO is degraded, leaving the plastics behind.

All controls and treatments were prepared with a moisture content of 60 % of maximum water holding capacity (MWHC) of respective, dried soils prior to experimental assessment. The treatments were incubated in either 4 L or 1 L borosilicate glass jars with stainless steel lids in a

temperature controlled room (23.5±2.8^{o}C) for a period of 1 week (unincubated or 0 month soil treatments), as well as for 3 and 9 months. All treatments were incubated in the dark to minimise potential plant growth in the soils as well as to prevent photolytic degradation of contaminants or plastics within the soil mixtures. Throughout the incubation period soils were aerated every two days to prevent anaerobic conditions occurring, mixed on a weekly basis to maintain homogeneity and moisture content maintained at 60 % MWHC throughout the incubation period. All soil and MWOO-amended soil treatments were prepared in triplicate.

Table 1. Summary of dosing rates of MWOO and microplastics used for experimental treatments.

Treatment	Soil (g)	MWOO (g)	Microplastic (g)	Assessments[a]
Soil + MWOO (control)	100	1	-	All
Soil	100	-	-	All
Soil + MWOO + plastic	100	1	0.5 (50 t/ha equiv.)	All
Soil + MWOO + plastic	100	1	1 (100 t/ha equiv.) 0.25 (25 t/ha equiv.) 0.1 (10 t/ha equiv.) 0.01 (1 t/ha equiv; PVC only)	Soil microbial toxicity (SIR and SIN) Batch sorption Soil solution distribution (metals + organic)
Soil + plastic	100	-	1 (100 t/ha equiv.) 0.5 (50 t/ha equiv.) 0.25 (25 t/ha equiv.) 0.1 (10 t/ha equiv.) 0.01 (1 t/ha equiv; PVC only) 0.01 (1 t/ha equiv; PVC only)	Soil microbial toxicity (SIR and SIN) Batch sorption Soil solution distribution (metals + organic)

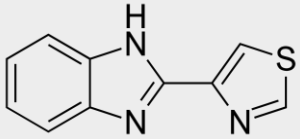
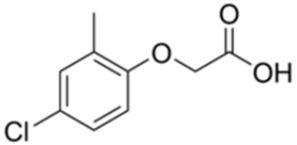
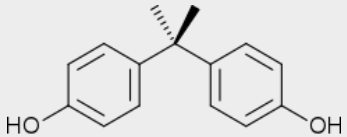
[a]Chemical and ecotoxicological assessments

3.4 Chemical assessment

3.4.1 Effect of microplastics on batch sorption of selected organic contaminants

The sorption behaviour of three chemicals, 2-methyl-4-chlorophenoxyacetic acid (MCPA), bisphenol A (BPA) and thiabendazole, was assessed (Table 2). These three chemicals were chosen because they had been identified in previous studies as priority organic contaminants in MWOO based on frequency of occurrence, concentrations in MWOO and/or evidence for persistence in (Wilson et al. 2014, NSW OEH 2015). A NSW OEH (2015) study found thiabendazole, dicamba, MCPA and MCPP were the most frequently detected organic contaminants and were found in greater than 50% of the solid samples of MWOO tested. They also found elevated concentrations of BPA in 100% of the solid samples tested. MCPA is a phenoxy-acid herbicide used to control broadleaf weeks which is mostly insoluble in water, has a field half-life of 14 d to one month and has a low affinity for soil. Thiabendazole is a systemic benzimidazole fungicide used to control fruit and vegetable diseases such as mould, rot and blight. It has a strong affinity to bind to soil particles and is highly persistent. It is stable to both photolysis and hydrolysis in soils (Walters et al. 2010). BPA is used by manufacturers as an intermediate in the production of polycarbonate and epoxy resins, flame retardants, and other speciality products. Final products include adhesives, protective coating, powder paints, automative lenses, protective window glazing, building materials, compact disks and for encapsulation of electrical and electronic parts (Staples et al. 1998).

Table 2. Selected properties of organic chemicals assessed in batch sorption study.

Chemical	Class	Log P[a]	Water solubility (mg/L)[b]	pK_a[c]	Concentration reported in MWOO[d]
Thiabendazole[1] 	Fungicide	2.39	30	4.73 (base)	0.028 mg/kg (Mean)
MCPA[1] 	Herbicide	-0.81	29390	3.73 (acid)	0.36-1.8 mg/kg (Range) [2] 0.75 mg/kg (Mean) [2]
BPA 	Industrial chemical (e.g. plastics manufacture)	3.32	120-300[3]	9.59, 10.2 (acid) [3]	4-100 mg/kg (Range) [2] 26 mg/kg (Mean) [2]

[1]Data from Pesticide Properties Database http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm

[2] NSW Office of Envrironment and Heritage (2015)

[3]Staples et al. (1998)

Sorption coefficients for the three chemicals were determined using the OECD 106 standard protocol for the adsorption – desorption of chemicals using a batch equilibrium method (OECD 2000a). Sorption was assessed after 0, 3 and 9 months of incubating soils with microplastics and MWOO. In unincubated (0 months) treatments, the soil treatments were sub-sampled before being wetted for the incubation experiment. For the 3 and 9 month incubated treatments, a sub-sample was taken at harvest, immediately freeze-dried, then sieved <2mm and weighed. Briefly, the procedure involved weighing 3 g of soil samples into glass vials which were pre-equilibrated for 24 h with 0.01 M $CaCl_2$. After the pre-equilibration a known volume of a 20 mg/L spiking solution (2% methanol) of the chemical being assessed was added to give a final volume of 15 mL and a soil:solution ratio of 1:5. The spiking solution was made from 1000 mg/L stock solution in 100% methanol. The spiked solution was then shaken for 24 h, centrifuged at 1200 rpm for 45 min and an aliquot was withdrawn using a glass pipette for analysis by liquid chromatography-mass spectrometry- mass spectrometry (LC/MS-MS). Previous studies have indicated that all three chemicals rapidly reach equilibrium in batch sorption assessments, such that 24 h is a suitable time period for this equilibration period (Ying et al. 2003, Hiller et al. 2012, Neto et al. 2017). In each batch the spiking concentration was measured as a blank (spiked sample with no soil) and this concentration was used in the determination of sorption. The initial concentrations for thiabendazole, MCPA and BPA were measured with each batch. Blanks (0.01M $CaCl_2$ only with no spike) were also run with every batch. These blank measurements were used to determine that no thiabendazole, MCPA and BPA were released from the soil, MWOO or plastics and that no other chemicals extracted with the 0.01M $CaCl_2$ interfered analytically with the measurement of these chemicals. In addition, at each sampling time blanks (0.01M $CaCl_2$ unspiked, no soil) for respective chemicals were assessed. After centrifuging pH was measured on all the blank samples and on one of the batches spiked with one of the chemicals to assess the effect of low (0.1%) methanol in solution. All samples were run in triplicate.

The sorption coefficient (K_d) values were calculated by:

$$K_d = \frac{C_s}{C_{aq}} \quad (1)$$

where K_d is the sorption coefficient, C_s is the concentration (mg/kg) of the chemical sorbed by the soil and C_{aq} is the concentration (mg/L) of the chemical measured in solution. The concentration of the chemical sorbed by the soil was measured indirectly from C_{aq} and was determined by:

$$C_s = \frac{(C_{aqi} \times Vol._{aqi}) - (C_{aq} \times Vol._{aq})}{M_s} \quad (2)$$

where C_s is the concentration (mg/kg) of the chemical sorbed by the soil and C_{aqi} is the intial concentration (mg/L) of the chemical in solution, C_{aq} is the concentration (mg/L) of the chemical measured in solution at the end of the batch sorption experiment, *Vol.* is the volume of the respective solutions and M_s is the mass of soil. This indirect measurement of C_s is dependent on the stability of the chemicals to ensure that K_d values are not overestimated due to degradation of the chemical. The measured half-lives of BPA, MCPA and thiabendazole from previous studies in a range of soils indicated that the 24 h equilibration period was short enough to minimise potential losses through degradation (Ying et al. 2003, Hiller et al. 2012, Neto et al. 2017).

All batch sorption solutions were analysed using LC-MS/MS (Finnigan TSQ Quantum Discovery MAX triple-quadrupole mass spectrometer) operating in electrospray positive (thiabendazole) and negative (MCPA and BPA) ionisation mode. Calibrations standards and samples (10 µL) in 0.01M $CaCl_2$ were injected onto a Thermo Dionex UltiMate 3000 HPLC system with a Phenomenex Kintex 100 x 2.1 mm C18 column. The mobile phase was 5 mM ammonium acetate (A) and methanol (B) at a flow rate of 0.25 mL/min and column temperature 30°C. The mobile phase started at 95% A: 5% B, changing to 5% A : 95% B over 5 min; holding at this ratio until 7 min; transferring back to 95% A: 5% B until 8 min; and continuing in this ratio until 13 min.

3.4.2 Inorganic and organic chemicals in soil solutions

Soil solutions were extracted from each soil treatment following methods in McLaughlin et al. (1997). Triplicate 20-30 g sub-samples, composited representatively from respective replicates, for each soil treatment were weighed into 20 mL syringes containing acid-washed glass wool. Syringes and soil were placed in 50 mL centrifuge tubes and ultrapure water (resistivity 18 Ω.cm; Milli-Q water, Millipore) added to the syringes to pF 1.7 (50 cm tension wetness, field capacity) for each soil. After a 24 h equilibration period, the syringes were centrifuged at 1200 rpm for 40 min. The soil solutions were filtered through 0.45 μm syringe filters (Sartorius) and sub-samples collected directly in 2 mL amber vials for organic contaminant analysis and stored at -18^{o}C until analysis. The remaining soil solutions were stored in a refrigerator at 4^{o}C until analysis for selected inorganic chemicals.

Selected metals (iron (Fe), manganese (Mn), cobalt (Co), nickel (Ni), copper, (Cu), zinc (Zn), arsenic (As), molybdenum (Mo), cadmium (Cd), chromium (Cr), tin (Sn), and lead (Pb)) were determined directly in soil solutions using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Thermo) or inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700).

For soil solution and solvent extract analysis of organic contaminants a SCIEX Exion LC AD autosampler with column oven, LC pumps and degasser was used for liquid chromatography (LC) separation. A Phenomenex Kinetex 2.6 μm C18 column (100x2.1 mm) column was used with a binary mobile phase at a flow rate of 0.45 mL/min. The first 2.5 min of the flow was sent to waste via a 10 port-2-position valve installed post-column in order to prevent the ion source from contamination with matrix components. The optimized separation conditions consisted of a mobile phase of 0.1% formic acid and 10 mM ammonium formate (A) and methanol (B). The gradient elution was as follows: 0–2 min: 5% B, increasing to 45% within 5 min, then to 95% in 7 minute, held at 95% for 1 minute, then was re-equilibrated at 5% B for 8 min with a total run time of 16 min. The column oven and autosampler temperature were set at 30^{0}C and 10^{0}C, respectively. The sample volume injected was 10 μL. For mass spectrometric analysis a SCIEX TripleTOF™ 5600+ system with a DuoSpray™ Source was used for data acquisition, over a mass range of 100 – 500 (m/z). Automated calibration was performed using an external calibrant delivery system (CDS) which infuses calibration solution

prior to sample introduction. The iInformation dependent acquisition (IDA) methods consisted of a TOF MS dependent scan (m/z 100-500) followed by a multiple reaction monitoring (MRM) scan was performed with collision energy of 30 eV and a spread of ±15eV for each targeted compound. The quantification was processed using MultiQuant^{TM} 3.0.2 Software. The 39 organic contaminants targeted by this analysis and their respective limits of quantification (LOQ) in each treatment matrix are summarised in Table F1. These analytes were selected based on these chemicals (or chemicals within the class of compounds outlined in Table F1) being detected on a previous assessment of MWOO (NSW OEH 2015).

3.4.3 Soil extraction for organic contaminants

Soil samples were extracted for analysis of organic contaminants using an exhaustive solvent extraction of each treatment. Soils were sampled for extraction following their respective incubation periods, with 1 g of soil collected in triplicate from each treatment in glass tubes for freeze drying at -50^{o}C. Dried soil replicates were mixed with clean sand at 1:1 ratio and added to 15 mL borosilicate glass tubes, along with 5 mL methanol, vortexed for ~1 minute and placed in an ultrasonic bath heated to 50^{o}C and ultrasonicated for 15 min. Tubes were centrifuged for 30 min at 1500 rpm and the supernatant was removed into a separate glass tube. This was repeated twice, using 5 mL methanol, then 5 mL acetone/dichloromethane, pooling the solvents. The solvents were then blown to dryness under a gentle stream of N_2 before being reconstituted in 1 mL of 0.1% formic acid and 10 mM ammonium formate (90 %) and methanol (10 %) for analysis. For assessment of recoveries from the extraction procedure, 100 μL of a 1 mg/L mixture of the target organic compounds was added to the soils prior to extraction. Analysis of the solvent extracts was undertaken as for the soil solution extracts.

3.5 Ecotoxicological assessments

A number of ecotoxicological assessments were undertaken on the incubated soil and MWOO-amended soils (Table 3). These standard ecotoxicological tests were selected for this study to cover a range of important soil biota, sensitivities and functions. Unless otherwise stated, all ecotoxicological assessments used triplicate treatments within each assay.

3.5.1 Soil microbial community structure

The soil microbial (bacteria, fungi and archaea) community structure and dynamics were assessed using terminal restriction fragment length polymorphism (TRFLP) as well as estimation of total bacterial numbers using quantitative polymerase chain reaction (qPCR) (see Section 3.5.2). TRFLP is a technique used to study complex microbial communities based on variations in the 16S rRNA gene and how they change in response to variations in environmental parameters and conditions. Briefly, the technique involves PCR amplification of a near complete 16S rRNA gene using fluorescently-labelled primers resulting in a mixture of labelled fragments representing different species. The PCR products are digested with restriction enzymes to produce labelled terminal restriction fragments (TRFs) of various sizes. The TRFs are separated via capillary electrophoresis with an internal size marker and are sorted based on fragment size to give an individual profile for each sample. Although there is the potential for fragments not being completely resolved between different microbial species, therefore underestimating population diversity, this technique offers a relatively rapid means of assessing microbial community structure and composition. Microbial diversity of bacteria, fungi and archaeal populations in the soils were assessed using multiplex TRFLP (m-TRFLP) targeting 16S (bacteria) with HEX-, ITS (fungal) with FAM- and archaeal genes with NED-labelled primers. Total DNA was extracted as described in Section 3.5.2. Genes were co-amplified in a multiplex PCR reaction following Singh et al. (2006). The 25 μL total reaction volumes contained 12.5 μL of Multiplex PCR MasterMix (Qiagen) providing a final concentration of 10mM dNTP; 3mM $MgCl_2$; 1X NH_4^+/K^+ buffer and 1U HotstartTaq polymerase. The PCR amplification was performed in an Eppendorf Gradient thermal cycler using the following program: 10 min hotstart at 95°C followed by 30 cycles of 95°C for 30 s,

55°C for 30 s and 72°C for 1 min; and a final extension of 10 min at 72°C. The lengths of the final products generated were verified via separation on a 2% e-gel (Life Technologies) with ethidium bromide staining.

Table 3. Overview of soil ecotoxicological assessments undertaken on soil and MWOO-amended soil following incubation for up to 9 months.

Assessment	Test	Expected outcome	Samples assessed
Soil microbial community structure	Soil microbial diversity (using TRFLP and next generation sequencing) Nitrification gene expression (*nif*H, *nir*K, AOA, AOB) using qPCR techniques.	To determine the effect of microplastics on the broad diversity of soil microorganisms and function relating to nitrogen cycling in soils	Soil Soil + MWOO Soil + MWOO + microplastics (added at 50 t/ha MWOO equivalent)
Soil microbial toxicity	Substrate induced respiration (based on OECD 217) Substrate induced nitrification (based on OECD 216)	To measure the effect of microplastics on the ability of soil microorganisms to perform key transformation activities of carbon or nitrogen.	All treatments
Nematode toxicity	*Caenorhabditis elegans* toxicity assay (based on ASTM, 2001)	To determine the pore water-based toxicity of microplastics to a soil invertebrate	Soil Soil + MWOO Soil + MWOO + microplastics (added at 50 t/ha MWOO equivalent)
Earthworm toxicity	Earthworm toxicity and reproduction (based on OECD 207/222) Earthworm avoidance (based on ISO 17512-1)	To determine the effects of microplastics on the soil-based toxicity, reproduction and behaviour to a soil invertebrate.	Soil Soil + MWOO Soil + MWOO + microplastic (added at 50 t/ha MWOO equivalent)
Plant toxicity	Wheat seedling emergence assay (based on OECD 208)	To determine the early life-stage toxicity of microplastics on an agricultural plant species.	Soil Soil + MWOO Soil + MWOO + microplastic (added at 50 t/ha MWOO equivalent)

The PCR products were purified using Agencourt AMPure XP PCR purification solution and approximately 100 ng of product was digested with 20 U of MspI, HaeIII and TaqI for 3 h at 37^{o}C or 65^{o}C. The digest reactions were diluted in 20 μL water and cleaned up with MinElute 96 UF PCR purification kit. The samples were processed by the Australian Genome Research Facility (AGRF) on an ABI 3730 Genetic Analyzer. Aliquots of 5 μL for each digest were mixed with 4 μL of formamide and 1 μL of the internal size standard (GeneScan-500 LIZ, ABI). The samples were denatured at 94^{o}C for 5 min then placed on ice prior to capillary electrophoresis.

The TRFs were analysed using GeneMarker AFLP/Genotyping software program (SoftGenetics LLC Version1.8) at a detection limit of 200 fluorescent units (FU). TRFs that deviated by less than 1 base pair in length were considered to be within the same bin set. Peak heights were automatically calculated by the software and used as a measure of abundance while richness was based on the number of individual peaks obtained. TRFs were standardized based on the relative peak area and removed from analysis if < 2% of total peak contribution (Mou et al. 2005).

In addition to mTRFLP analysis, total bacterial populations was estimated using amplification of 16S rRNA gene copies as described for the nitrogen cycle genes (see Section 3.5.2).

3.5.2 Soil microbial function

This assessment comprised of two assessments to determine the effect of the microplastics on the key soil processes relating to carbon and nitrogen cycling. Substrate induced respiration (SIR) and substrate induced nitrification (SIN) were used as a means of measuring these two key soil nutrient cycling processes. For these assessments, a source of nitrogen (SIN) and carbon (SIR) are added to the treatment soil and the production of nitrate/nitrite (NO_3^-/NO_2^-; SIN) or carbon dioxide (CO_2; SIR). The methodology used for these assessments is adapted from OECD protocols for SIR (OECD 2000b) and SIN (OECD 2000c). It is worth noting for SIN and SIR, these assessments were also the most comprehensive of the ecotoxicological assays, where effects were measured at different rates of microplastic addition rates both in the presence and absence of MWOO (Table 3). This was to account for a scenario where the

organic matter present in the MWOO had been completely degraded, with residual microplastics remaining in the soil.

In the case of SIN, 2.9 mg $(NH_4)_2SO_4$ was added to 7 g soil in 50 mL polypropylene tubes and maintained at 25^{o}C for 28 days, before being extracted with 35 mL of 1 M potassium chloride (KCl) solution and the extract solution analysed for nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) concentrations by ion chromatography. In the case of SIR, 10 g of soil was incubated at 25^{o}C for 14 days in polypropylene vials before 50 mg of ^{14}C-labelled glucose was added to the soil. Following addition of the glucose, 3 mL of 1 M sodium hydroxide (NaOH) solution was added to another vial and the soil and NaOH were sealed in a 250 mL glass jar for 6 h. The amount of $^{14}CO_2$ collected in the NaOH traps was then measured using beta scintillation counting.

For both SIN and SIR assessments, soils were aerated daily during the test incubation period. All soil samples collected for the SIN and SIR assessments for the unincubated (0 months) treatments were incubated prior to the assessments for a period of 14 days at 60% MWHC.

The SIN and SIR assessments were selected to have the full range of treatments applied to them (Table 3) since soil microbial communities can be highly sensitive to contaminants and their function is key to soil processes such as soil nutrient cycling and overall fertility (OECD 2000b, 2000c).

In addition to the SIN assessment, the quantification of three genes relating to key soil nitrogen cycle processes was also assessed using quantitative polymerase chain reaction (qPCR). Molecular techniques such as qPCR can be used to link microorganisms to key processes in soil and the analysis of the abundance and structure of functional genes involved in the biogeochemical cycling of N in soils offer an approach to directly link microbial groups to soil characteristics and ecosystem processes. The majority of N entering ecosystems is biologically-driven from fixation of atmospheric N_2 and use the nitrogenase reductase (*nifH*) marker gene. Ammonia-oxidising bacteria (AOB) oxidise ammonia (NH_3) to NO^-_3 as the first step of nitrification and are studied using the ammonium monooxygenase (*amoA*) marker. There are several genes within the denitrification pathway that result in N_2O release and the nitrite reductase (*nirK*) marker was selected to assess the denitrification potential in these soils (Table 4).

For gene quantification assays aliquots of soil were collected after 0, 3 and 9 months incubation. To ensure a homogenous sample was taken from each sample, each jar was well mixed and multiple soil aliquots were taken from various points within the soil for a 2 g composite samples. This was done in duplicate for each treatment, which were stored at -0°C prior to processing.

DNA was extracted in duplicate with MoBio PowerSoil DNA Isolation kit and a FastPrep bead beater (MP Biochemicals) at 6 m/s for 30 s for Kirby Sand and Warialda Loam and 2 x 6 m/s for 30 s for Kirby Clay. DNA purity was assessed using Nanodrop ND-1000 spectrophotometer and quantified with PicoGreen ds DNA quantification reagent (Invitrogen). DNA samples were adjusted to a standard concentration (5 ng/µL), where necessary, and stored at -20°C until further processing.

The copy numbers of 3 genes involved in key steps of the geochemical cycling of N were quantified in the DNA extracts, along with 16S rRNA subunit to assess the total bacteria numbers. The genes, the enzymes they encode and the functions of these enzymes are given in Table 4.

Table 4. Functional genes measured for this study and their related enzymes and nitrogen cycle function in soil.

Gene	Enzyme	Nitrogen cycle function
*nif*H	dinitrogenase reductase	N_2 fixation
*amo*A	ammonia monooxygenase	Nitrification / N conversion
nirK	nitrite reductase	Denitrification

Quantitative PCR (qPCR) of the 16S rRNA, *amoA*, *nifH* and *nirK* genes were carried out using an Agilent AriaMX RealTime PCR system. qPCR reactions consisted of DNA template, 0.2 µM forward and reverse primers, and Brilliant III UltraFast QPCR Master Mix with SYBR Green (Agilent Technologies). Primer pairs used were Bact1369F/PROK1492R (Suzuki et al. 2000), *amoA*-1F / *amoA*-2R* (Rotthauwe et al. 1997, Stephen et al. 1999), *nifH*-F-Rosch / *nifH*-R-Rosch (Rosch et al. 2002), F1aCu / R3Cu (Braker et al. 1998, Throback et al. 2004). For 16S qPCR conditions were 95 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 56 °C for 1 min,

and 72 °C for 30 s. For *amoA* the annealing temperature was 50°C and *nifH* and *nirK* was 60°C. Verification of PCR specificity was performed via dissociation curve and agarose gel. Functional gene quantification was based on real-time PCR amplification against appropriate standard curves containing known copy numbers of each gene.

3.5.3 Nematode (*Caenorhabditis elegans*) mortality and reproduction

Aquatic toxicity tests with the soil based nematode, *Caenorhabditis elegans*, measured the acute toxicity of leachates, based on immobilisation/mortality of *C. elegans* (ASTM 2001). *C. elegans*, wild-type strain N2, was used in the tests. The culture had been kept on NGM agar plates, with a bacterial lawn of a uracil-deficient strain of *Escherichia coli* (OP50) as food source and maintained at 20 °C. Tests were conducted with age-synchronized 3-4 day old adult organisms obtained using a standard alkaline hypochlorite treatment method. The acute bioassay was performed in soil pore water solutions collected for contaminant analysis (see Section 3.4.3) and followed the methodology previously outlined in (Boyd and Williams 2003).

Briefly, 2 mL of leachate was added to wells of a 6-well tissue culture plate. Each well had 5 worms added and it was incubated for 24 h at 20°C with no food. K media was used as the blank control and $CuSO_4$ as a reference toxicant positive control. The number of worms alive was counted to give a percentage of mortality. For reproduction testing one worm was added to each well of a 6-well tissue culture plate containing 2 mL of leachate and 50 μL of OP50 culture. Plates were incubated with shaking at 20°C for 72 h and the number of worms in each well counted.

3.5.4 Earthworm (*Eisenia fetida*) avoidance

The avoidance assay, using the earthworm *Eisenia fetida*¸ represents a relatively rapid (48 h) assessment relating to a behavioural endpoint. This assessment protocol followed that of ISO 17512-1 (ISO 2008), where 10 adult (fully clitellate) *E. fetida* were added to a polypropylene

container half filled with a control soil and a treatment. Immediately prior to the addition of the worms, the divider was removed and a perforated polypropylene lid sealed the container to prevent escape. *E. fetida* were added at the mid-line of the container where the control soil and treatment met and after 48 h incubation under a 16:8 light:dark cycle at 22^{o}C the divider was replaced between the control and treatment and the number of worms present on each side was counted. For Kirby Clay and Warialda Loam, 250 g was used for the control and treatments, respectively, while 350 g was used for all Kirby Sand treatments. Avoidance was calculated using the equation:

$$\boldsymbol{Avoidance} = \left(\frac{\boldsymbol{n_C - n_T}}{\boldsymbol{N}}\right) \quad \textbf{(3)}$$

where n_C is the number of earthworms present on the control side after 48 h, n_T is the number of earthworms present in the treatment side after 48 h and N is the total number of worms added at the beginning of the treatment period. Based on Equation 3, an equal number of earthworms in the control and treatment side is equivalent to no avoidance. Where there were more earthworms present on the treatment side to give a negative equation, this was also given a value of 0 in that no avoidance of the treatment was deemed to have occurred.

Positive controls included the addition of boric acid (H_3BO_3) at a rate of 750 mgkg^{-1} dry soil, which were compared with control soils, containing MWOO-amended soil.

3.5.5 Earthworm (*Eisenia fetida*) mortality, growth and reproduction

The earthworm mortality, growth and reproduction assay was adapted from OECD chemical testing protocols 207 and 222 (OECD 1984, OECD 2004). The three assessments were combined within the same container, with the mortality and weight of adult worms (*Eisenia fetida*) determined 28 days following the addition of 10 adult (fully clitellate) *E. fetida* to 500 g of soil treatments in glass jars, incubated under a 16:8 light:dark cycle at 22^{o}C. Prior to weighing, worms were placed in a Petri dish containing a filter paper moistened with 2 mL deionised water and allowed to depurate for 24 h. After counting and weighing adult worms,

the soils were replaced in their respective glass jars and re-incubated for a further 28 days under the same conditions before counting the number of juveniles and unhatched cocoons present in the treatments. Unhatched cocoons were identifiable as they sank in a beaker of deionised water.

At weekly intervals throughout the duration of the incubation, worms were fed with 5 g of autoclaved horse manure and the soil moisture was maintained at 60 % MWHC with high purity (18 MΩ.cm) water. Glass jars were covered in perforated polyethylene film to prevent the escape of worms.

3.5.6 Wheat (*Triticum aestivum*) seedling emergence and growth

The assessment of seedling emergence and growth was undertaken using the Axe variety of wheat (*Triticum aestivum*) following the OECD 208 protocol (OECD 2006). For each replicate sample, seven wheat seedlings were evenly distributed in a glass jar containing 200 g of soil and planted at a depth of 0.5 cm, oriented so that the shoots would grow directly upwards. Seeds were lightly covered with soil, watered in with 4 mL of high purity (18 MΩ.cm) water and incubated within a purpose-built plant growth chamber under a 16:8 light:dark cycle (intensity > 75 Wm^{-2}) at 21^{o}C. After 4 seedlings (> 50%) of seedlings emerged within the control soils, the seedlings were allowed to grow for a further 14 days, after which the number of emerged seedlings were counted and the wheat was harvested by cutting the stems with sharp scissors at the soil surface. The seedlings were then freeze-dried at -50^{o}C to a constant weight and the dry biomass of the tissue was recorded.

Soils were maintained at 60 % MWHC with the addition of high purity (18 MΩ.cm) water every second day.

3.6 Statistical analysis

The majority of statistical analyses related to the comparison of treatment means against the relevant control sample, which was undertaken by a one-way analysis of variance (ANOVA).

The statistical significance was set at p <0.05, with Dunnett's multiple comparison test used to verify the differences between the treatment and control groups.

TRFLP data was analysed with PRIMER v6 mulitvariate statistics program. Similarities between microbial community structures were determined using a Bray-Curtis algorithm on 4th root transformed abundance data. Cluster plots were generated using the group average method and the significance of grouping tested with a SimProf routine. The effects of specific treatments on community structure were tested by 1 or 2-way analysis of molecular similarities (ANOSIM). Principal component analysis (PCA) was similarly used to group the community types.

The diversity of each microbial domain was assessed using Shannon's diversity index (H'), a commonly used ecological measure for assessing abundance and distribution of a community and was calculated from the relationship:

$$H' = \sum_{i=1}^{S} p_i \ln(p_i) \qquad (4)$$

where S is the number of species in the community and p_i is the relative abundance of species i.

Statistical analyses were undertaken using SigmaPlot v12.5 (Systat Software Inc. 2013).

4 Results and Discussion

4.1 Chemical assessments

4.1.1 Effect of time and soil on batch sorption of selected organic contaminants

Sorption of organic contaminants to soil OC through hydrophobic mechanisms is an important mechanism, especially for unionised contaminants, with an increasing %OC of a soil generally leading to an increase in its sorption capacity (Della Site 2001, Franco and Trapp 2008). Kirby Sand and Warialda Loam have a similar and low %OC, so hydrophobic sorption mechanisms are likely to be relatively weak in both soils.

The extent of sorption of a contaminant to soil is also dependent on the physicochemical properties of the contaminant, which is highly dependent on the physicochemical properties of the soil environment. For example, the pH of the soil can have an important effect on the properties of contaminants with ionisable functional groups. When the pH is within 2 pH units of the acid-base dissociation constant (pK_a) value of a contaminant it can dissociate (or ionise), with 50% of the contaminant in its ionised state when the pH is equivalent with its pK_a value (Della Site 2001, Franco and Trapp 2008). For contaminants containing an acidic functional group, such as MCPA and BPA, increasing the pH above their pK_a will lead to a greater extent of ionisation until it is completely ionised 2 pH units above its pK_a. Conversely, decreasing the pH below its pK_a value will decrease the extent of ionisation until the contaminant is fully unionised 2 pH units below its pK_a. For contaminants containing a basic functional group, such as thiabendazole, the opposite case is true. When ionised, basic functional groups have a positive charge and acidic functional groups have a negative charge, which has important implications for interactions of the contaminant with charged functional groups within the soil. For example, electrostatic sorption of contaminants containing a cationic functional group to negatively charged clays can mean contaminants with ionised cationic functional groups can associate more strongly with soils with high clay content compared with the unionised functional group (Della Site 2001, Franco and Trapp 2008).

With respect to the physicochemical properties of the selected compounds, thiabendazole contains a weakly basic imidazole nitrogen with a pK_a of 4.17, which means that a relatively small proportion of the thiabendazole present would have been positively charged in the Kirby Clay and Kirby Sand soils only. MCPA, with an acidic carboxylic acid group with a pK_a of 3.73 would have been completely in its ionised form for all soils. The weakly acidic BPA, on the other hand, with pK_a values much greater than all of the soil pH values would have been unionised for all treatments.

Of the 3 soils, Kirby Clay has the highest clay content along with an associated high cation exchange capacity (CEC), which is a measure of a soil's ability to associate with positively charged cations, and would therefore be expected to have a high affinity with positively charged functional groups. This, along with the greater affinity of Kirby Clay for unionised contaminants due to a high %OC, means the comparatively high K_d values for thiabendazole were expected (Figure 3). While Warialda Loam also has a high clay content it has a low CEC, comparable with Kirby Sand, which has a considerably smaller fraction of clay (Table A1). This, along with the low %OC of Warialda Loam and Kirby Sand, means sorption of contaminants would be expected to be correspondingly weaker. Although Warialda Loam and Kirby Sand were similar with respect to their %OC and CEC, the affinity of thiabendazole and MCPA was in the order Kirby Clay > Kirby Sand > Warialda (Figures 3 and 4). This, however, was not the case for BPA where there was a higher degree of variability in K_d values obtained with the greatest K_d values noted in the Kirby Clay and Warialda soils, especially after the soils were incubated for 3 and 9 months (Figure 5).

Of the three organic contaminants assessed in the batch sorption experiments, MCPA had the lowest affinity to all three soils while thiabendazole had the highest K_d values (Figures 3 and 4). As MCPA was a predominantly or wholly negatively charged species in all three soils, the influence of hydrophobic interactions with soil OC would have been substantially reduced. It should be noted that for MCPA in Warialda Loam treatments, the amount of MCPA in solution at the conclusion of the batch sorption experiments were >80 % of the added amount, which suggests there needs to be some degree of caution employed in the absolute K_d values for MCPA in this soil (OECD 2000a). Its K_d values in Warialda Loam (<1 L/kg), however, suggest that MCPA is likely to be highly mobile in Warialda Loam due to their weak association. Even K_d values of ~10 L/kg in the Kirby Clay demonstrating a low affinity with this soil is consistent

with previous work, especially where low amounts of organic matter is present (Shang and Arshad 1997, Weber et al. 2004, Cabrera et al. 2011). Because of this high mobility in soils, MCPA is a pesticide of concern to the USEPA (Extoxnet 2017). Conversely, thiabendazole was strongly associated with the test soils, with K_d values ranging from 32-1768 L/kg which is also consistent with previous literature (Cayley and Lord 1980)

The K_d values obtained for BPA, on the other hand, were more variable than those of MCPA and thiabendazole. All Kirby Sand treatments had consistently low K_d values for BPA ranging from 5 to 35 L/kg (Figure 5). As may be expected from MCPA and thiabendazole K_d values, the K_d values of BPA were greater in the Kirby Clay treatments (range 52-456 L/kg) than the Kirby Sand treatments (range 5-36 L/kg). The K_d values of BPA in Warialda Loam, however, were not expected based on the relative properties of the soils with the K_d values equivalent to those observed in the Kirby Clay treatments (Figure 5). Previous studies have found a strong relationship between sorption of BPA and the OC content of soils (Fent et al. 2003, Ying and Kookana 2005, Zeng et al. 2006), while this did not seem to be the case when comparing K_d values in Kirby Clay and Warialda Loam. BPA has also been previously found to have a low to moderate affinity with soils (Fent et al. 2003, Ying and Kookana 2005, Zeng et al. 2006). As BPA was unlikely to be ionised at the experimental pH of the soils, ionic interactions with clay in the Warialda Loam is unlikely to have played a role in its enhanced sorption. The independence of BPA sorption from soil pH has also been established in other work (Ying and Kookana 2005). The biodegradation of BPA in soils has been found to be relatively rapid with a half-life of only a few days, compared with the more stable MCPA and thiabendazole (Caux et al. 1995, Cousins et al. 2002, Walters et al. 2010). It is therefore possible that the observed high K_d values for BPA, and the variability associated with them, in the Warialda Loam and Kirby Clay may have been in part due to degradation of BPA during the 24 h batch equilibrium shaking period. In this case, degradation of BPA would lead to lower measurable concentrations in solution, giving an apparent higher K_d value (Equation 2). An increase in apparent K_d values for BPA after the soils were incubated for 3 and 9 months may indicate that this incubation period allowed enough time for maturation of the soil microbial community to rapidly degrade BPA.

With respect to the effect of incubation time on the sorption of the spiked compounds the K_d values of thiabendazole generally increased, particularly for the treatments Kirby Sand and

Kirby Clay incubated for 9 months (Figure 3). Conversely, MCPA had a generally reduced affinity with Kirby Clay although this was only in a few treatments, as well as the MWOO control.

4.1.2 Effect of microplastic and MWOO on batch sorption of organic contaminants

Within each time period and soil type there were also significant differences found between the treatment K_d values. A notable exception was observed for thiabendazole in Kirby Sand, for all treatments, and Kirby Clay, for all treatments in the absence of MWOO, for all soil incubation periods (Figure 3). There were, however, no clear trends relating to the addition of microplastics or MWOO to the soil and the effect on K_d values. For example, the addition of HDPE increased the K_d values of thiabendazole relative to respective controls in Kirby Sand after 3 months incubation (p=0.016), although not after 9 months incubation or in unincubated soils (Figure 3). In Kirby Clay, the K_d value of thiabendazole significantly increased with the addition of HDPE after 9 months incubation in soil only (p< 0.001), while there was no difference in K_d values following addition of MWOO for the same incubation period. In the case of BPA, HDPE increased the K_d value after 3 months incubation in Kirby Sand (p< 0.001) and Kirby Clay (p< 0.001) although the K_d values decreased in Warialda Loam after 3 and 9 months incubation (p< 0.001). HDPE is often used in environmental passive sampling devices for integrative monitoring of water samples due to the ability of HDPE to effectively capture a range of organic contaminants, although it is more effective with contaminants with a high log K_{ow} value (Sacks and Lohman 2011, Aminot et al. 2017). Of the three plastics selected for this assessment, HDPE has been previously demonstrated to have the highest affinity for organic chemicals (Rochman et al. 2013) although this was not reflected in the results, where K_d values in the presence of HDPE were comparable with the other plastics treatments and controls (Figures 3-5). Without any consistent trends in the batch sorption experiments relating to the presence of HDPE, PET or PVC, it is not possible to conclude that the presence of the microplastics in the soil, both with and without MWOO, enhanced the degree of sorption for the selected organic contaminants at the rates added.

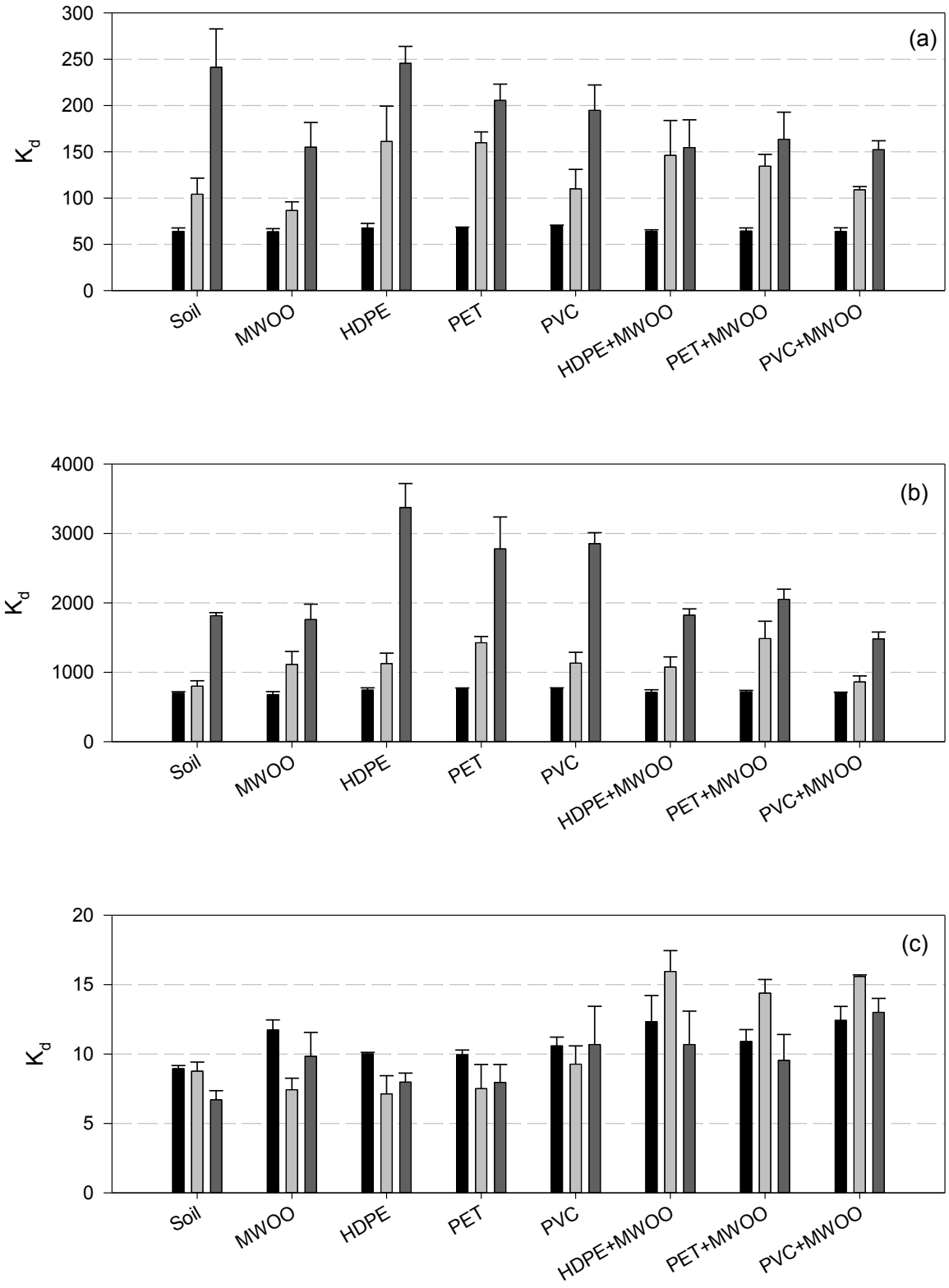


Figure 3. The sorption coefficient (K_d) determined from batch sorption experiments for thiabendazole in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (■) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples).

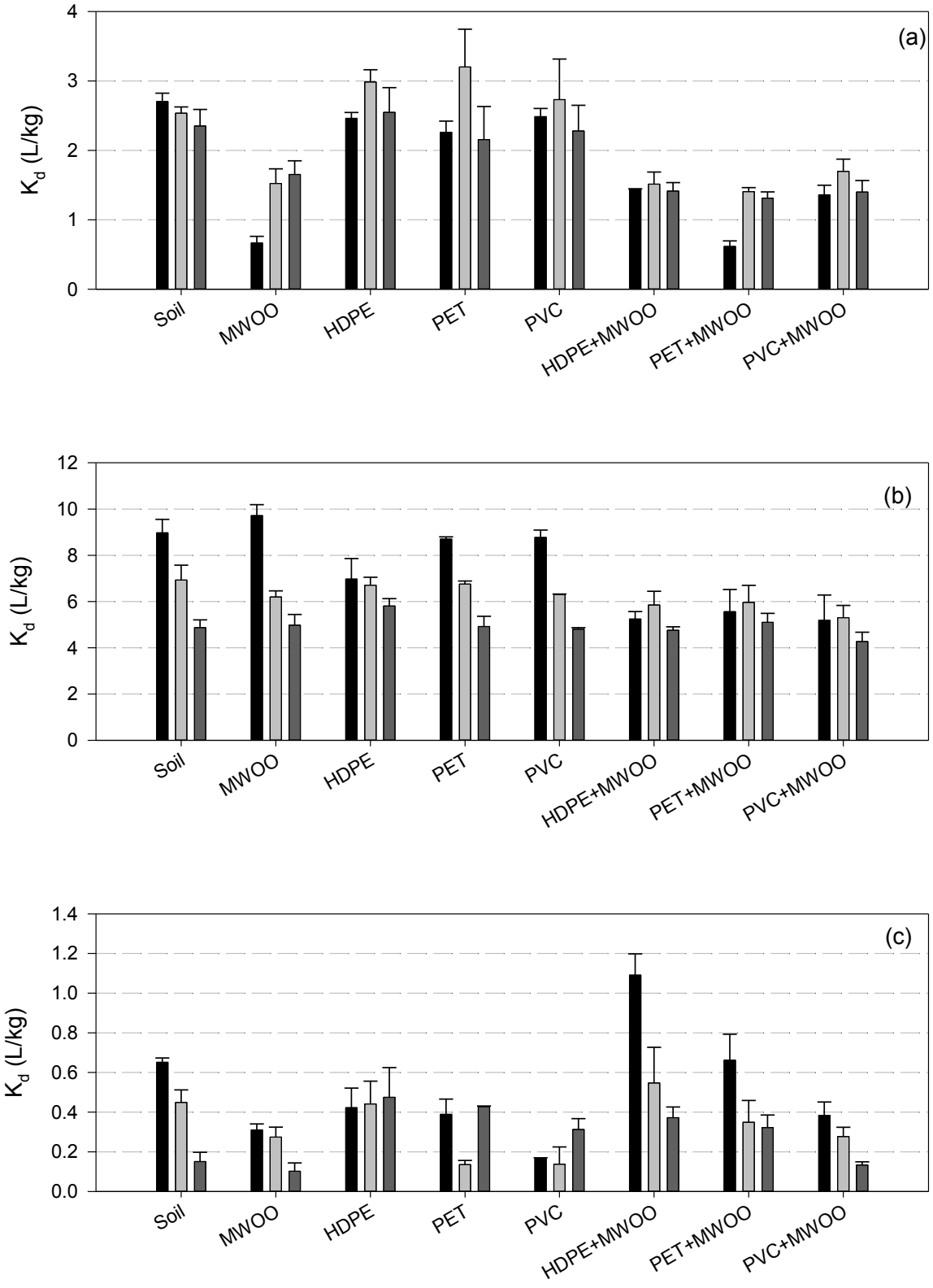


Figure 4. The sorption coefficient (K_d) determined from batch sorption experiments for MCPA in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (■) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples).

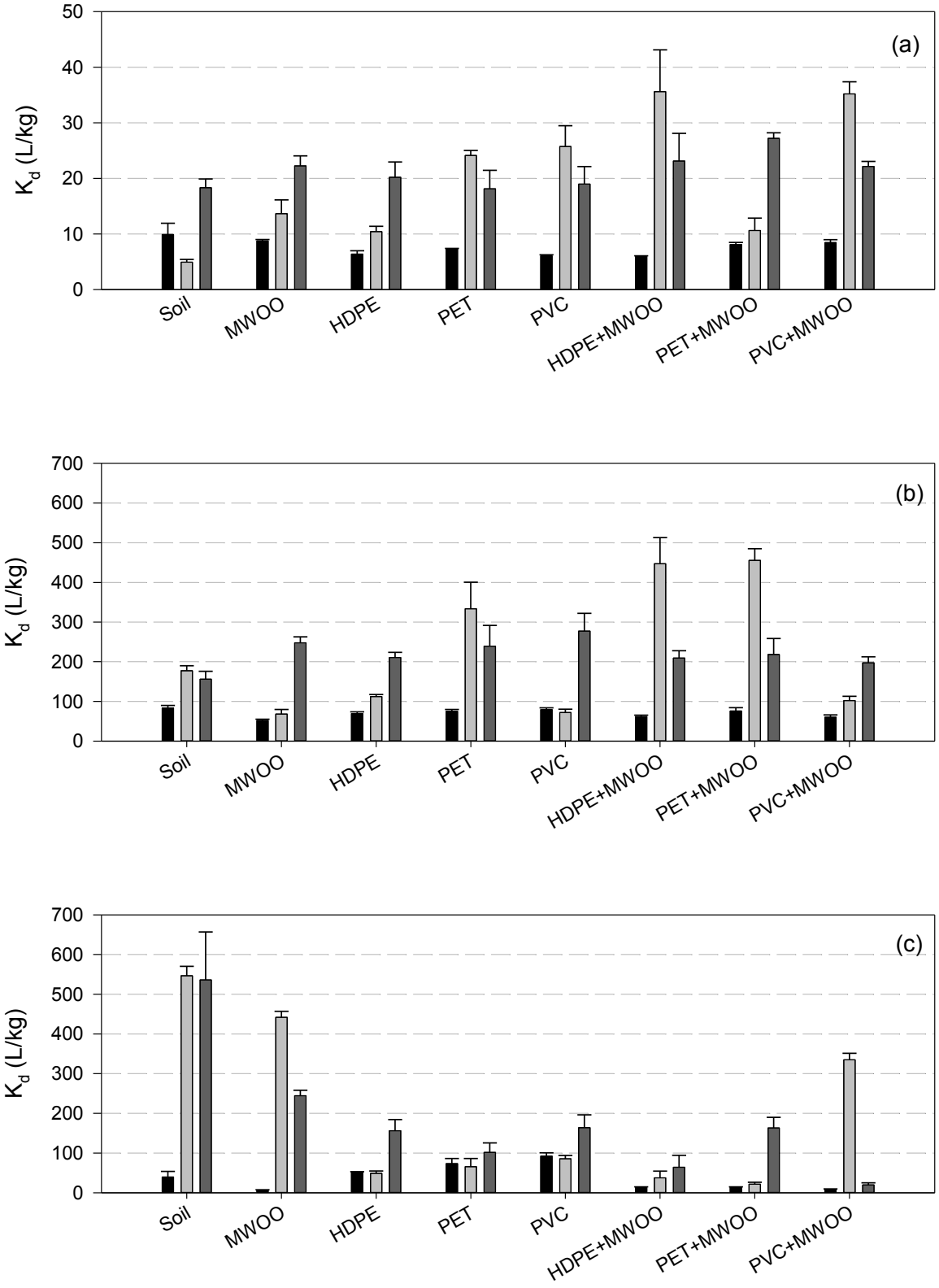


Figure 5. The sorption coefficient (K_d) determined from batch sorption experiments for BPA in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (■) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples).

4.1.3 Contaminants in soil solutions - effect of incubation on soil solution pH and electrical conductivity

The acidity of soil solutions, measured from pH values, were found to vary throughout the incubation period with the addition of MWOO having a negligible effect on pH values of the soils (Appendix D). The EC of the soils were found to increase in the Kirby Sand and Kirby Clay, while decreasing during incubation in the Warialda Loam. Addition of MWOO to soils caused an increase in soil EC, while having no apparent effect on soil pH (Appendix D). The addition of microplastics, however, to the soils had minimal effect on these two soil parameters, even at the highest rates (Tables D1 and D2).

4.1.4 Effect of the addition of MWOO on soil solution trace metal concentrations

Trace metal concentrations ranged from ng/L to mg/L in the soil solutions both in the absence and presence of MWOO (Figures 6-9, Appendix E).

Concentrations of the majority of metals in soil solutions for unincubated (0 months) soils were similar in soil only and MWOO controls (Figure 6; Tables E1 and E2). Some metals, such as Fe, As (Kirby Sand) and Zn (Kirby Clay), were however significantly lower (p <0.05) in MWOO control soil solutions compared to their respective soil controls (Figure 6; Tables E1-E3). The significantly lower concentrations of these metals in samples containing MWOO could be due to their presence as insoluble (non-labile) water soluble phases in MWOO through greater partitioning of metals into non-labile fractions possibly associated with Al-, Fe-, and Mn-oxides and organic matter present in soils and MWOO. Conversely, concentrations of metals such as Fe (Warialda Loam), Cu, Zn (Kirby Sand and Warialda Loam) Mn, Co, Ni and Cd (all soils) in soil solutions were significantly (p <0.05) higher in unincubated MWOO controls relative to soil only (Figure 6; Tables E1 and E2). This may be due to their association with readily water soluble phases associated with MWOO that can be easily mobilised into soil solutions. The fate and lability and, therefore, potential bioavailability of metals released from MWOO (and associated microplastics) is highly dependent on soil physical and chemical properties, including pH, Fe/Mn-oxide, %OC, CEC and anion exchange

capacity (Adriano 1986, McBride 1994, Sumner 2000, National Research Council 2003, Hooda 2010). Higher metal concentrations were usually found in soil solutions from the acidic Kirby Sand soil followed by more organic and clay rich Kirby Clay and alkaline Warialda Loam, independent of microplastics addition (Figures 6-8; Tables E1-E3). The fate and availability of metals in soils is known to be influenced by physical and chemical properties (Adriano 1986). For example, cationic metals such as Cu, Ni, Pb and Zn often show increased solid phase partitioning and decreased lability with increasing clay content, cation exchange capatity, soil pH, organic carbon and Fe/Mn oxide contents in soils (McBride 1994, Sumner 2000, Hooda 2010).

With respect to the effect of soil incubation time on soil solution concentrations of selected metals, the majority of metals were found to be at similar or lower concetrations after 3 months incubation and decreased further from 3 to 9 months incubation (Figures 7 and 8). Conversely, the concentrations of some metals, such as Cu (Kirby Clay only), Pb (KS Kirby Sand only), Mn, Ni, Zn (Kirby Sand and Kirby Clay only and MWOO control) and As (Kirby Sand and Kirby Clay, Warialda Loam only and MWOO) were found to be significantly higher ($p< 0.05$) in soil solutions after 9 months compared to 3 month incubation (Figures 6 and 7). The observed increase in Mn concentrations in soil solutions of soil only and MWOO-amended soil controls after 9 months compared to 3 months incubation suggests there may have been the presence of anaerobic site in soil and soil+MWOO treatment, despite ongoing aeration and mixing of incubated soils to prevent this from occurring e.g. reductive dissolution of Mn phases following reaction such as:

$$MnO_2(s) + 4H + 2e^- \rightarrow Mn^{2+}(aq) + 2H_2O(aq) \quad (4)$$

Anaerobic sites may still have occurred in soil clumps during incubation in some soil treatments, especially clay rich Kirby Clay and Warialda Loam which tended to clump in incubation containers. The influence of anaerobic conditions on metal contaminant fate and lability has been well studied, especially for paddy soils (Adriano 1986, Kirk 2004). A reduction in redox potential as a result of anaerobic conditions has been shown to cause changes in oxidation states, leading to the formation of low-soluble minerals phases and reductive

dissolution of Fe and Mn phases. This can result in the release of associated metals into solution (Masscheleyn et al. 1991, Amrhein et al. 1994, Chuan et al. 1996, Kirk 2004). In this study, anaerobic conditions within soil clumps may have resulted in reductive dissolution of Fe and Mn phases in some soil treatments and consequently the release of associated metals such as Zn, As, Cu and Pb into soil solutions.

4.1.5 Effect of microplastics on trace metals in soil solutions

The addition of individual plastics at 0.1 % w/w into soil and MWOO in unincubated treatments resulted in similar or significantly lower ($p \leq 0.05$) concentrations of metals such as Fe, Cr, Cd, Mo, Cu, and Sn in soil solutions (Figure 9; Tables E4-E12). The lower metal concentrations in soil solutions of some soil treatments suggests the added microplastics were able to remove some metals into fractions, possibly through adsorption or precipitation reactions, that could not be readily released into soil solutions. Holmes et al. (2012) reported that metals such as Cr, Co, Ni, Cu, Zn, Cd, and Pb had a high adsorption affinity to virgin and field collected polyethylene pellets that could have implications for transport and bioaccumulation of metals. In contrast, metals such as Mn, Co, Ni, Zn, and Pb could be found at significantly ($p < 0.05$) higher concentrations in soil solutions of some treatments in unincubated soils (Tables E4-E12). This observed increase in soil solutions concentrations may be due to their association with readily soluble phases on microplastics, related to their manufacturing and handling, that can be mobilised into soil solutions.

The availability to soil organisms of metals released from or because of microplastics and MWOO will depend on the physical and chemical properties of soils, such as pH, Fe/Mn oxide and organic carbon contents and cation and anion exchange capacities (Adriano 1986, National Research Council 2003, Hooda 2010). There was observed high variability in metal concentrations in soil solutions from incubated soils following addition of increasing amounts of individual plastics to treatments (Tables E4-E12). In general, metal concentrations in soil solutions from all treatments were similar or significantly ($p < 0.05$) lower following addition of increasing amounts of individual plastics compared to the lowest addition rate for the different soil incubation periods (Tables E4-E12).

The effect of chemical ageing, through incubation in soils over time, on the bioavailability and toxicity of metal contaminants in soils is well known (Ma et al. 2006a, 2006b, Oorts et al. 2006, Wendling et al. 2009). When metal contaminants are added into soils, there is an initial fast reaction for sorption onto solid phases. This initial fast reaction is followed by slower reactions that can remove metals from labile pools into a pool or pools from which desorption is slow. The immobilised metal can often still be measured using conventional techniques for bulk chemistry analysis but has essentially become unavailable to soil biota. Consequently, lability and toxicity of metals in soils can decrease with time as the amount of actual metal exposure is reduced.

The total concentration of a metal in soil or soil solution is frequently a poor indicator of its potential biological availability and, hence, toxicity (Lock and Janssen 2003, Fendorf et al. 2004). It is widely recognised that a number of physical, chemical and biological properties such as the type of species, soil properties, ageing processes and speciation play a major role in determining the fate and effects of metal contaminants in soils (Adriano 1986, National Research Council 2003, Hooda 2010). In this study, metal concentrations in soil solutions represent the most readily available pool of metals in soil to organisms. The bioavailability, and subsequent toxicity, of metals in soil solutions is highly dependent on the physical and chemical properties of the soil pore water and the uptake mechanisms and detoxification strategies of exposed soil organisms. For example, complexation of Cu in soil solution by dissolved, colloidal organic matter is well known to reduce its lability and potential bioavailability (Waller and Pickering 1990). The ecotoxicological effects of metals associated with colloidal particles, however, is poorly known. Colloids are known to have an important role in the bioavailability and transport of inorganic and organic contaminants in soil (Lombi et al. 2003, Bin et al. 2011).

4.1.6 Effects of microplastics on organic chemicals in soil and soil solutions

With respect to organic contaminants, a suite of 39 compounds screened for in pore water and soil solvent extracts were not detected in any of the extracts. An assessment of respective recoveries from the soil and MWOO matrix, representing the most complex matrix for analytical recoveries, ranged from very low for the pyrethroids up to >100 % for BPA and a number of the pesticides (Table F1). This group of organic compounds represents a diverse range of physicochemical properties in terms of, for example, water solubility, hydrophobicity and ionisable functional groups. All of these physicochemical properties have a bearing on the degree of contaminant association with soil and its subsequent release into an extractive solution. Also, these physicochemical properties play a role in contamination interaction with soil matrices during ionisation for mass spectrometry analysis, which can affect the measurable analytical response (Niessen et al. 2006).

A previous assessment of organic contaminants in MWOO revealed a number of organic contaminants both in the solid material, as well as in leachates of soil solutions (NSW OEH 2015). Compounds such as DEHP, MCPA, BPA and phenol were detected with mean concentrations ranging from 550 μg/kg for MCPA up to 124 mg/kg for DEHP in MWOO. DEHP and other phthalates, however, were not detected in MWOO in the present study (Figure B3). The measured concentrations of these organic chemicals also had a high degree of variability around mean values (relative standard deviation >58 %) in the NSW OEH study. The highest frequency of concentrations for dioctyl phthalate, for example, in MWOO were at low mg/kg concentrations despite its mean value being 100 mg/kg. In the MWOO leachates, organic chemicals such as MCPA and 2,4-D were at low to mid-μg/L concentrations, with relative standard deviations also being similarly high (NSW OEH 2015).

In the present study, MWOO was added to the soil at a rate of 1 % w/w, which would have the effect of diluting the concentrations of any organic contaminant within the MWOO by 100-fold. Furthermore, the high variability of organic chemicals concentrations measured in MWOO in previous work can imply the material was highly heterogenous (including from batch to batch of produced MWOO), suggesting the 39 organic chemicals in the extracts and soil solutions may have plausibly been below the method limit of quantification (LOQ).

The method LOQs for the soil and soil solution analysis would suggest that is was likely that there was a comparatively low risk from the 39 compounds, where soil toxicity values would be expected in the mg/kg concentration range (e.g. Jansch et al. 2006, Hartnik et al. 2008, Weeks et al. 2012, NSW OEH 2015, Ma et al. 2017). For example, soil toxicity values have previously been estimated using criteria concentrations for the protection of ecological organisms from chemicals previously measured in MWOO (NSW OEH 2015). With the derived criteria concentrations having an assessment factor making them 1000 times less than the lowest literature ecotoxicity values, these criteria concentrations are highly conservative and the majority of them are in the mg/kg range (NSW OEH 2015). A notable exception for this was thiabendazole, which had a criteria concentration of 4.2 μg/kg, slightly higher than the method LOQ of 1 μg/kg for thiabendazole in the present study (Table F1).

It is, however, highly likely for there to be considerably greater than the 39 targeted organic chemicals in the MWOO (NSW OEH 2015). The possibility that the combination of a number of such organic chemicals, along with trace metals, may lead to mixture toxicity effects (Sousa et al. 2008, van Gestel 2012) was evaluated further using a range of soil ecotoxicity assessments.

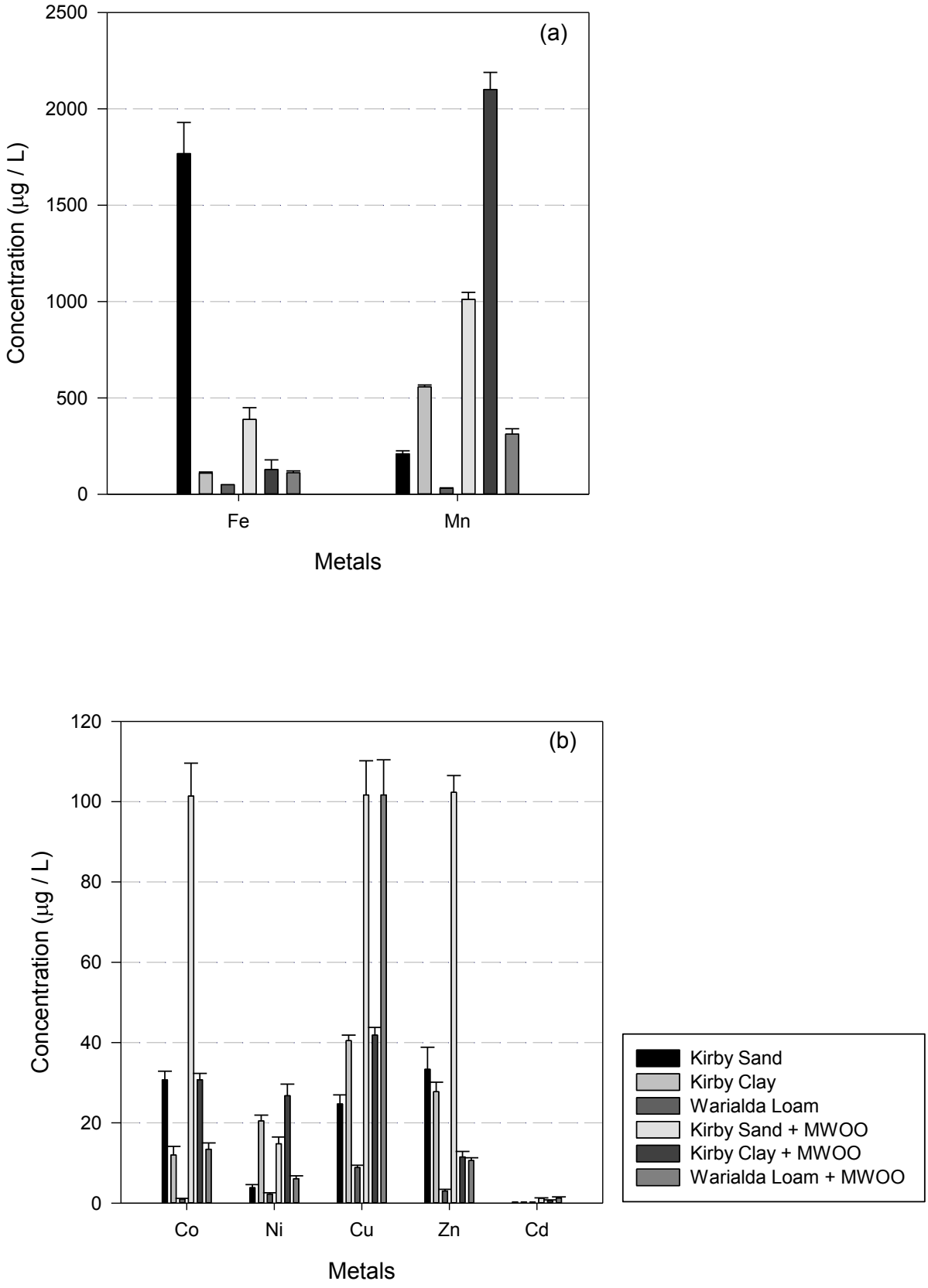


Figure 6. Selected concentrations of (a) Fe and Mn and (b) Co, Ni, Cu, Zn and Cd in soil solution from soil and MWOO-amended soil controls in unincubated treatments**.** Values represent mean ± standard deviation (n=3 samples).

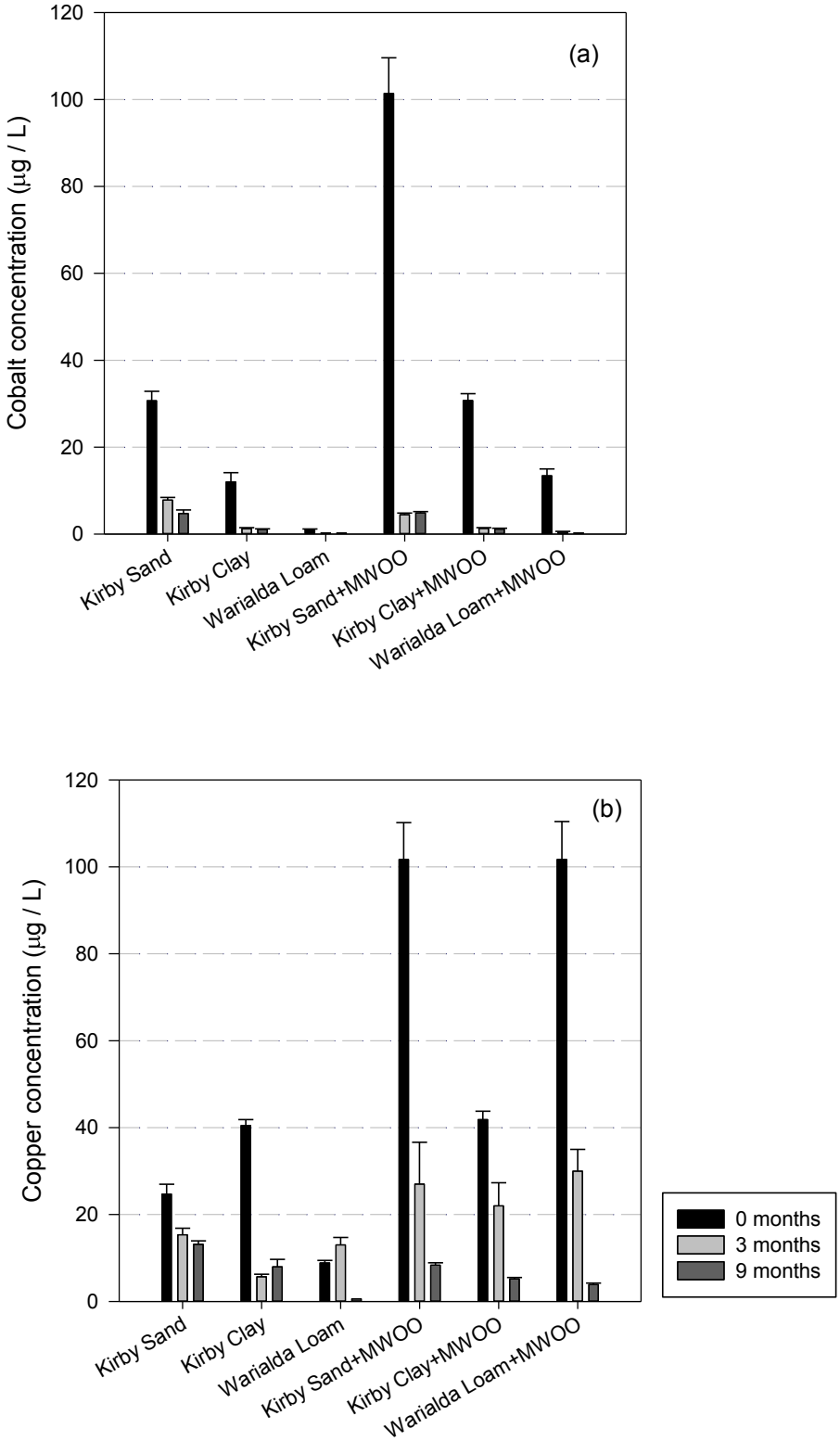


Figure 7. Examples of the influence of soil incubation for up to 9 months of soil and soil+MWOO controls on (a) Co and (b) Cu concentrations in soil solutions. Values represent mean ± standard deviation (n=3 samples).

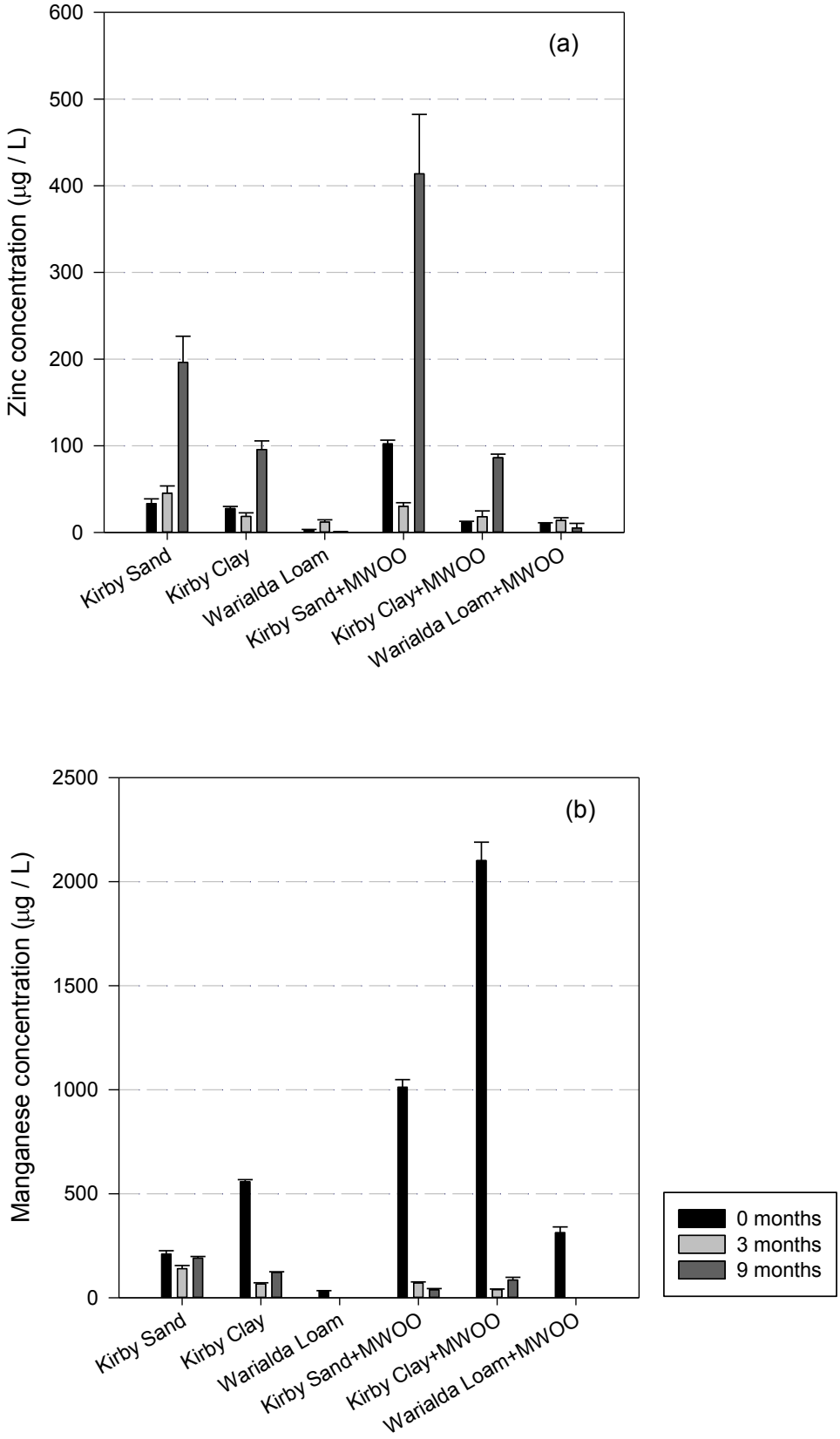


Figure 8. Examples of the influence of ageing of soil and soil+MWOO controls on (a) Zn and (b) Mn concentrations in soil solutions. Values represent mean ± standard deviation (n=3 samples).

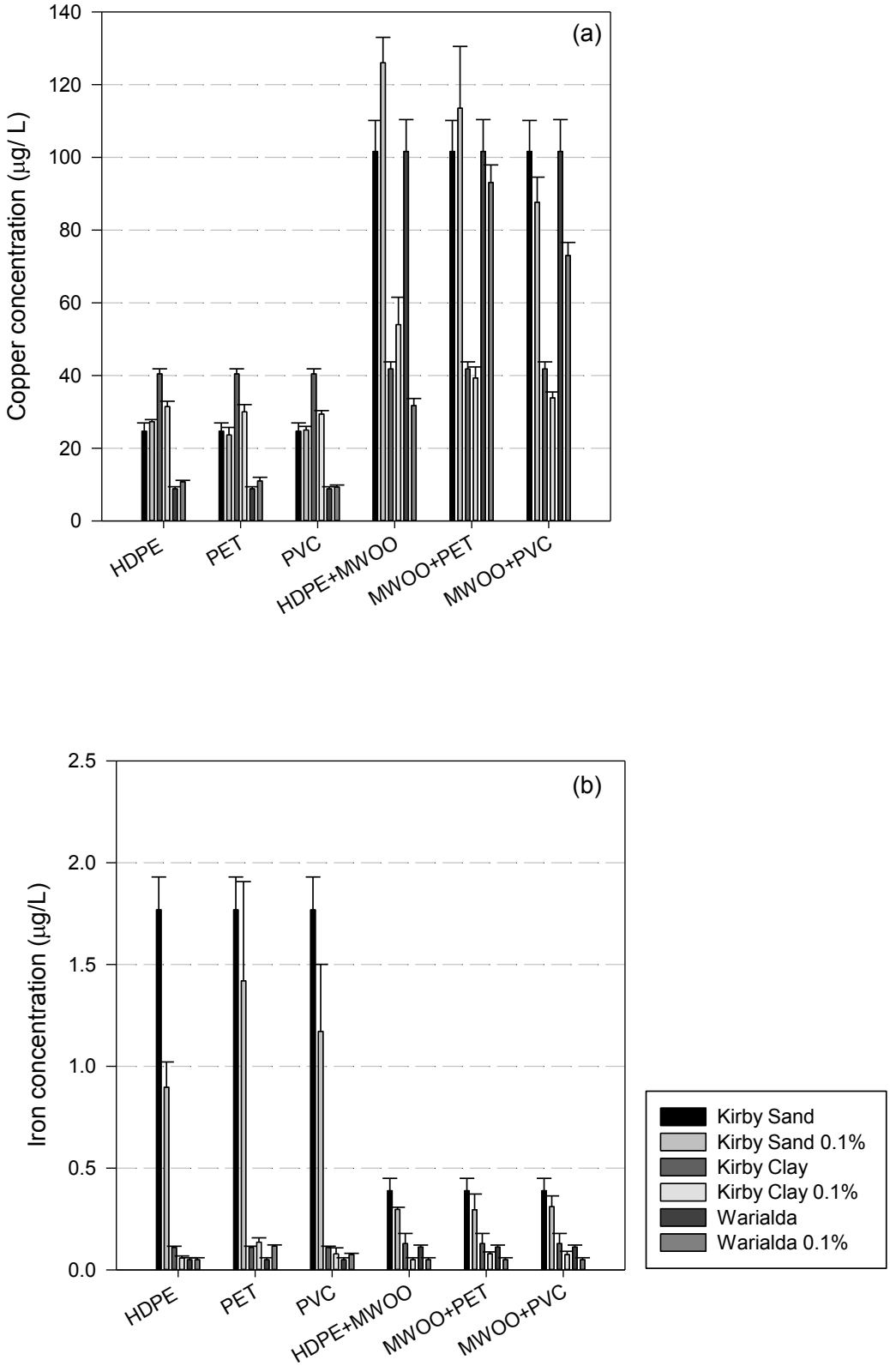


Figure 9. Example of the influence of added plastics (HDPE, PET, and PVC) at 0.1 % w/w to soil and soil+MWOO treatments in unincubated soils on (a) Cu and (b) Fe concentrations in soil solutions. Values represent mean ± standard deviation (n=3 samples).

4.2 Ecotoxicological assessments

As with the chemical analyses, there were clear differences between the selected soils in a number of the ecotoxicological assessments. For example, the biomass of the seedlings in the Kirby Clay was considerably higher compared with the other two soils, while the soil respiration, as measured in the SIR assessment, was also greatest in the Kirby Clay soil, which was expected based on its relative nutrient content (Table A1). Warialda Loam has a similar nutrient content compared with the Kirby Sand but the respiration and nitrification rates of Warialda Loam were considerably lower than the other soils (see Section 4.3.2).

The main emphasis of these ecotoxicological assays related to the effect that the microplastics had on the selected endpoints in relation to the control soil within a soil type. The effect of the time of incubation was also an important factor to consider, as the microplastics may have, for example, leached contaminants into the soil over the 9 month incubation period.

The control soil for all ecotoxicological assays was where a respective soil was mixed with MWOO, with the soil alone considered to be an additional treatment for comparison with the control. The exception to this was for the SIN and SIR assays in treatments where there was no added MWOO, in which case the soil only was used as a control.

Statistical analyses for all ecotoxicological assays are summarised in Appendix G (Tables G1-G7).

4.2.1 Soil microbial community structure

Bacteria, fungi and archaea represent the majority of total biomass living in soils and, therefore, any impacts on their community structure and function can have important implications for soil health (Silva et al. 2012, Leff et al. 2015, Dong et al. 2017).

The soil microbial community was characterised based on the diversity of the bacterial, fungal and archaeal populations present and was determined using TRFLP.

It should be noted that the samples collected for the soil microbial structure assessment were pooled from the replicate treatments. This means that the results presented are therefore indicative of general trends and could not be meaningfully statistically analysed, in terms of comparisons between treatments, due to lack of replication. It was considered that representativeness of the treatments through pooling them was more important than replication for a highly diverse and variable endpoint. The results presented here, however, still give an important indication of the potential impacts that the addition rates of the microplastics may have had on the soil microbial community structure. A quantitative assessment of the total bacterial populations in the soil are covered in the following section, along with specific functional genes (see 4.3.2 Soil microbial function).

The TRFLP analysis produces a chromatogram which separates TRFs based on fragment size and intensity, which is associated with the number of fragments of this size (Figure H1). The TRFLP data was also analysed using principal component analysis (PCA), where the TRFs are grouped according to their similarity using abundance variations. From the PCA plots, it is notable that the main driver in microbial diversity was related to soil type and incubation periods, which is evident from the grouping of these treatments (Figures 10 -12). There were a number of exceptions to this, where a lesser degree of similarity between treatments at each incubation occurred. For example, this occurred for bacteria and archaea in Warialda Loam treatments after 3 and 9 months, respectively. (Figures 10 and 12). An additional analysis of the TRFLP data by ANOSIM, however, did not suggest addition of the microplastics may have affected microbial community diversity. This analysis revealed that the main factor relating to the similarity of the microbial communities was soil type, especially for bacteria and fungi, and time, especially for bacteria and archaea, based on the r^2 values (Table 5).

Table 5. Summary of analysis of similarity (ANOSIM) for microbial communities in all treatments containing microplastics and incubated for a period of 0, 3 and 9 months.

	SOIL		TIME		TREATMENT	
	r^2	*p*-value	r^2	*p*-value	r^2	*p*-value
Bacteria	0.371	0.008	0.735	0.001	0.006	0.366
Fungi	0.598	0.001	0.235	0.024	0.098	0.1
Archaea	0.075	0.184	0.508	0.001	-0.096	0.896

The microbial diversity analysis is summarised in Figures 13 to 15. These figures show there was little change in the diversity of the bacterial species, a moderate increase in the diversity of fungi and a greater increase in archaea over the 9 months of soil incubation. Furthermore, the treatments for each respective domain were found to be similar to the MWOO-amended soil control, suggesting there was minimal effect due to the addition of microplastics (Figures 13-15). An exception was noted for the abundance of archaea in the HDPE treatment in all soils and for PVC in Kirby Sand was low relative to the MWOO control, although this was not evident in the incubated soils (Figure 15).

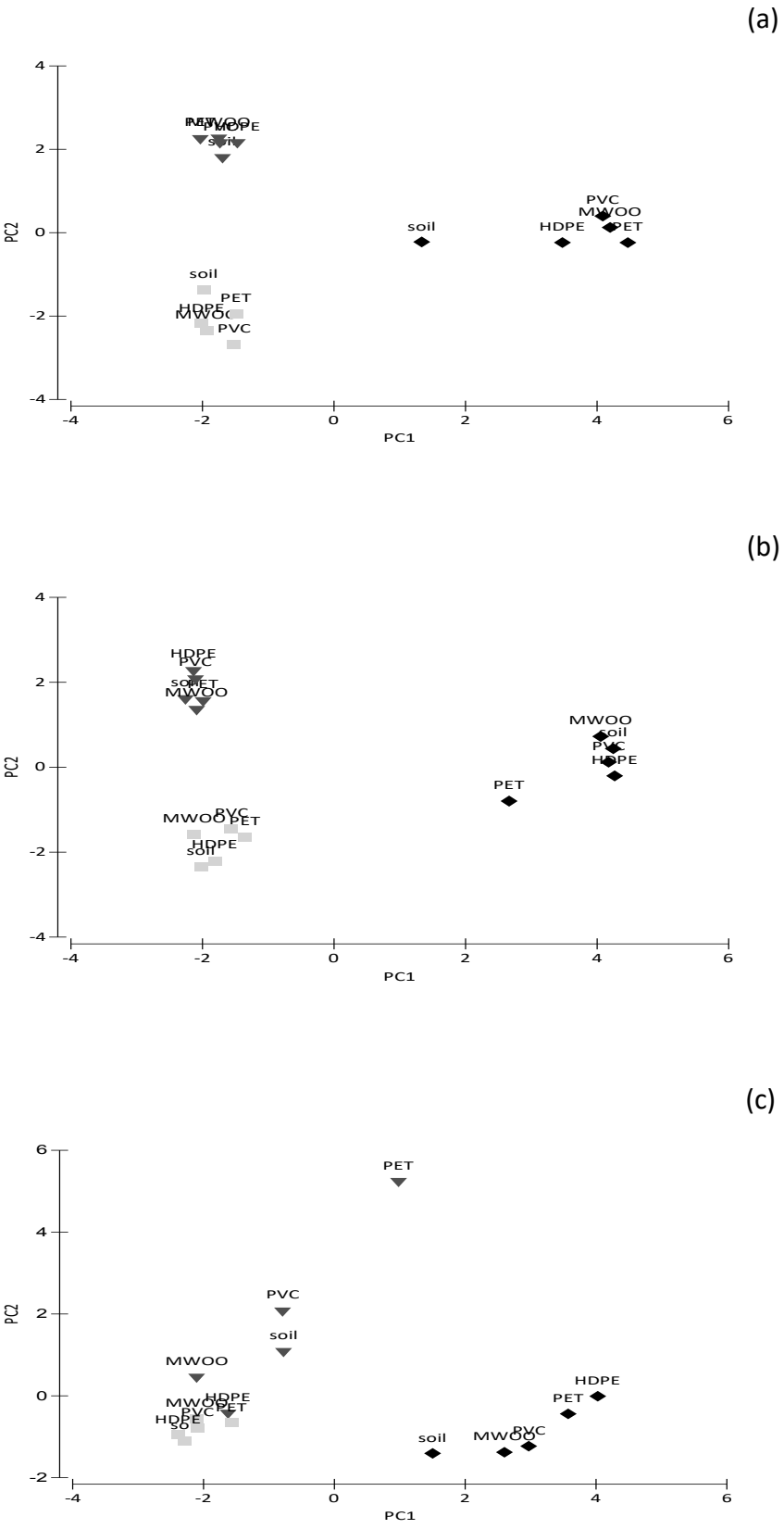


Figure 10. Principal component analysis (PCA) plots of TRFLP analysis for bacteria in soil treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (◆), 3 (▼) and 9 (▒) months incubation of the soil. Values represent samples pooled from three replicate treatments.

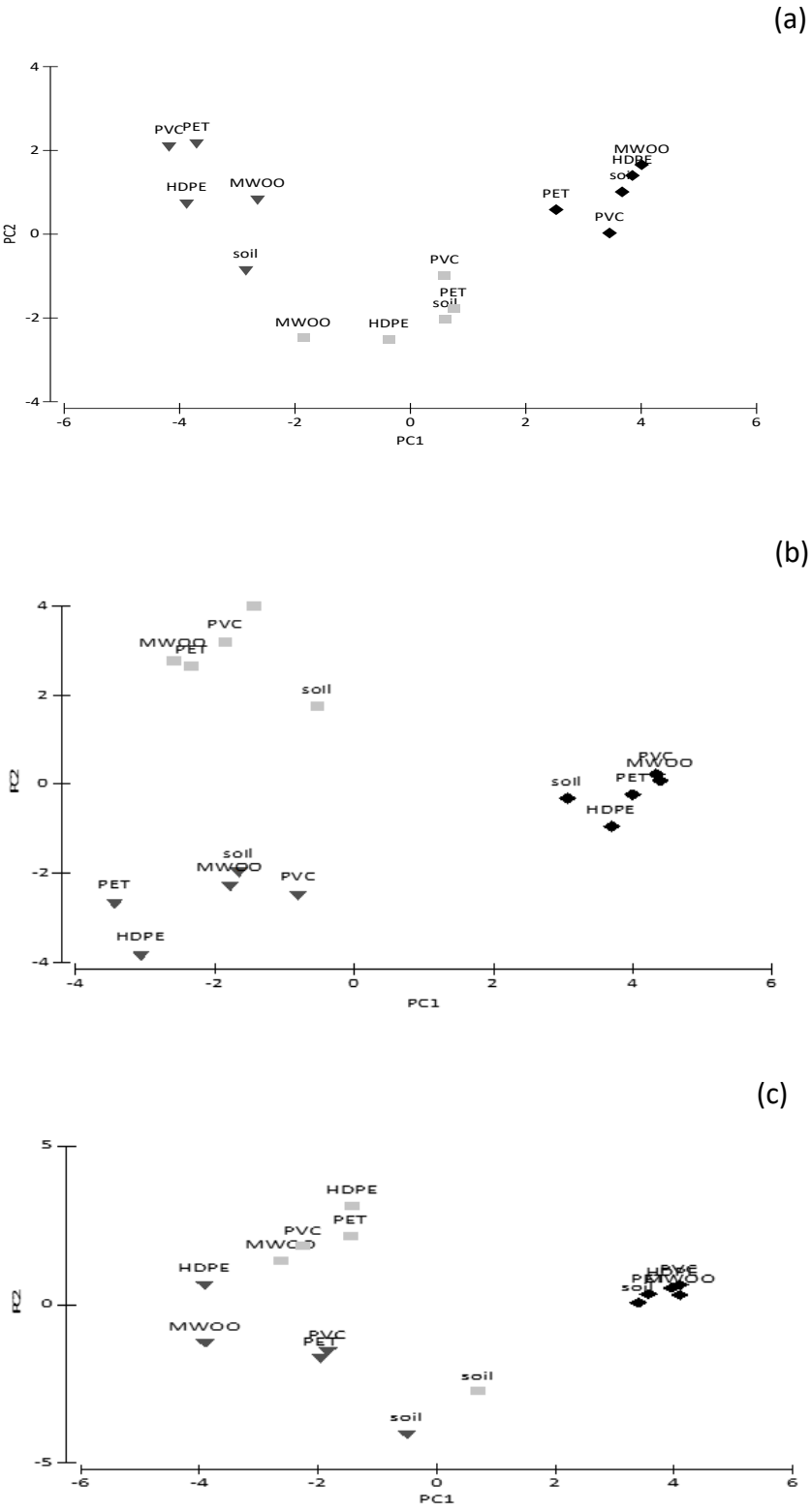


Figure 11. Principal component analysis (PCA) plots of TRFLP analysis for fungi in soil treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (◆), 3 (▼) and 9 ( ) months incubation of the soil. Values represent samples pooled from three replicate treatments.

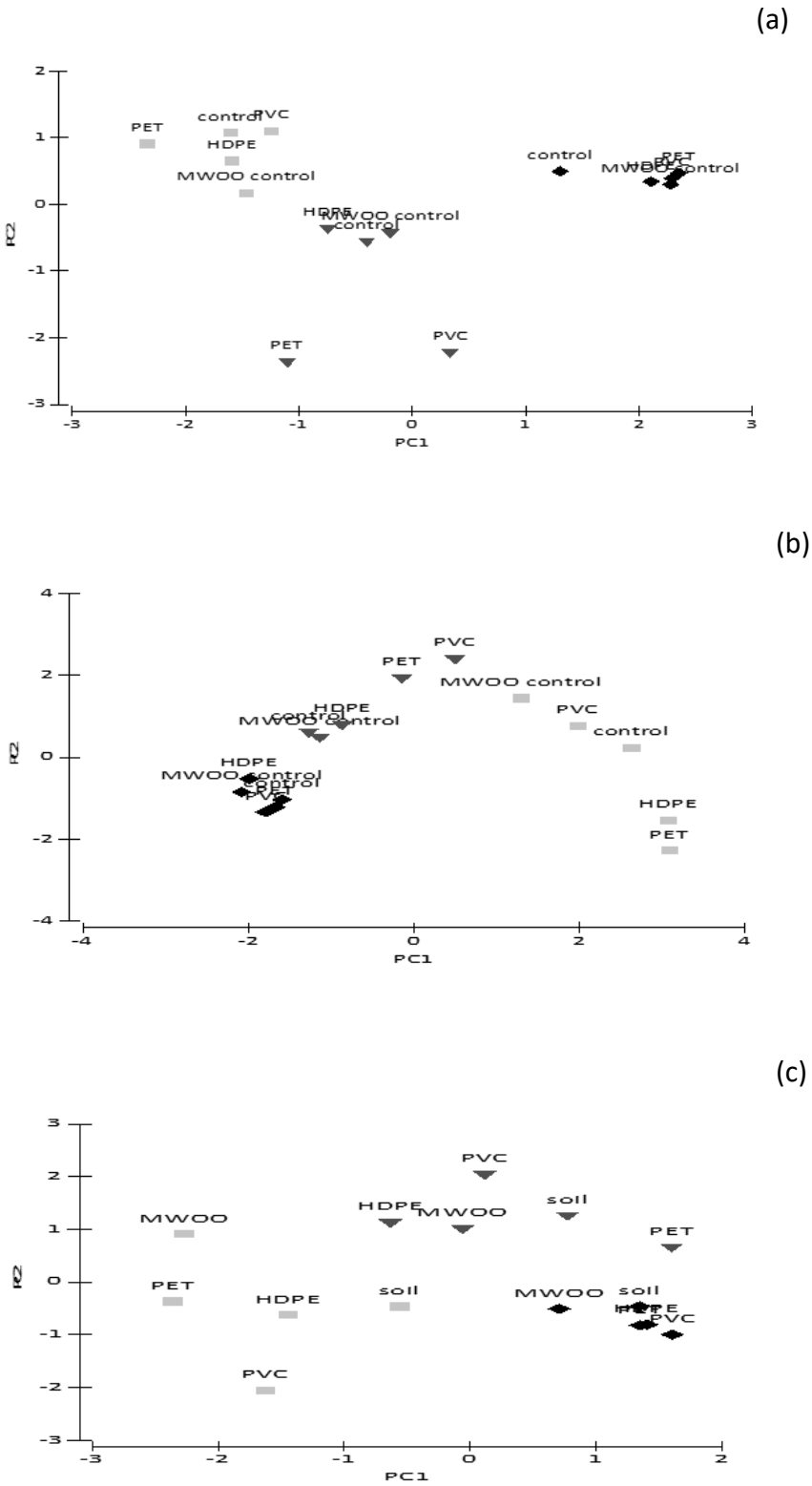


Figure 12. Principal component analysis (PCA) plots of TRFLP analysis for archaea in soil treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (◆), 3 (▼) and 9 (▩) months incubation of the soil. Values represent samples pooled from three replicate treatments.

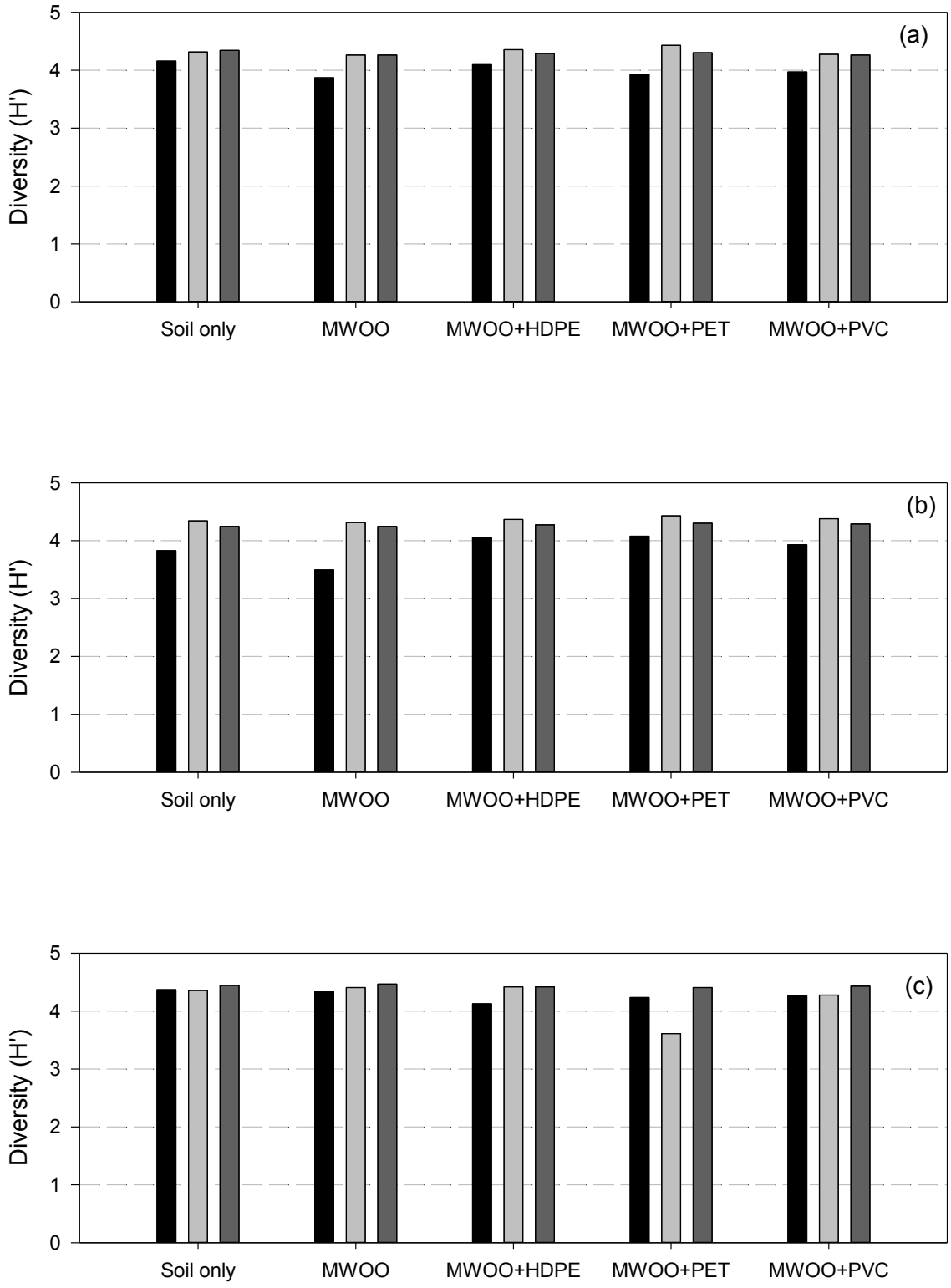


Figure 13. The diversity of bacteria, based on Shannon's Diversity Index, measured using TRFLP analysis in soil treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (■) months incubation of the soil. Values represent samples pooled from three replicate treatments.

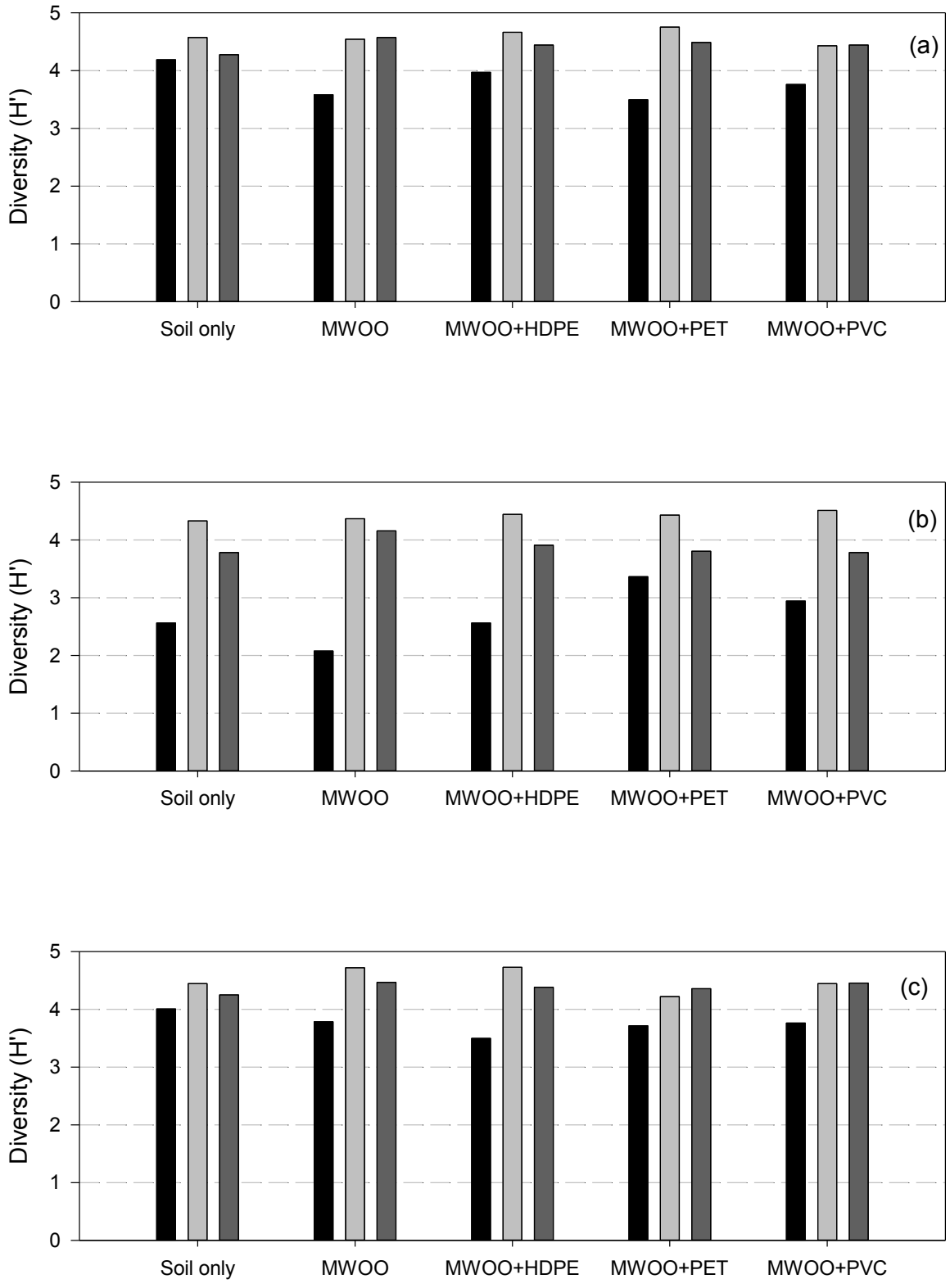


Figure 14. The diversity of fungi, based on Shannon's Diversity Index, measured using TRFLP analysis in soil treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (■) months incubation of the soil. Values represent samples pooled from three replicate treatments.

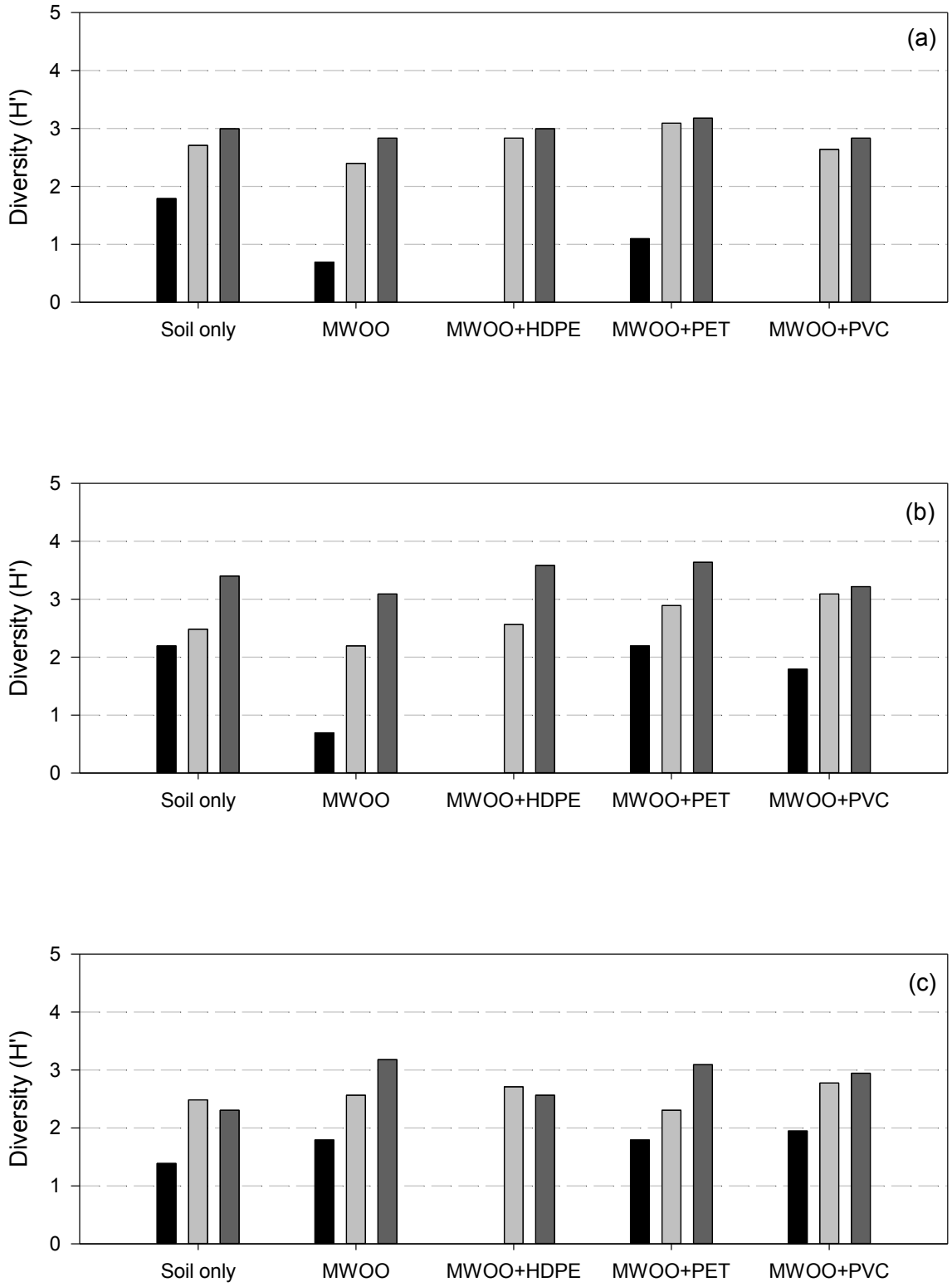


Figure 15. The diversity of archaea, based on Shannon's Diversity Index, measured using TRFLP analysis in soil treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (■) months incubation of the soil. Values represent samples pooled from three replicate treatments.

4.2.2 Soil microbial function – quantification of microbial biomass and key nitrogen cycle genes

The assessment of the effect of microplastics on the soil microbial function included substrate induced respiration (SIR), substrate induced nitrification (SIN) and quantification of the numbers of functional genes relating to nitrogen cycling, including *nifH*, *nirK* and *amoA*. Also, the total number of bacteria in the soil was estimated based on the quantification of 16S rRNA.

Real-time PCR was used to quantify the population size, through gene copy numbers, of nitrogen fixing bacteria (*nifH*), ammonia oxidizing bacteria (*amoA*), denitrifiers (*nirK*) and total bacteria (16S rRNA) of soils with MWOO plus 3 different plastics incubated samples taken at three different incubation time points in three different soils. Gene copy numbers tended to increase along with the period of incubation, although this was not the case for Warialda Loam 16S gene copies (Figure 16). This effect of time on increasing the number of gene copies for the targeted genes suggests that the development of the soil microbial population was likely to be ongoing throughout the incubation period. This suggests that a longer incubation period may have been desirable to cover the developing population dynamics.

For nearly all treatments, no significant differences in the number of gene copies of *amo*A, *nir*K and *nif*H or 16S rRNA between the MWOO control and microplastic treatments were found (Figures 16-19). Within the Warialda HDPE treatment after 9 months incubation there was a significant decrease in *amoA* gene copies (p <0.03; $3.36x10^3 \pm 5.8x10^3$ compared with $1.4x10^5 \pm 9.5x10^3$), relative to the MWOO control, but this did not occur in any of the other soils (Figure 17, Table G4). Also, *nifH* numbers were significantly reduced (p <0.022; $1.43x10^3 \pm 3x10^3$ compared with $1.42x10^5 \pm 1.02x10^5$) in Kirby Clay soil incubated for 9 months with PVC (Figure 18, Table G4).

It should be noted that Figures 16 to 19 are depicted on a logarithmic scale, which reduces the perception of variability between samples and the high degree of variability in the non-transformed values accounted for no significant differences being found in the majority of treatments.

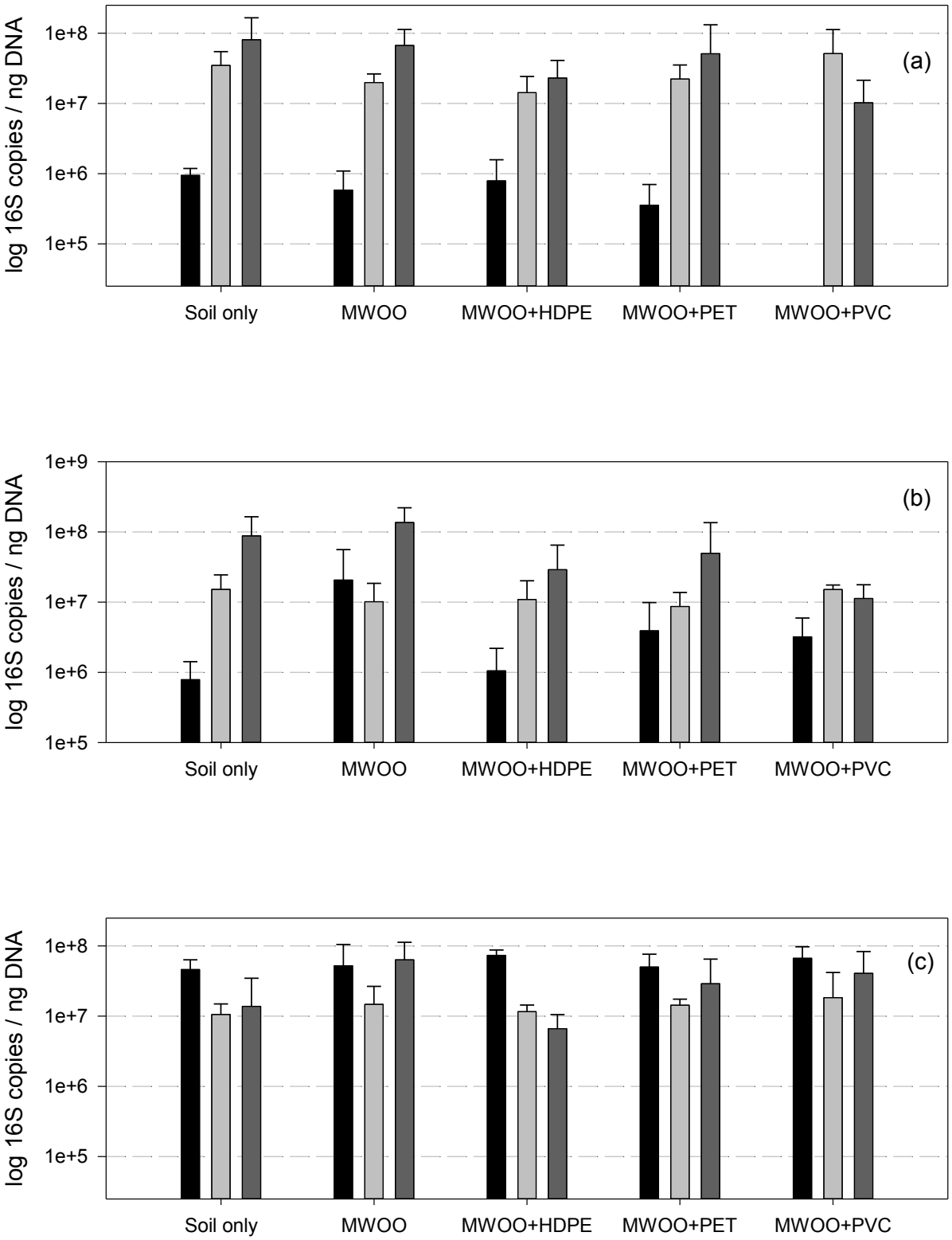


Figure 16. Logarithmic number of copies, using qPCR, of 16S rRNA extracted from treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples).

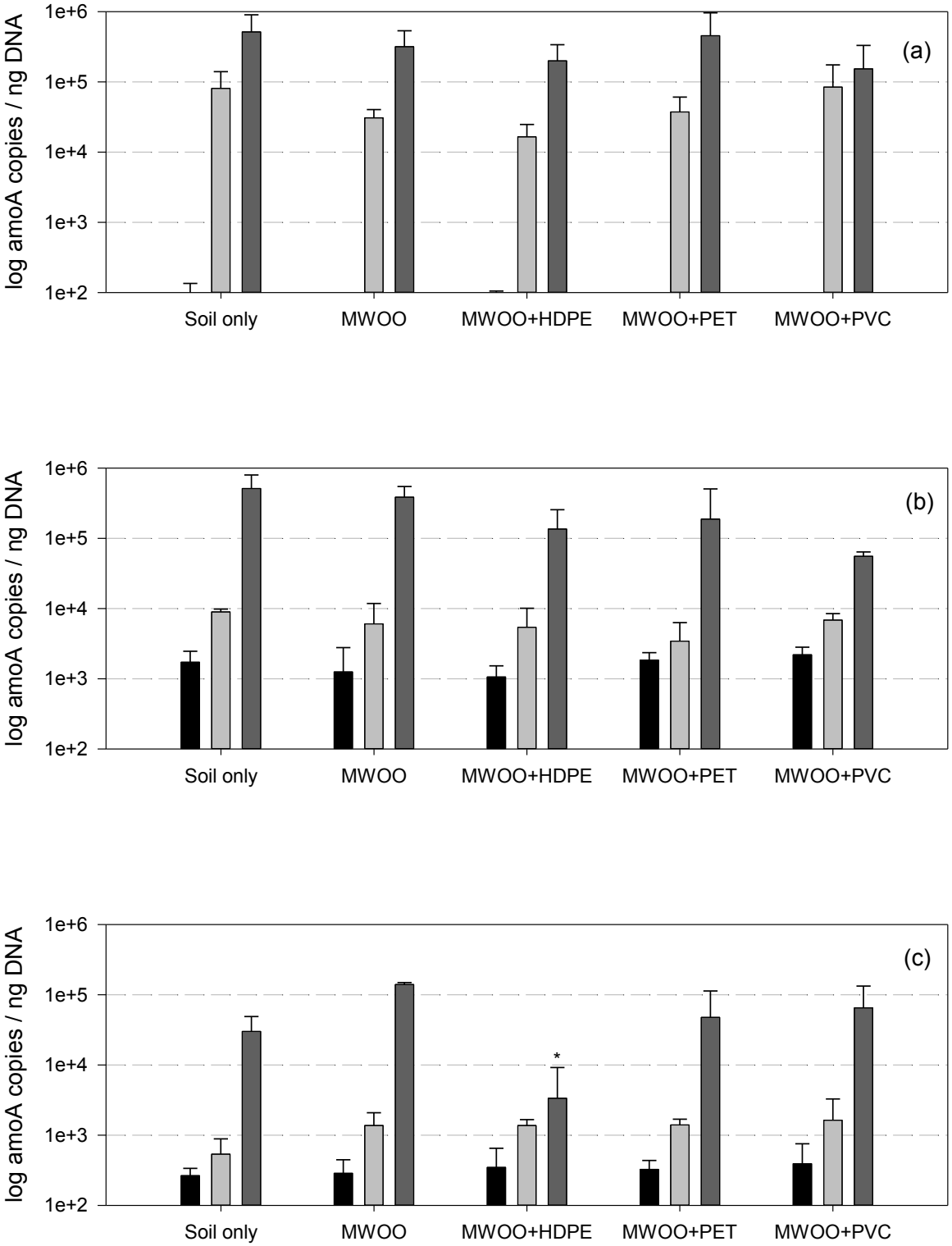


Figure 17. Logarithmic number of copies, using qPCR, of *amoA* (ammonia monooxygenase) genes extracted from treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes treatment significantly different (p<0.05) from MWOO control.

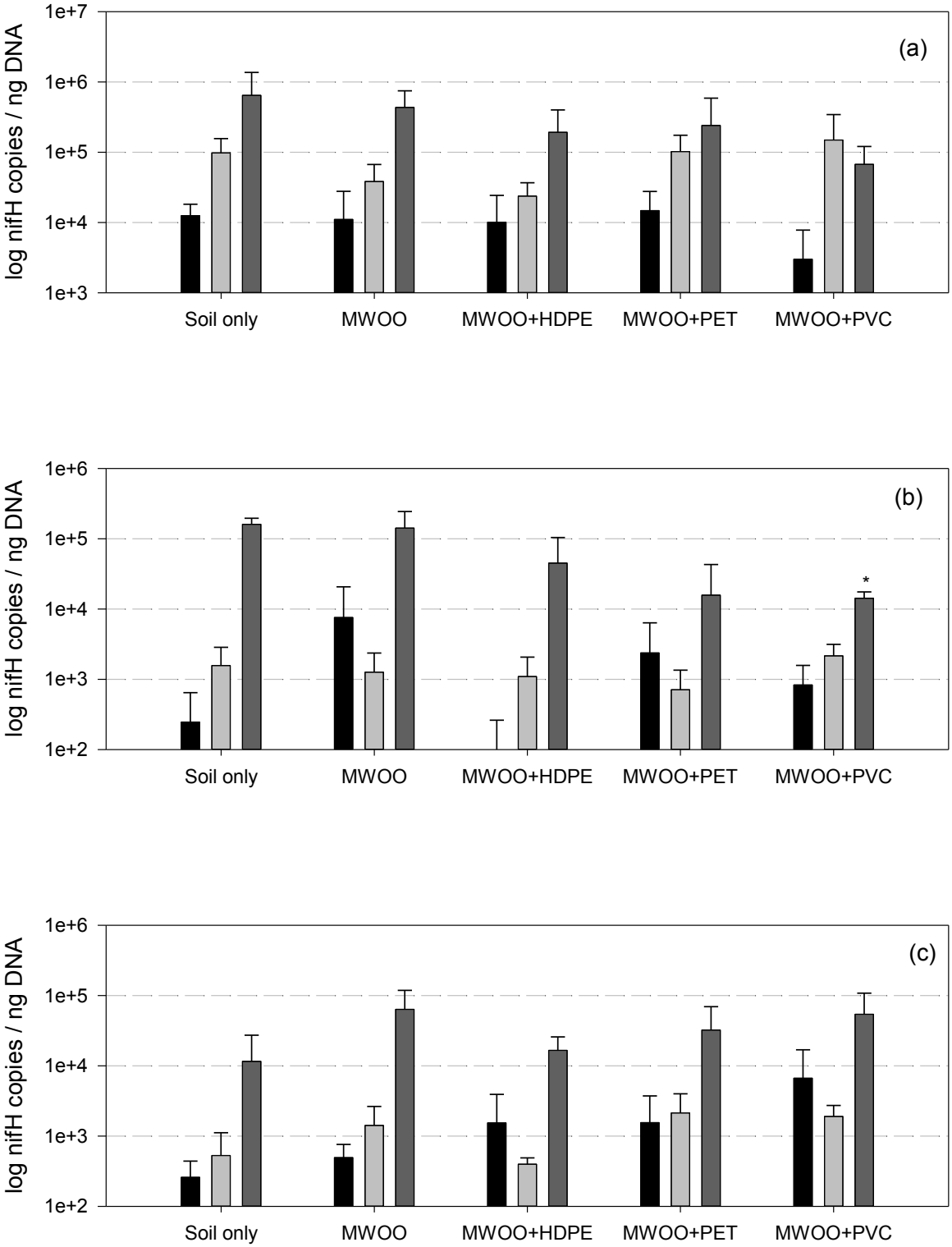


Figure 18. Logarithmic number of copies, using qPCR, of *nifH* (nitrite reductase) genes extracted from treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (■) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes treatment significantly different (p<0.05) from MWOO control.

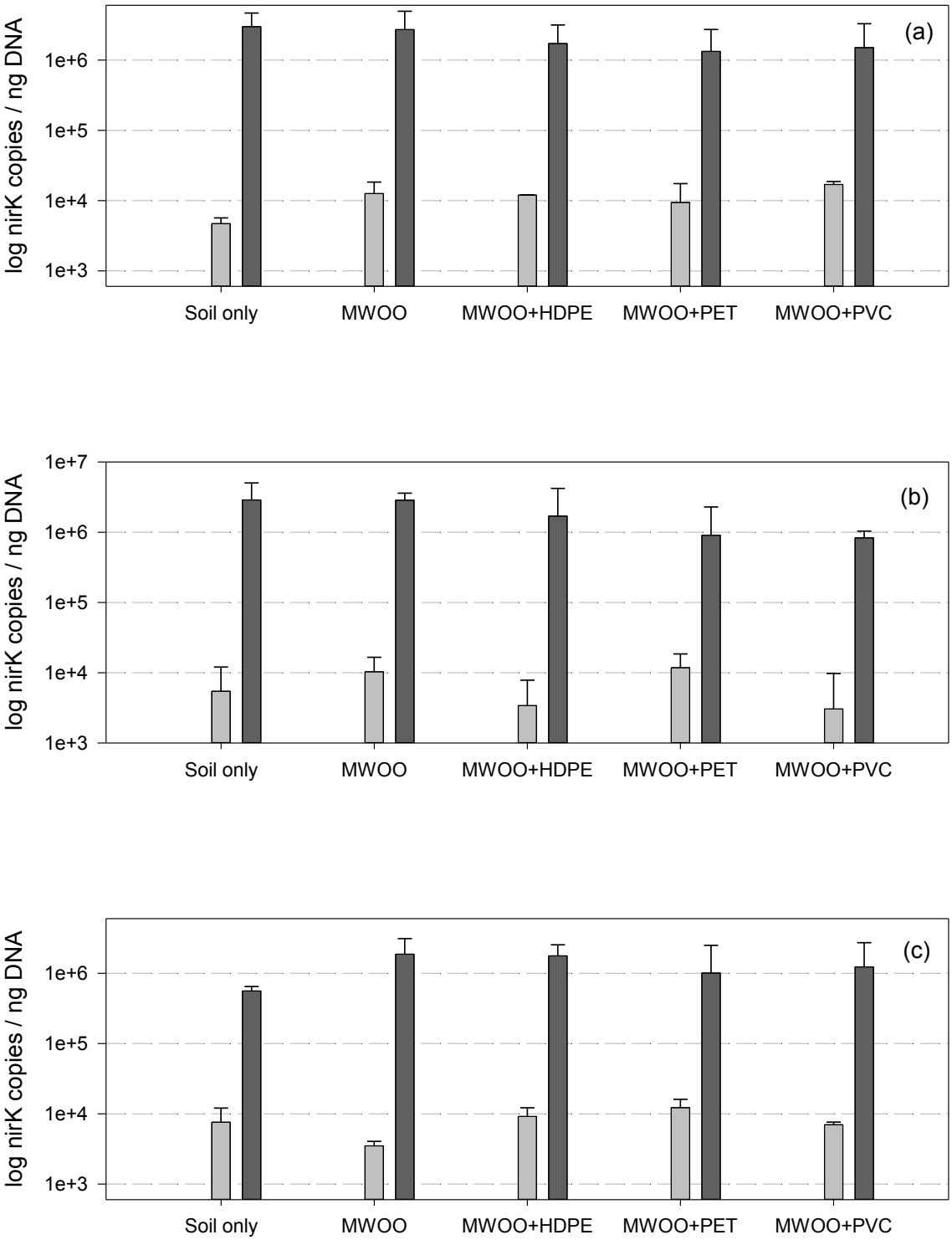


Figure 19. Logarithmic number of copies, using qPCR, of *nirK* (dinitrogenase reductase) genes extracted from treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (■) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples).

4.2.3 Soil microbial function - substrate induced respiration (SIR) and nitrification (SIN)

The SIN and SIR assays represented the most comprehensive toxicological assessment of the microplastics in the soils, in that the full spiking rates of the microplastics were employed including the addition of microplastics to soil without the addition of MWOO.

There were inconsistent effects on SIR following addition of microplastics, depending on the incubation period of the soils. For example, the rates of respiration were unchanged in the 3 month Kirby Clay samples for soils spiked at 0.5 % w/w rates of the three microplastics (Figure 20) but plastics spiked at both higher and lower concentrations increased the rate of respiration (Figure 22). Conversely, for the SIN assessment the 3 month Kirby Clay samples nitrification rates decreased relative to the unincubated (0 months) and 9 month samples, for the majority of treatments (Figures 23-25). There were, however, no clear trends of effects related to the addition of microplastics on the SIR and SIN in all of the soil treatments. For SIR, the rate of respiration was similar to treatments where no MWOO was added, with a significant increase relative to soil only controls noted in the 9 month Kirby Sand samples with no MWOO added (Figure 21). The addition of PET at 0.1, 0.25 and 0.5 % w/w significantly increased the rate of SIR but this did not occur in the 1 % w/w PET treatments (Figure 21). The only decrease on SIR in treatments where MWOO was not added occurred in the 0.25 % w/w HDPE treatment, although there was again no concentration-dependence observed for HDPE with no change in SIR rates (Figure 21).

When MWOO was applied to the soils in conjunction with the microplastics significant decreases in the SIR rates were observed in a number of the treatments. There was an inconsistency in trends, however, related to the effects on SIR in soil treatments from incubation time. For example, addition of 0.1 % and 1 % w/w PET in the Kirby Clay soil significantly reduced the SIR rates in the unincubated (0 months) and 9 month incubated soils, while in the 3 month incubated soils there was an increase in respiration rates (Figure 22). This also occurred in a number of other MWOO-amended soil treatments where PVC was added to the Kirby Clay. There was, however, no concentration-dependence found between the amount of microplastic spiked into the soils and the effect on SIR. One exception to this was noted in the Warialda Loam soils mixed with MWOO, incubated for 9 months and spiked

with PVC. In all the PVC treatments, apart from where PVC was spiked at 0.5 % w/w, there was a significant decrease in the SIR rates although these lower rates were not significantly different at each PVC concentration (Figures 20 and 22). In the case of the 0.5 % w/w PVC spiked treatment, there was a non-significant increase in the SIR relative to the MWOO control in the treatment incubated for 9 months. A concentration-response effect following addition of PVC in the Warialda Loam soils incubated for 9 months in the presence of MWOO was therefore not able to be established.

In the case of the SIN assessments, the only significant decrease on nitrification rates were noted in the Kirby Clay soils incubated for 9 months without the addition of MWOO (Figure 24). This was mainly related to the addition of PVC, where significant decreases occurred at addition rates of 0.25, 0.5 and 1 % w/w and the SIN rates increased with an increasing rate of PVC addition. Without additional treatments containing higher rates of PVC, it is not possible to establish whether a concentration-response relationship existed. It is also worth noting that the addition of PVC at the highest rate in the unincubated (0 months) Kirby Sand also significantly decreased SIN, relative to the control, but there was no significant difference observed for soils incubated for 3 and 9 months (Figure 24). Also, the addition of MWOO to the Warialda Loam reduced the SIN rates, relative to samples where no MWOO was added (Figure 25). This effect of the addition of MWOO was not observed with SIR rates for Warialda Loam, however, or for both SIN and SIR rates in any other treatments.

The only significant increases in SIN rates were found in treatments where MWOO was added (Figures 23 and 25), which is in contrast with the SIR assessments. There was a high degree of variability associated with the SIN values, especially in the Kirby Sand treatments, which would reduce the ability to ascertain whether any significant effects on SIN rates occurred in the treatments. Despite this, the lack of any clear trends relating to microplastic addition rates and incubation time suggest that under the conditions represented by these treatments there was little effect on SIN and SIR in the three agricultural soils following addition of MWOO and microplastics. This is consistent with the previously discussed expression of genes related to soil nitrification, specifically, as well as 16S related to total numbers of bacteria, where no significant differences were observed at each respective incubation period, relative to their controls.

In the case of PVC, where significant negative effects were found on SIR in Kirby Clay and Warialda Loam in the presence of MWOO and on SIN in Kirby Clay after 9 months incubation in the absence of MWOO suggests further investigation should occur for this microplastic, especially for longer incubation periods. Where increased incubation rates are used, it would be worth introducing other processes that may induce accelerated ageing of the microplastics, such as sunlight.

It is worth noting, however, that the range of addition rates in these exposures was 100-fold, from 0.01 % to 1 %. Furthermore, the highest rate of addition for PVC is considerably greater than what would be expected to be present in MWOO at currently legislated rates of MWOO application, particularly where PVC is likely to be a minor component of the total plastics load of MWOO.

Overall, the assessment relating to the potential effects of microplastics on soil function, related to SIR and SIN, indicated there is no clear effect under the experimental conditions of exposure.

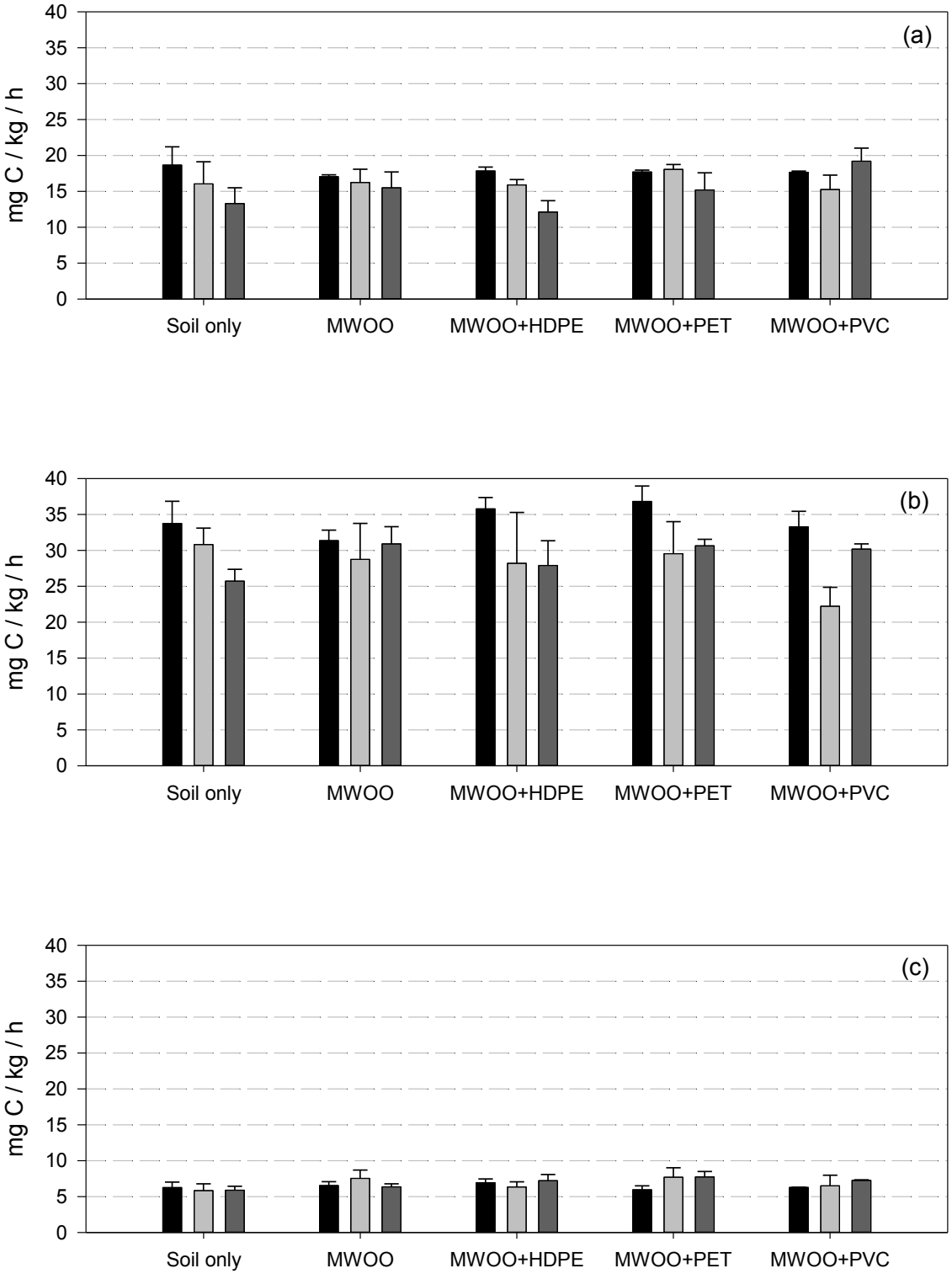
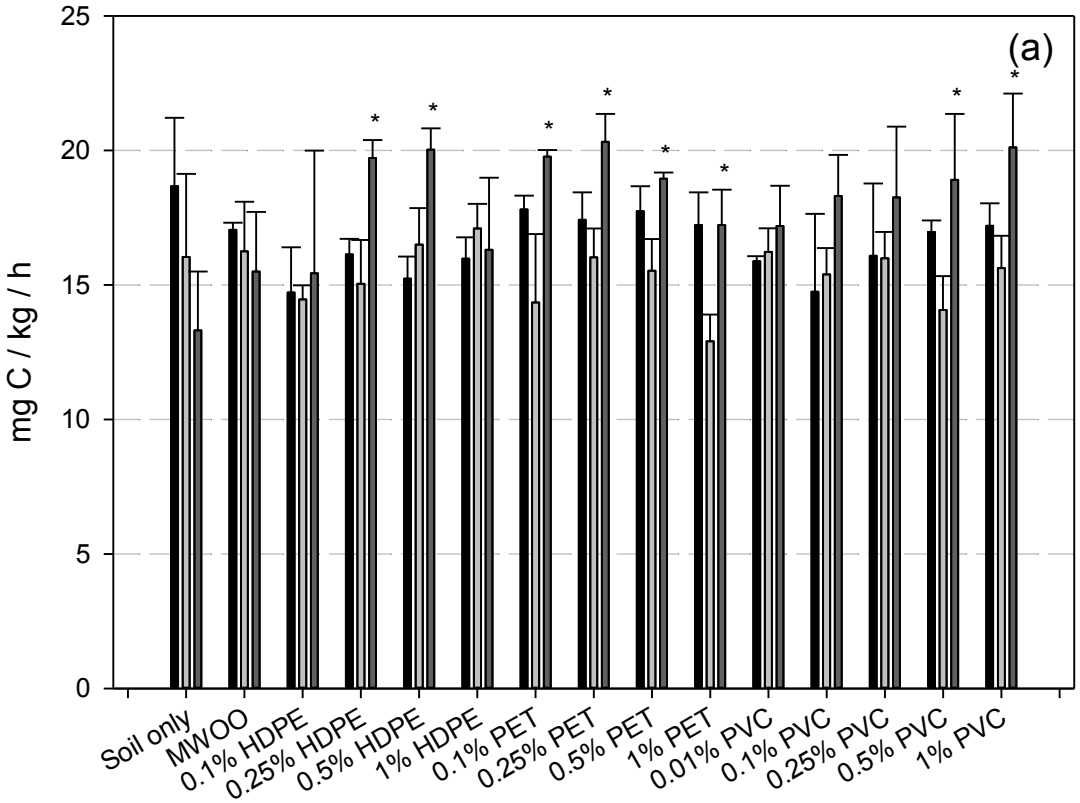
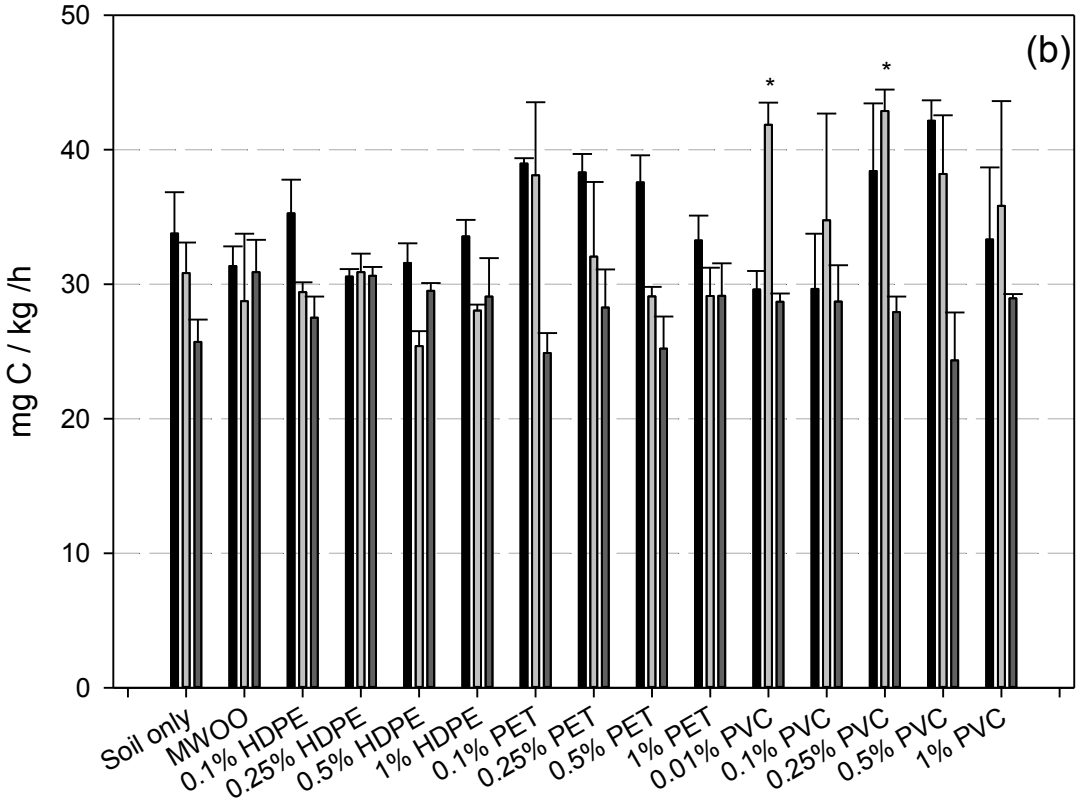


Figure 20. Rates of substrate induced respiration (SIR) of added glucose from treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples).


(a)
mg C / kg / h
25
20
15
10
5
0
Soil only
MWOO
0.1% HDPE
0.25% HDPE
0.5% HDPE
1% HDPE
0.1% PET
0.25% PET
0.5% PET
1% PET
0.01% PVC
0.1% PVC
0.25% PVC
0.5% PVC
1% PVC


(b)
mg C / kg /h
50
40
30
20
10
0
Soil only
MWOO
0.1% HDPE
0.25% HDPE
0.5% HDPE
1% HDPE
0.1% PET
0.25% PET
0.5% PET
1% PET
0.01% PVC
0.1% PVC
0.25% PVC
0.5% PVC
1% PVC

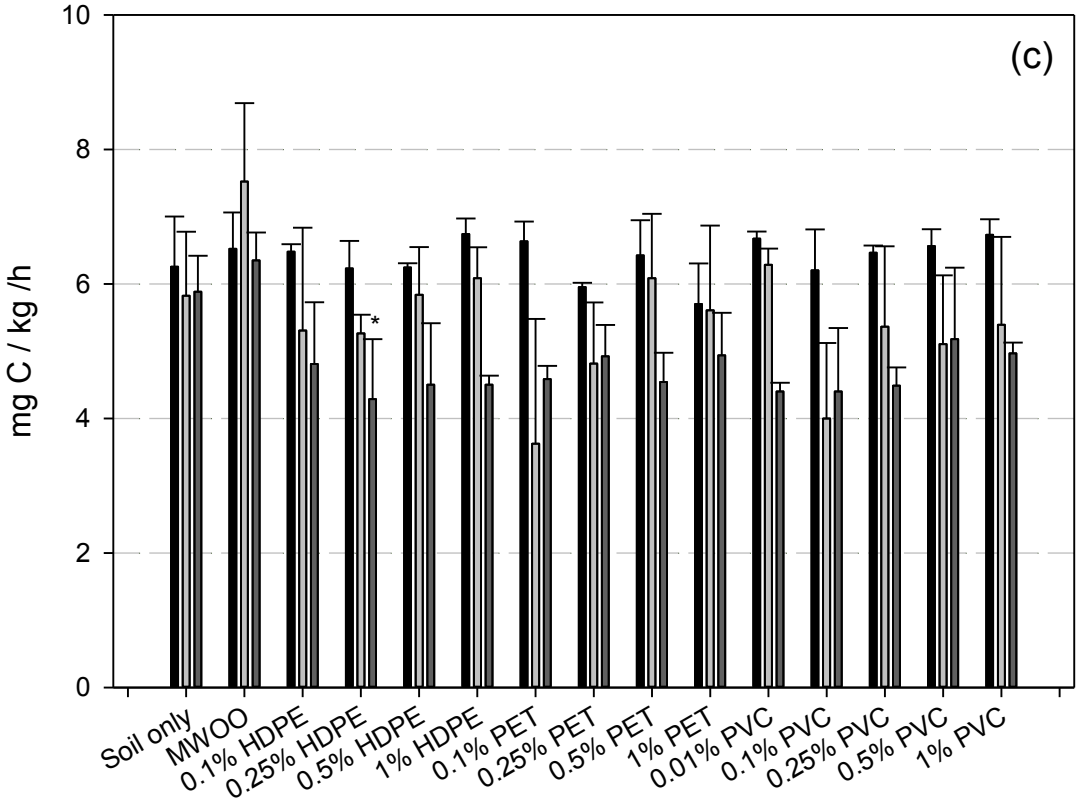
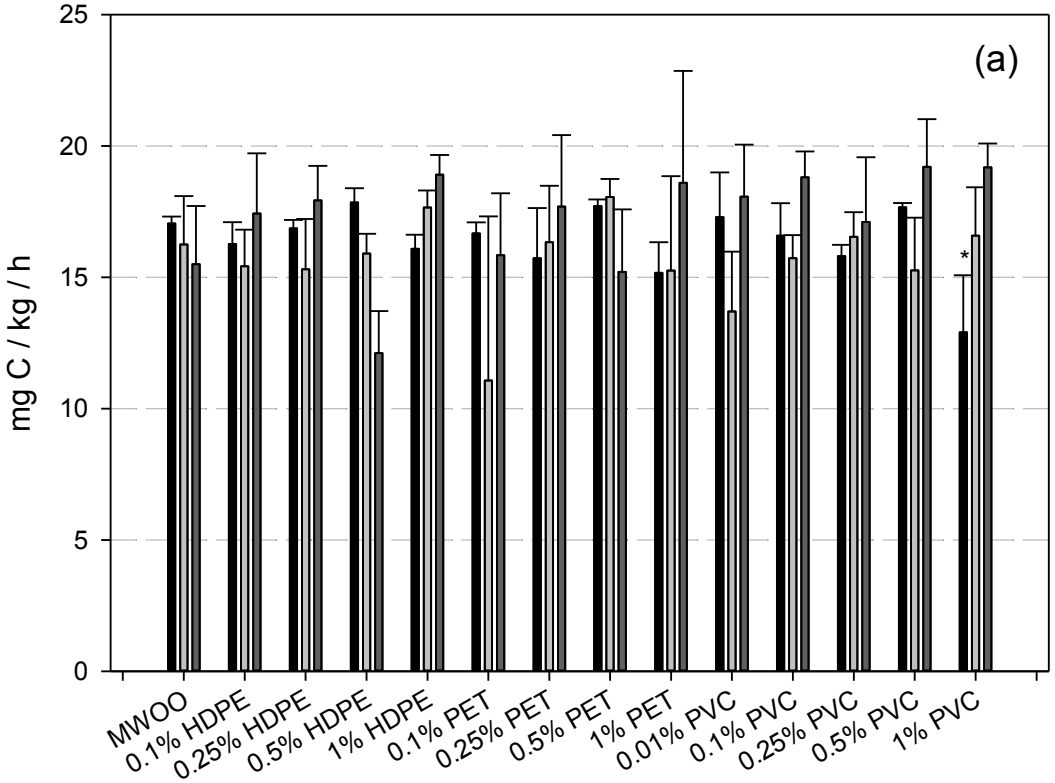
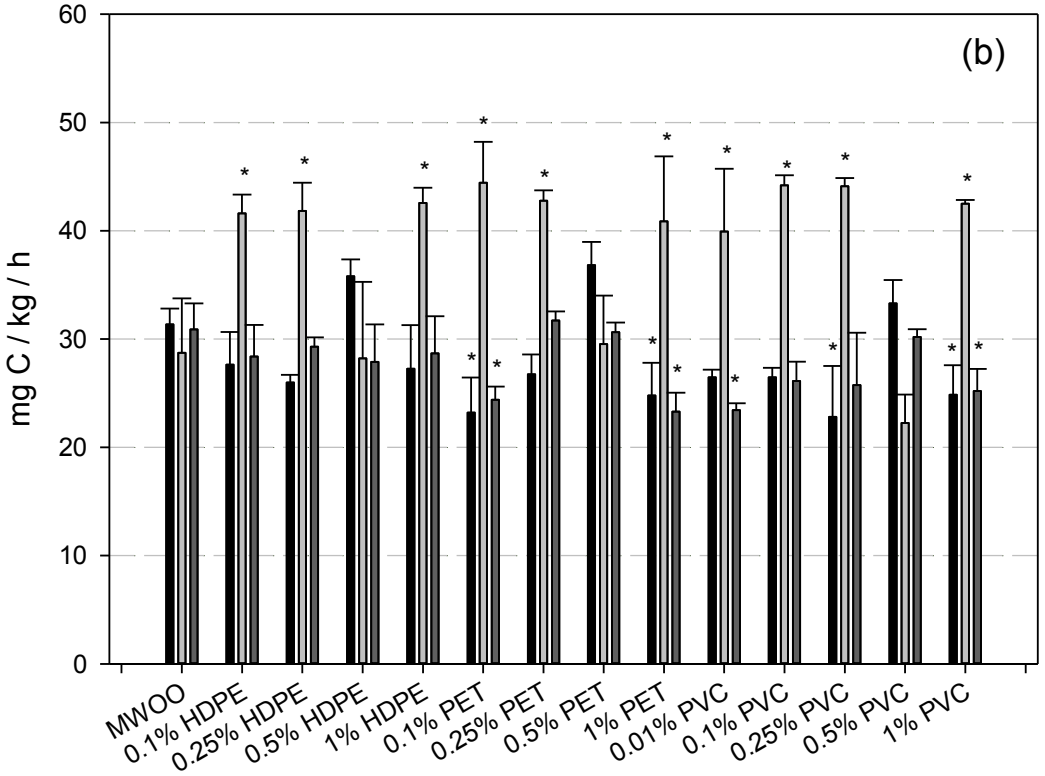


Figure 21. Rates of substrate induced respiration (SIR) of added glucose from treatments containing soil only and microplastics spiked at all concentrations in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes samples significantly different (p<0.05) from soil only control.


(a)
mg C / kg / h
25
20
15
10
5
0
MWOO
0.1% HDPE
0.25% HDPE
0.5% HDPE
1% HDPE
0.1% PET
0.25% PET
0.5% PET
1% PET
0.01% PVC
0.1% PVC
0.25% PVC
0.5% PVC
1% PVC


(b)
mg C / kg / h
60
50
40
30
20
10
0
MWOO
0.1% HDPE
0.25% HDPE
0.5% HDPE
1% HDPE
0.1% PET
0.25% PET
0.5% PET
1% PET
0.01% PVC
0.1% PVC
0.25% PVC
0.5% PVC
1% PVC

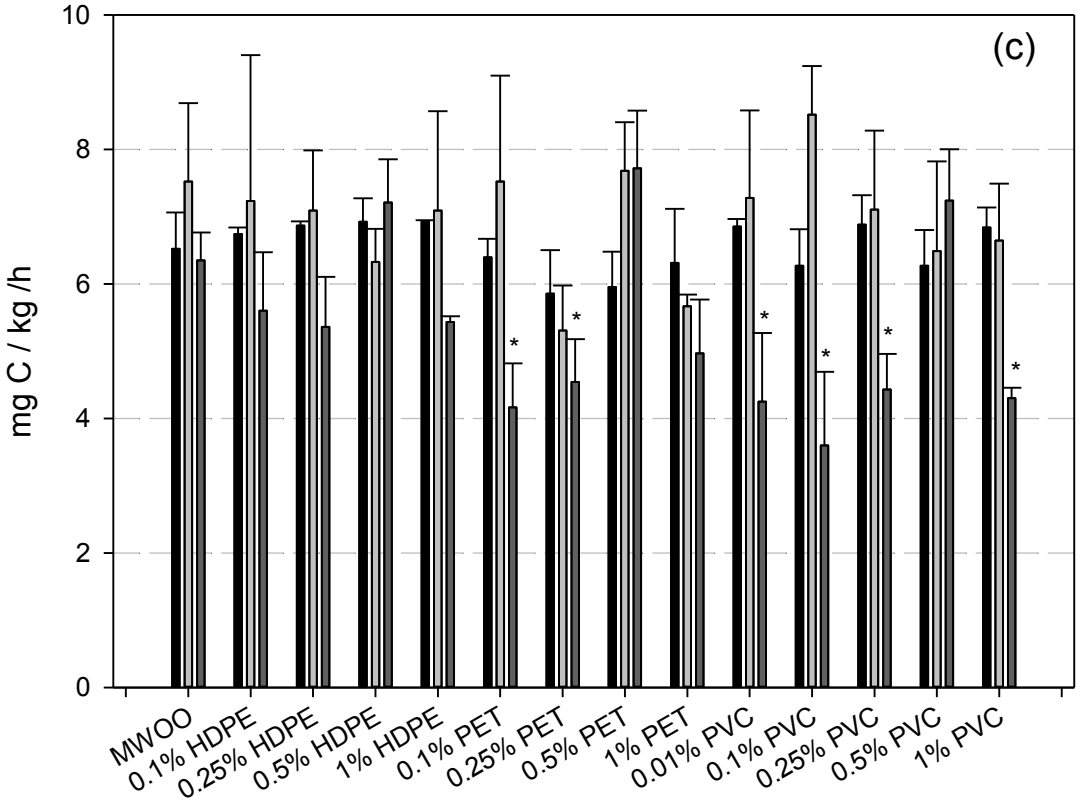


Figure 22. Rates of substrate induced respiration (SIR) of added glucose from treatments containing MWOO and microplastics spiked at all concentrations in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes samples significantly different (p<0.05) from MWOO control.

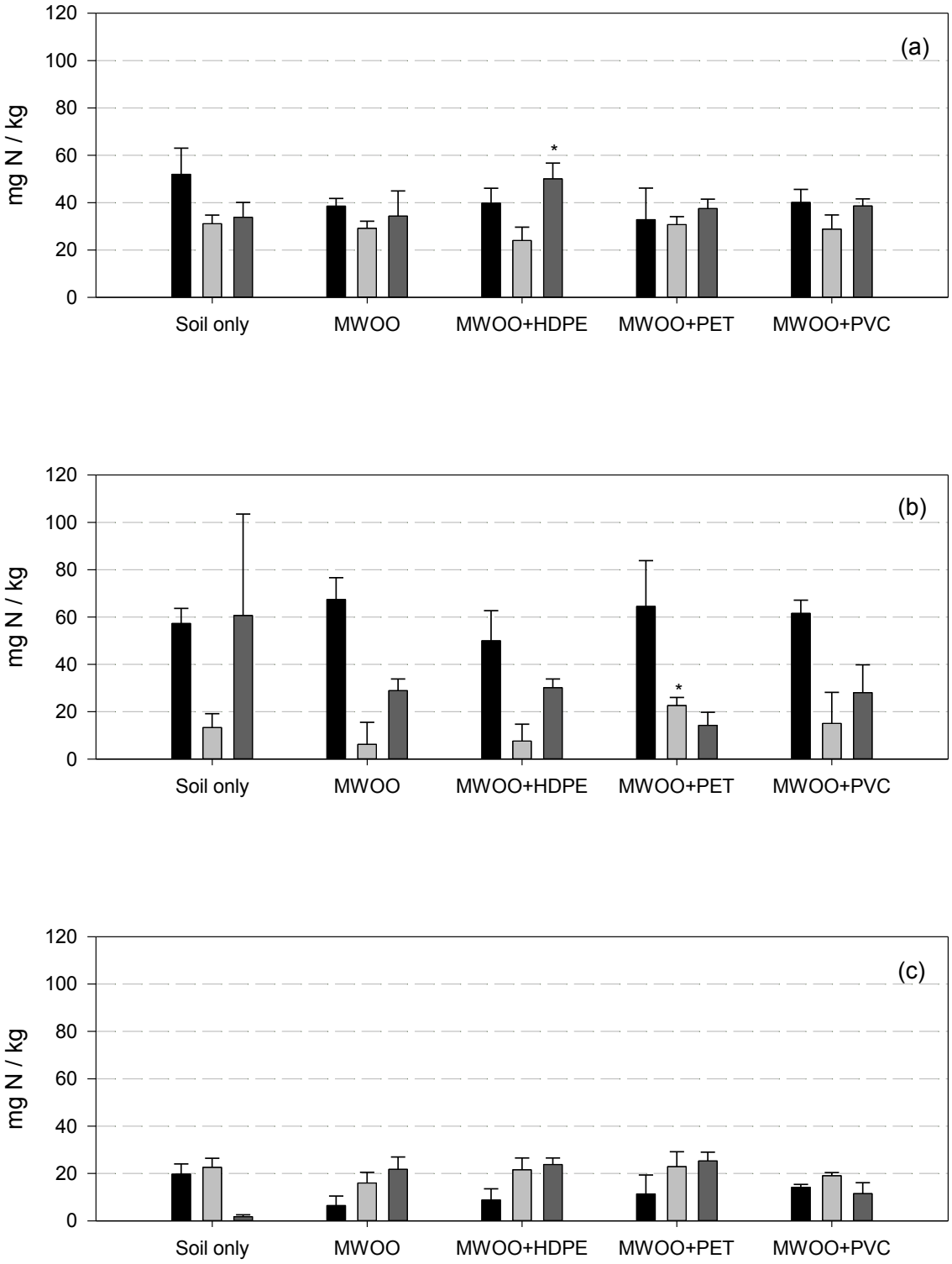
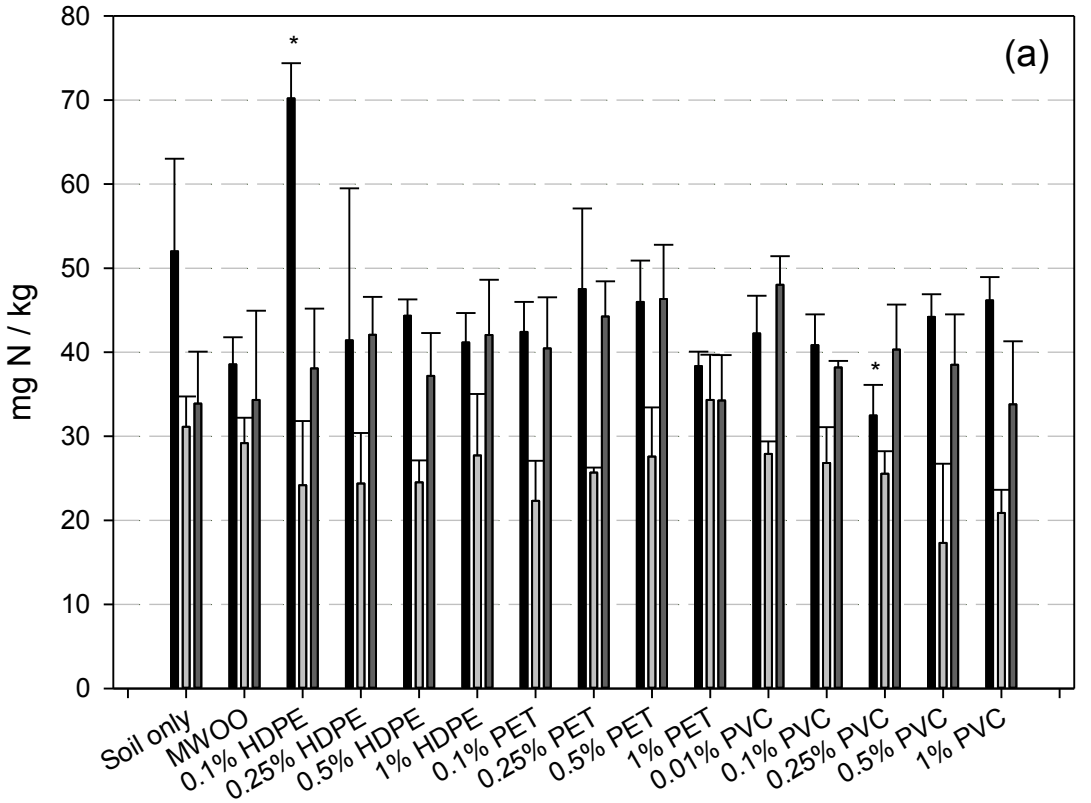
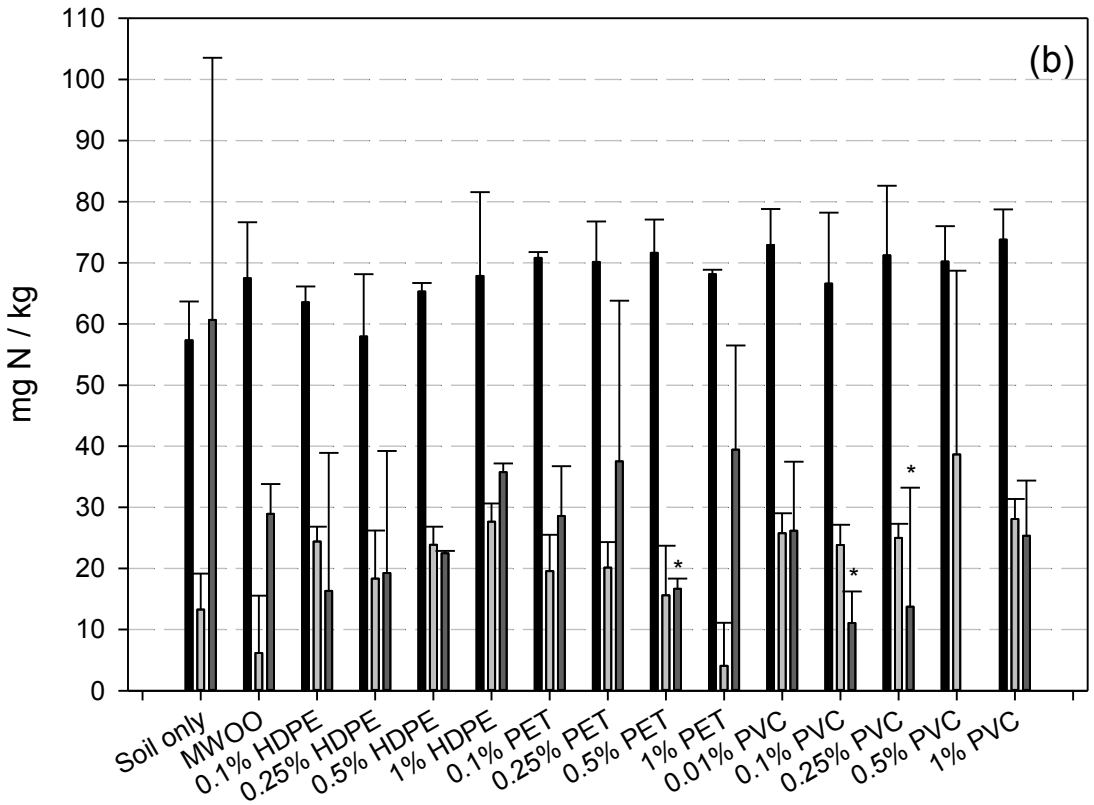


Figure 23. Rates of substrate induced nitrification (SIN) of added NH_4^+ from treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (■) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes samples significantly different (p<0.05) from MWOO control.


(a)
mg N / kg
80
70
60
50
40
30
20
10
0
*
*
Soil only
MWOO
0.1% HDPE
0.25% HDPE
0.5% HDPE
1% HDPE
0.1% PET
0.25% PET
0.5% PET
1% PET
0.01% PVC
0.1% PVC
0.25% PVC
0.5% PVC
1% PVC


(b)
mg N / kg
110
100
90
80
70
60
50
40
30
20
10
0
*
*
*
Soil only
MWOO
0.1% HDPE
0.25% HDPE
0.5% HDPE
1% HDPE
0.1% PET
0.25% PET
0.5% PET
1% PET
0.01% PVC
0.1% PVC
0.25% PVC
0.5% PVC
1% PVC

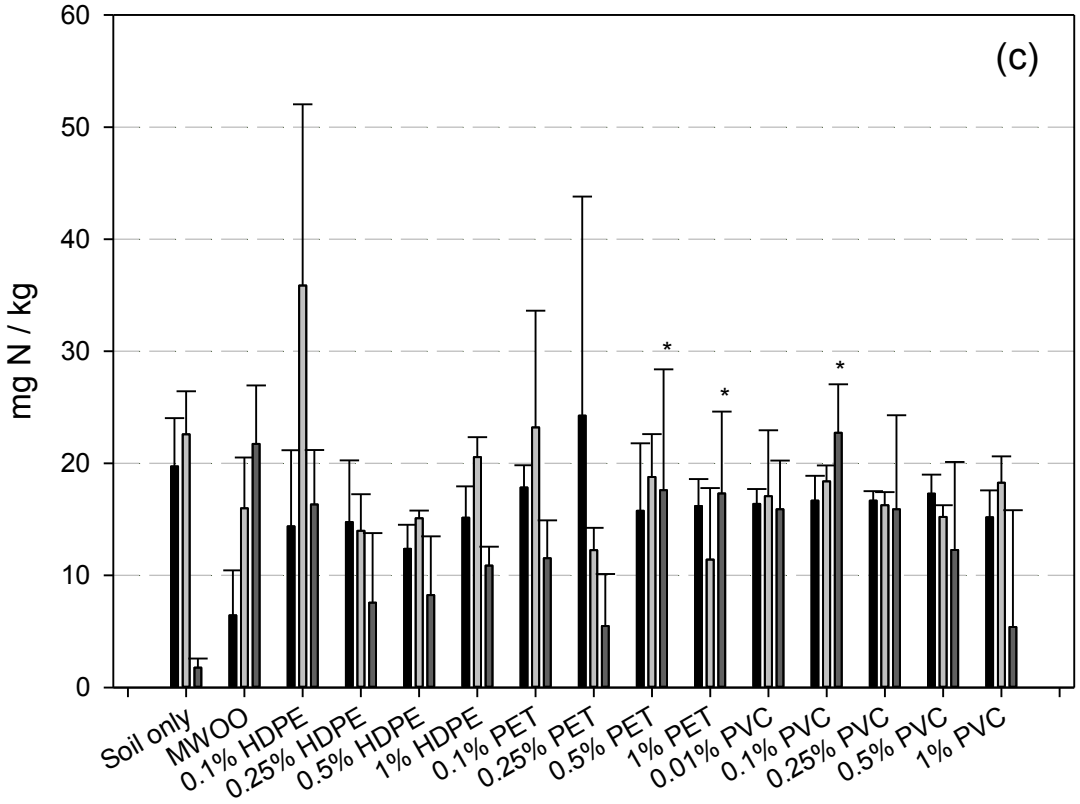
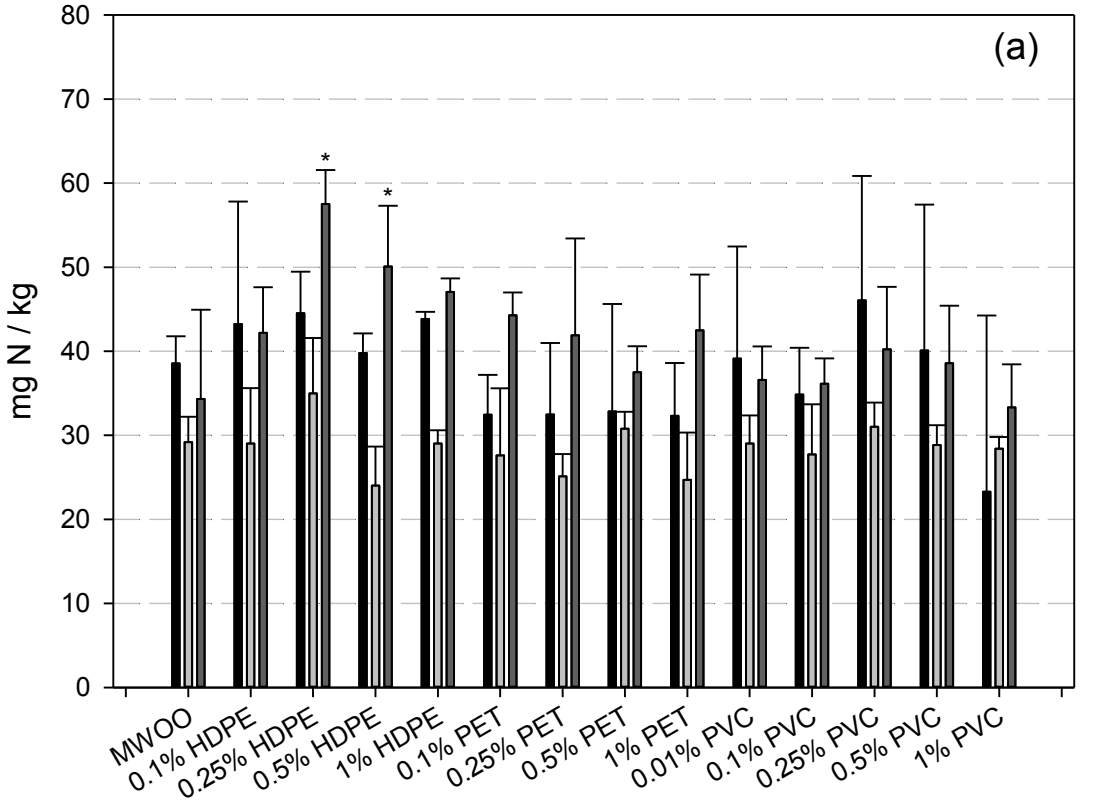
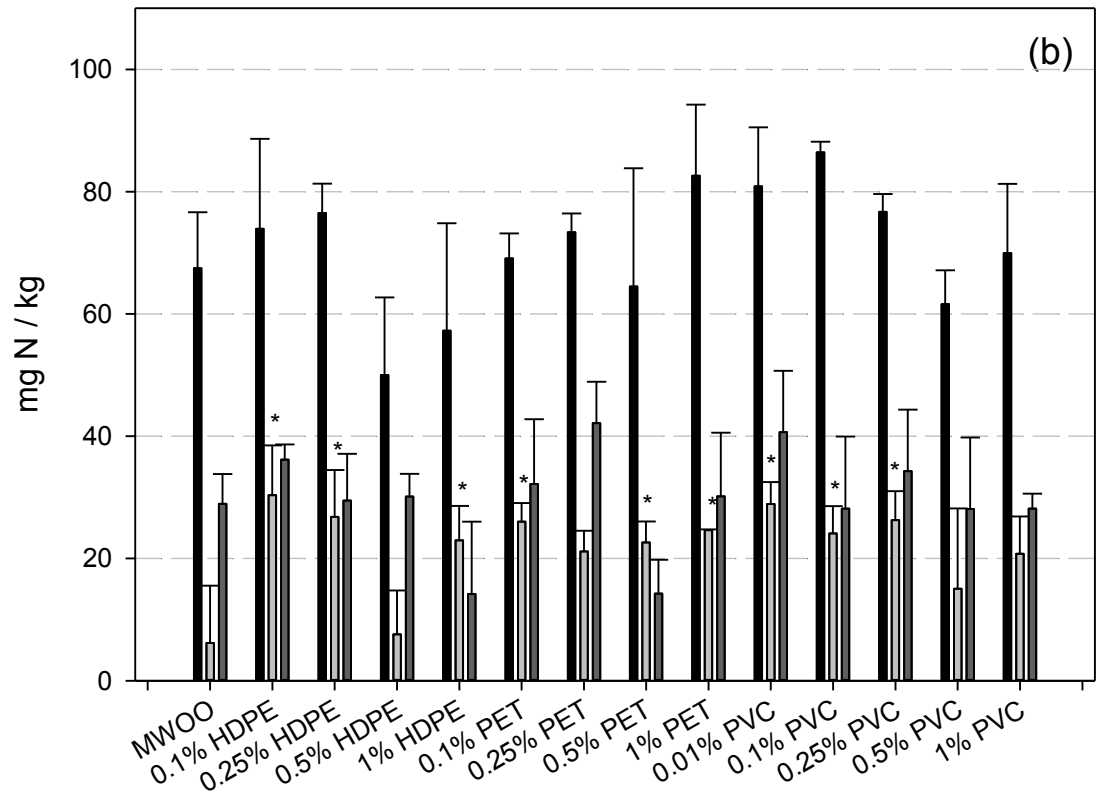


Figure 24. Rates of substrate induced nitrification (SIN) of added NH_4^+ from treatments containing soil only and microplastics spiked at all concentrations in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes samples significantly different (p<0.05) from soil only control.


(a)
mg N / kg
0
10
20
30
40
50
60
70
80
*
*
MWOO
0.1% HDPE
0.25% HDPE
0.5% HDPE
1% HDPE
0.1% PET
0.25% PET
0.5% PET
1% PET
0.01% PVC
0.1% PVC
0.25% PVC
0.5% PVC
1% PVC


(b)
mg N / kg
0
20
40
60
80
100
*
*
*
*
*
*
*
*
*
MWOO
0.1% HDPE
0.25% HDPE
0.5% HDPE
1% HDPE
0.1% PET
0.25% PET
0.5% PET
1% PET
0.01% PVC
0.1% PVC
0.25% PVC
0.5% PVC
1% PVC

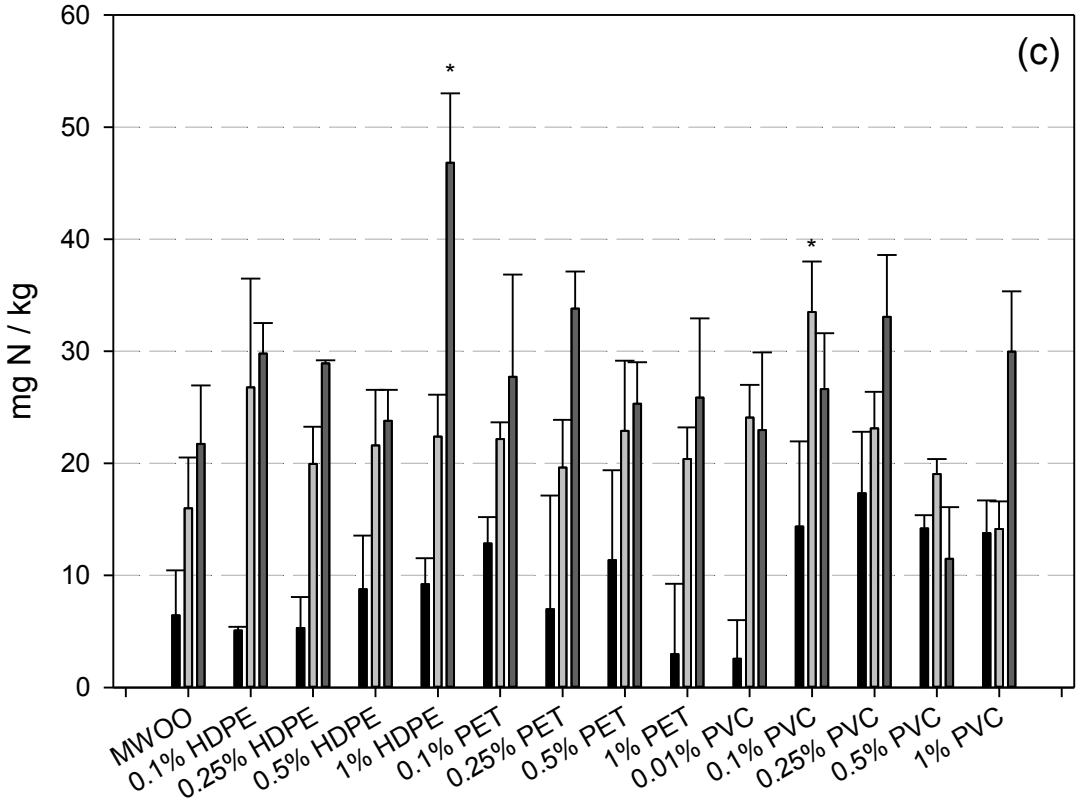


Figure 25. Rates of substrate induced nitrification (SIN) of added NH_4^+ from treatments containing MWOO and microplastics spiked at all concentrations in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes samples significantly different (p<0.05) from MWOO control.

4.2.4 Nematode mortality and reproduction

Nematodes, such as *C. elegans*, represent a highly abundant species of soil invertebrate that perform a range of critical soil functions, including energy flow, nitrogen and other nutrient cycling and primary production (Sochova et al. 2006). Furthermore, the permeability of their cuticle makes them especially vulnerable to soil contaminants present in pore water, although under the experimental parameters of the present study there was little toxicity evident.

In the MWOO control treatments, the survival was greater than 87±11 %, which was in accordance with the testing requirements (ASTM 2001). There were no significant effects found on *C. elegans* survival following the addition of MWOO or microplastics to soils with up to 9 months incubation (Figure 26). In treatments where mortality was observed, they were not significantly different from the MWOO controls. This was the case for the PET treatment in Kirby Clay incubated for 3 months (60±40 % survival) but there was a high degree of variability associated with this treatment (Figure 26). This degree of mortality was not observed in the 9 month incubated soil, where the survival rate was identical with that in the unincubated soil.

There was a high degree of variability observed in the *C. elegans* reproduction assay for the treatments incubated for up to 9 months (Figure 27). The majority of treatments were found to have no significant effects following the addition of MWOO and microplastics or following incubation in the soils. The only significant reduction in the number of juvenile nematodes counted occurred in the PVC (7±13; p=0.014) and HDPE (14±9; p=0.035) treatments in the Warialda Loam that had been incubated for 9 months, compared with the MWOO control (44±17) (Figure 27, Table G5). This was despite a high degree of variability in the treatment with PVC added to MWOO-amended soil, which was also noted in a number of the other treatments.

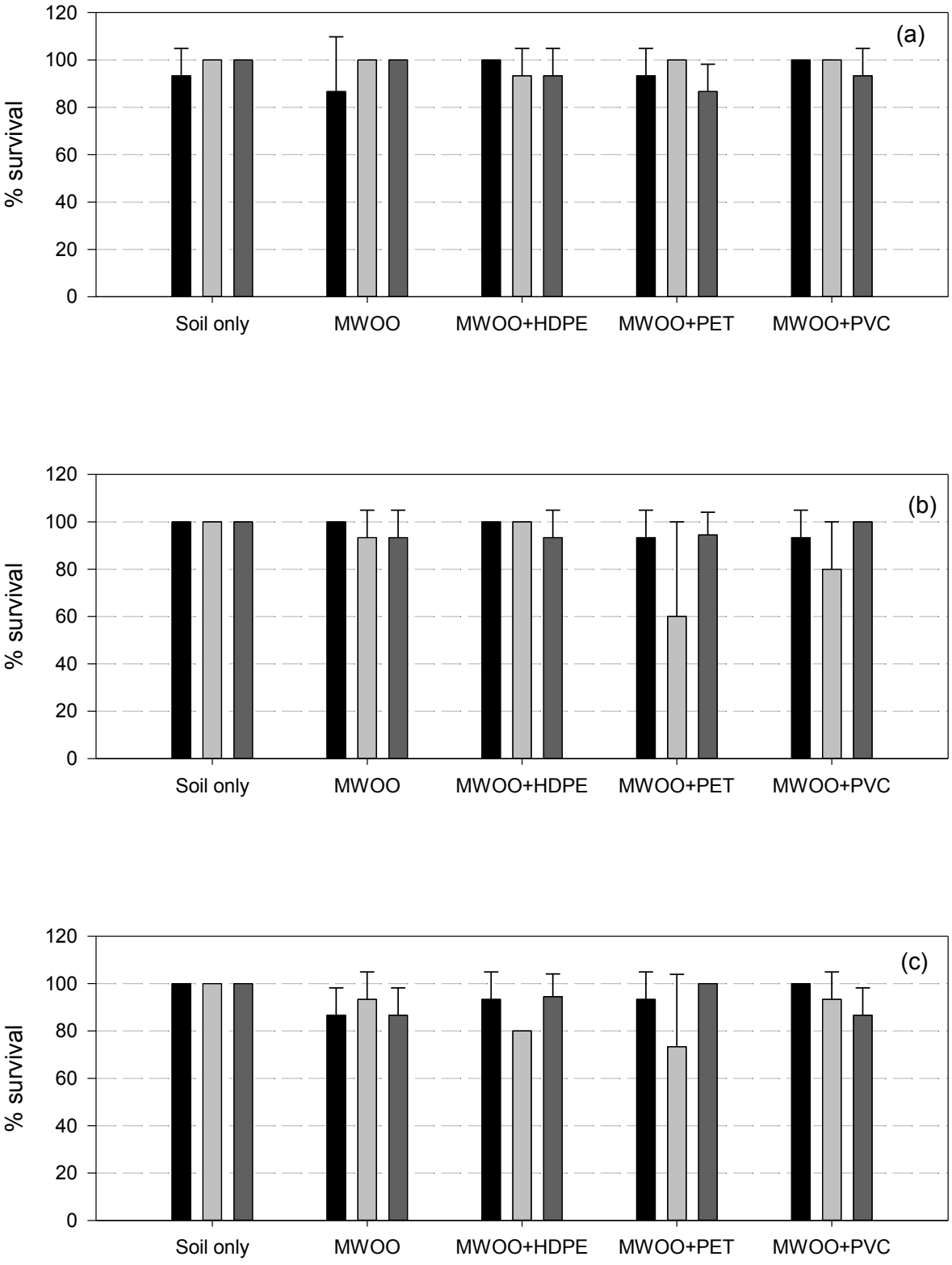


Figure 26. The percentage survival of nematodes in leachates from treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples).

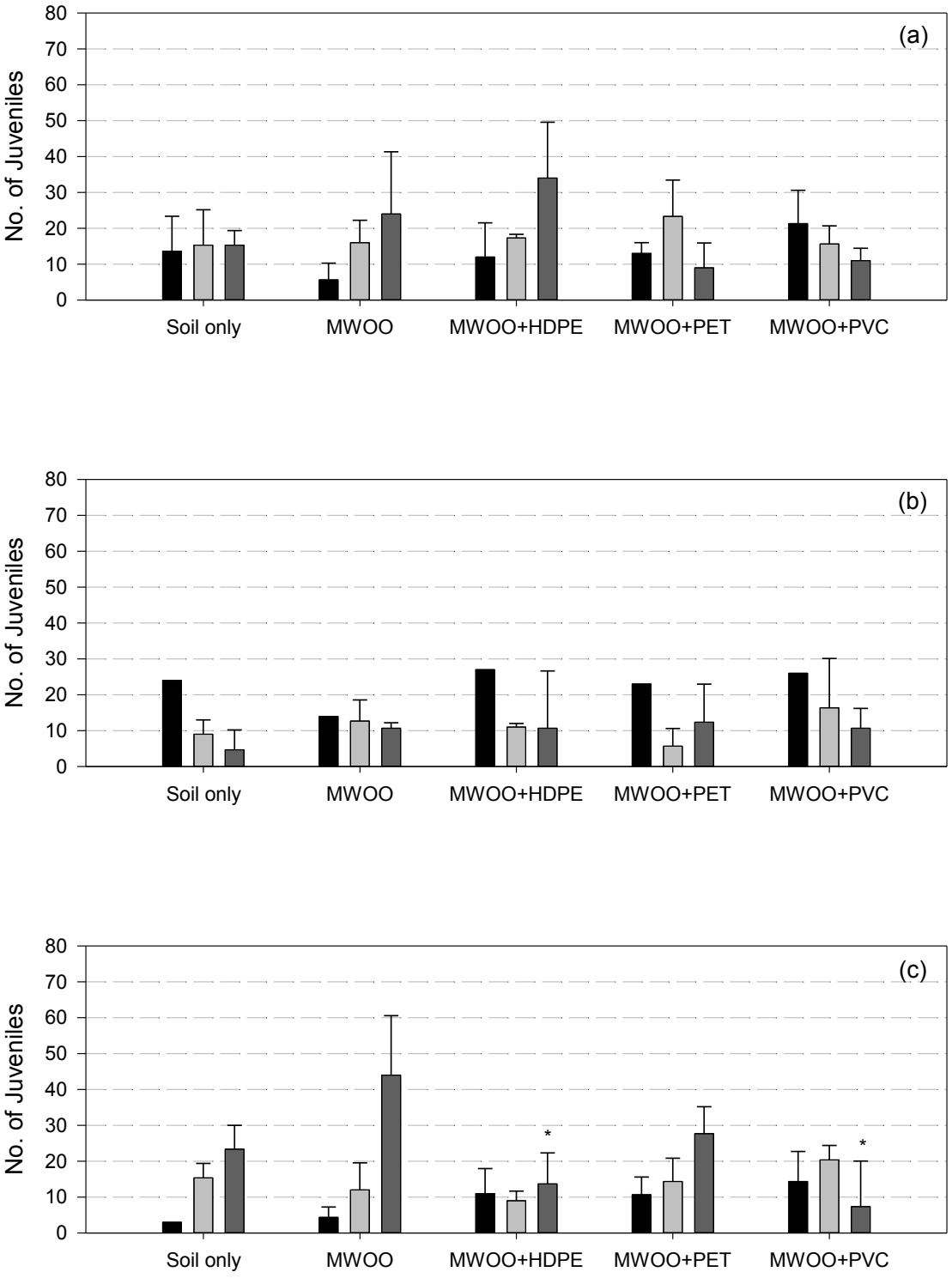


Figure 27. The number of juvenile nematodes counted after exposure of adults to leachates from treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes samples significantly different (p<0.05) from MWOO control.

4.2.5 Earthworm avoidance

The earthworm avoidance is a short-term but often highly sensitive assay examining behavioural impacts. Earthworms can exhibit a marked preference for avoiding areas that have been impacted by a number of contaminants and environmental stressors, including trace metals and organic contaminants (Sousa et al. 2008, Shoults et al. 2011, Lowe et al. 2016).

The positive controls containing boric acid and MWOO were effective in proving a reference point for avoidance induction (Figure 28) although avoidance was less in the Kirby Clay boric acid treatment (13-47% avoidance compared with 33-87%), which may have been related to the high %OC and clay content of the Kirby Clay. The validity of all the tests were found to be acceptable, with <10 % mortality or escapes observed in all treatments and MWOO controls (ISO 2008).

There was no significant difference found in earthworm avoidance behaviour for the majority of the soil treatments with MWOO and microplastics added (Figure 28). Avoidance in the Kirby Clay alone was comparable with that observed in the Kirby Clay amended with MWOO after 3 and 9 months incubation (Figure 28). For the Kirby Sand and Warialda Loam, however, the addition of MWOO made the soils more preferable for the worms, probably due to the related increase in organic matter in these relatively low %OC soils (Delgadillo et al. 2017). The extent of avoidance was similar or greater in these treatments compared with that observed in the treatments with plastics added in all the soils for all incubation periods.

Based on these results, it is unlikely that the addition of the three microplastics added at 0.5 % w/w would have an effect on earthworm behaviour. This is consistent with a recent study assessing earthworm (*Lumbriculus terrestris*) uptake of Zn-loaded microplastic HDPE added to soils at 0.35 % w/w (Hodson et al. 2017). In this study earthworms did not avoid the HDPE-treated soil, even when >4 g/kg Zn was loaded onto the HDPE. This would suggest that earthworm exposure to microplastics present in soil is likely to occur, despite the potential contaminant loading of the microplastic. In this case, longer term assays relating to earthworm toxicity and reproduction may be more useful to assess for effects following exposure to the microplastics.

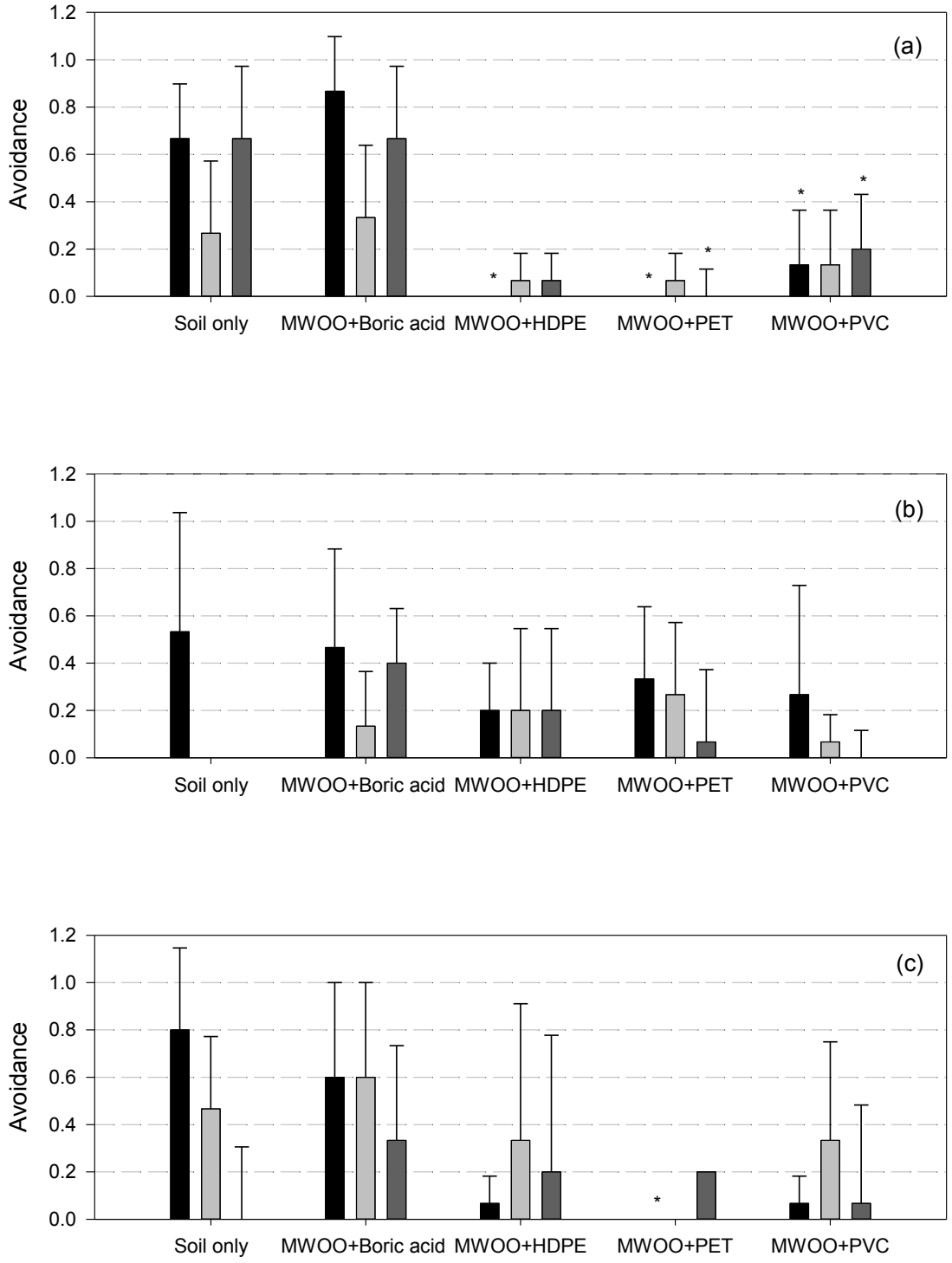


Figure 28. The proportion avoidance, measured for earthworms added to treatments, including the boric acid positive control, containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. The values (mean ± standard deviation, n=3 samples) are relative to a MWOO control where a value of 1 is equivalent to 100% avoidance. Asterix denotes samples significantly different (p<0.05) from MWOO control.

4.2.6 Earthworm mortality, growth and reproduction

There was no toxicity evident in the 28 d assay, where there was 100% survival in the majority of treatments with only 10 treatments (out of 135) having a 97±2% survival rate equivalent to one death per 30 worms (data not shown). Additionally, there were no significant differences in growth rates (as measured by relative mass of earthworms) and reproduction (as measured by the number of juveniles) in the MWOO controls and treatments (Figures 29 and 30). This finding is supported by the chemical analyses of the soils, which highlighted, for example, the addition of microplastics having very little effect on the trace metal concentrations of the soils and soil solutions (see Section 4.1.4). In the case of Zn, for example, the concentrations measured in the soils within the present study were considerably lower than those likely to elicit toxicity in earthworms, especially as ageing during soil incubation decreased its bioavailability (Spurgeon and Hopkin 1995, Hodson et al. 2017).

A study on toxicity to the earthworm (*Lumbriculus terrestris*) with micro-sized HDPE (> 150 μm) spiked in bulk soils at the equivalent of up to 1.2 % w/w demonstrated toxicity relating to a decrease in rate of growth (Lwanga et al. 2016). This decrease in growth rate occurred at an equivalent of > 0.4 % w/w HDPE, although in this study the microplastic was distributed in a concentrated form on the soil surface in leaf litter, with the assumption that bioturbation would equally distribute the HDPE throughout the soil column (Lwanga et al. 2016). This in contrast with the present study where microplastics were mixed throughout the soil treatment to attempt to attain homogeneity prior to addition of the worms. Despite the marked effect on the growth rate of the earthworms at a comparatively low HDPE concentration noted by Lwanga et al. (2016), there was no observed effect on the reproduction of the earthworms, based on the number of cocoons produced. This was consistent with the findings in our reproduction assay, where no significant effects on reproduction were observed, although the endpoint in the present study related to the total number of juveniles after an additional 28 d rather than cocoon production (Figure 30).

It is noteworthy that the greatest reproduction rates were found in the Kirby Sand, despite having a lower nutrient value and soil microbial activity and diversity, relative to Kirby Clay. This may have been related to the Kirby Sand having a lower bulk density than the Kirby Clay, which worms can have a preference for inhabiting (Eijsackers et al. 2005).

In summary, there were no negative effects or apparent trends for the earthworm mortality, growth and reproduction assays, relating to the addition of microplastics that could be observed for treatments from any of the incubation periods. Despite no effects being noted, there is evidence that exposure of earthworms to microplastics can result in their accumulation in an earthworm's gut (Lwanga et al. 2016). This may potentially lead to trophic transfer of microplastics due to earthworms being an important food source for many organisms, including vertebrates, which should be considered within future assessments for environmental hazards of microplastics.

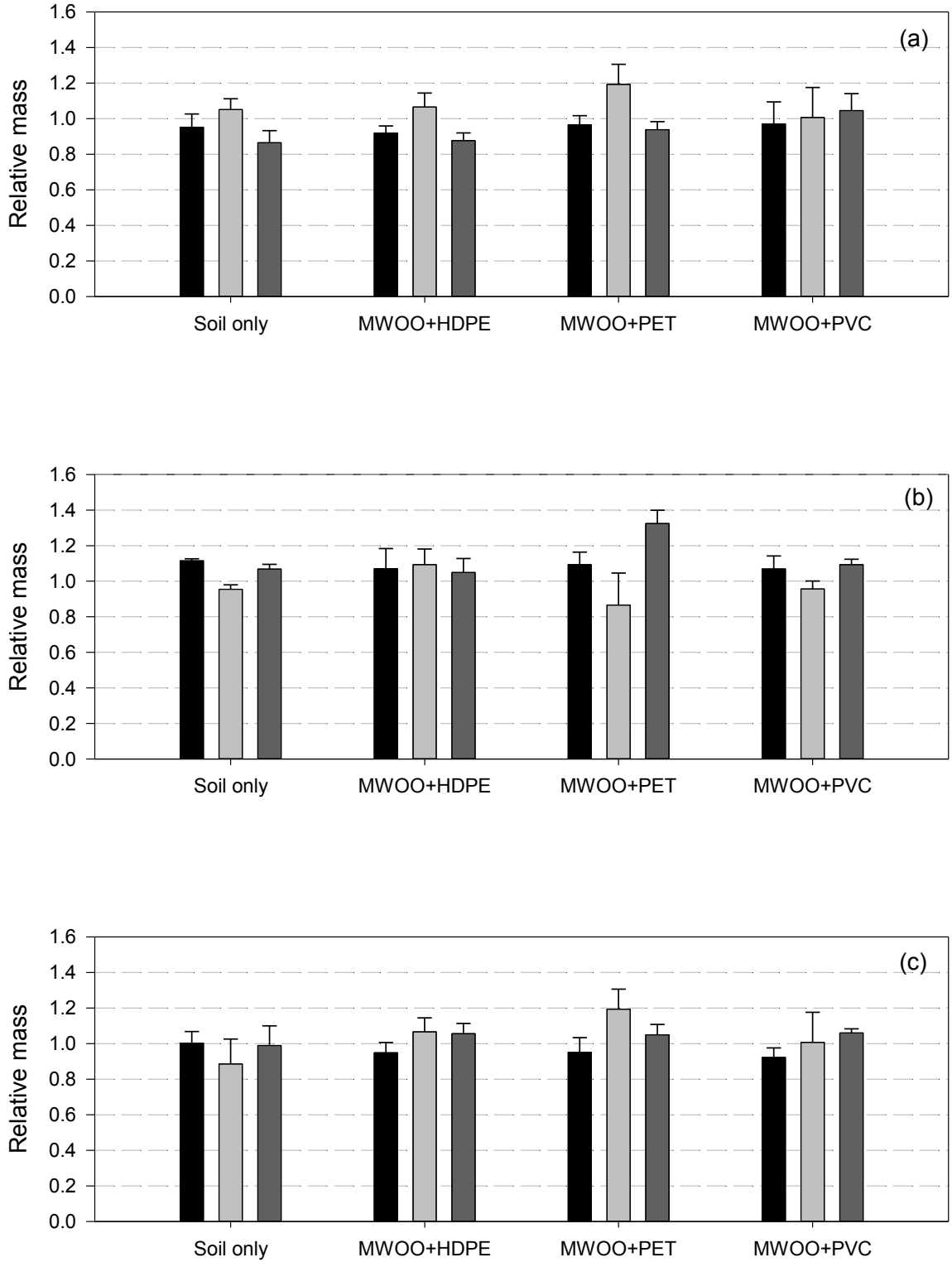


Figure 29. The mass of adult earthworms, relative to mass of adult earthworms (where 1 is equivalent mass) in MWOO + soil controls, removed after 28 days in treatments containing MWOO and microplastics spiked at 0.5% w/w (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples).

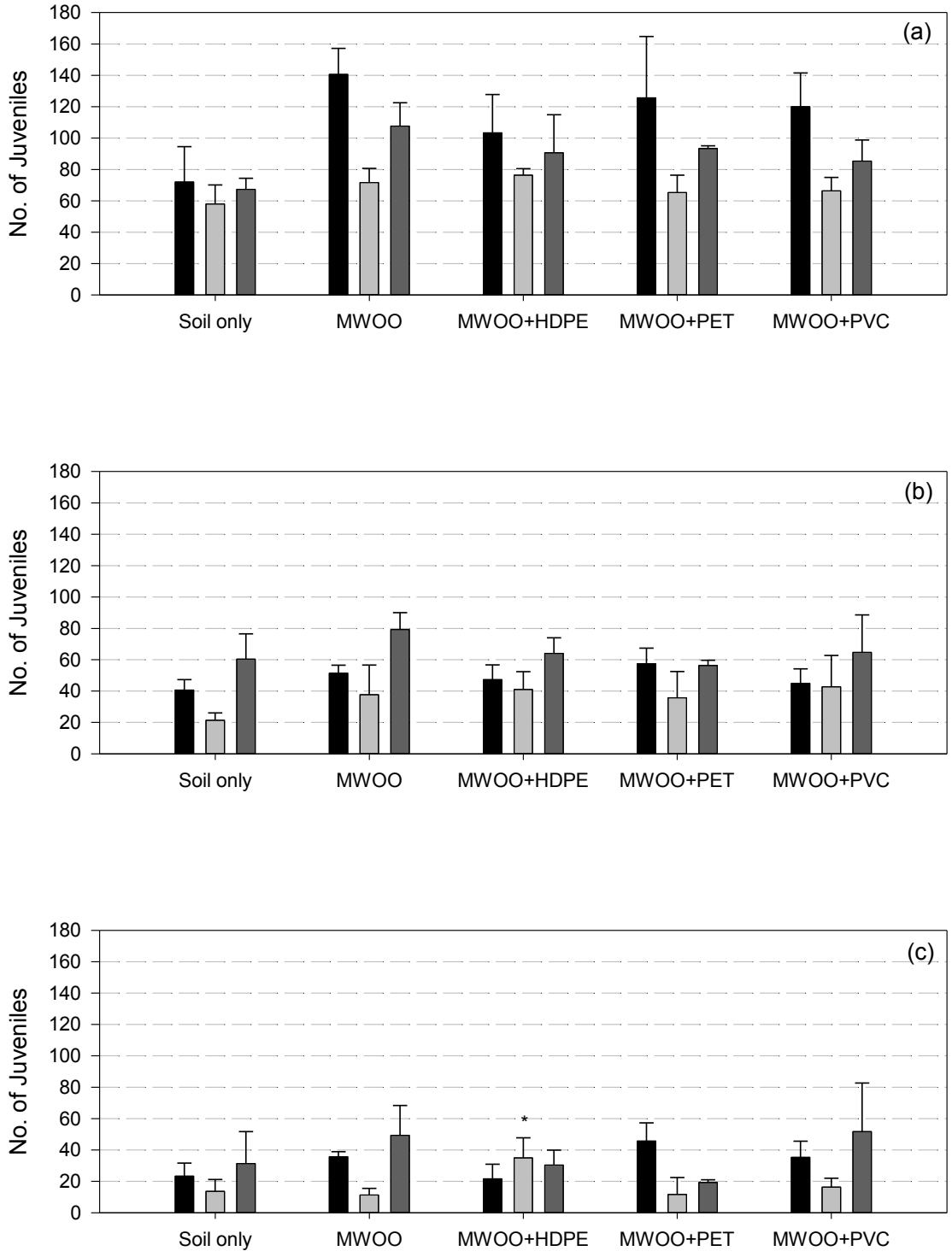


Figure 30. The number of juveniles counted 56 days after the addition and 28 days after the removal of adult worms in treatments containing MWOO and microplastics spiked at 0.5% w/w (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes samples significantly different (p<0.05) from MWOO control.

4.2.7 Wheat seedling emergence and growth

The influence of microplastics addition to the soil treatments on wheat seedling emergence and growth is summarised in Figures 31 and 32.

Even after incubation of the soils for up to 9 months, the addition of the three microplastics at 0.5% w/w (in addition to the microplastics already present in the MWOO) did not have an effect on the emergence of the wheat seedlings since germination of the seedlings was not significantly different from 100 %, irrespective of the treatment (Figure 31). With respect to the effect of microplastics on the growth rate of the seedlings over 14 d, the biomass of the seedlings were similarly unaffected with no significant differences observed between the MWOO-amended controls and treatments (Figure 32).

Plants such as wheat have been previously found to be a less sensitive species compared with other soil invertebrates when exposed to a number of different contaminants, including those which may have been expected to be present in MWOO or microplastics (Jansch et al. 2007, Amorim et al. 2010, Silva et al. 2014). This is consistent with the present study where the comparative sensitivity of the other ecotoxicity assays was greater than the plant assays. Of the metals measured in the soil solutions, Cu is considered to be more toxic than Zn to wheat seedlings (Wang et al. 2011), although the concentrations of Cu in pore water were likely to be much lower than toxicity thresholds previously reported for a range of endpoints in wheat (Paschke and Redente 2002).

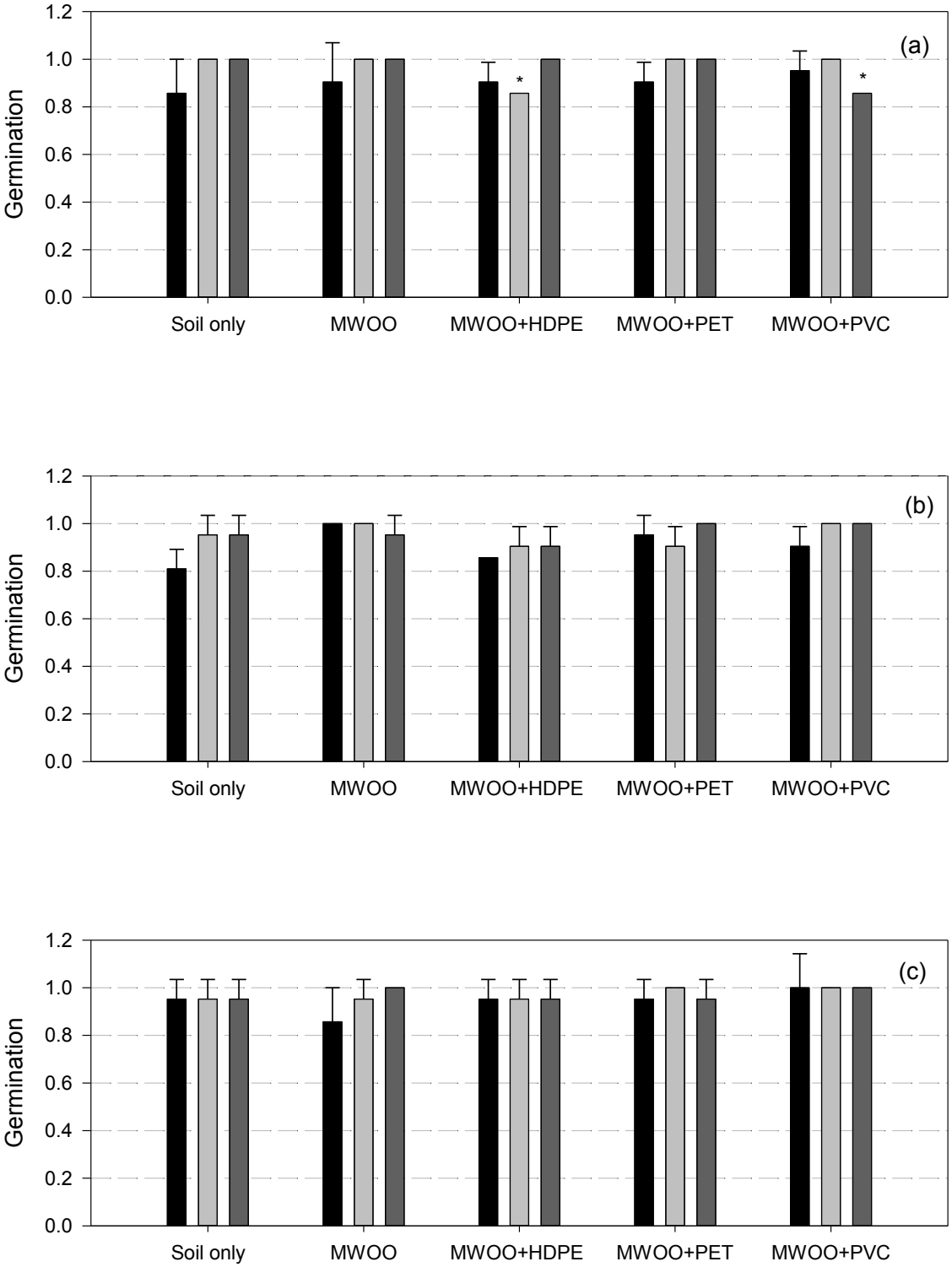


Figure 31. The proportion emergence of wheat (*Triticum aestivum*) seedlings in treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes samples significantly different (p<0.05) from MWOO control.

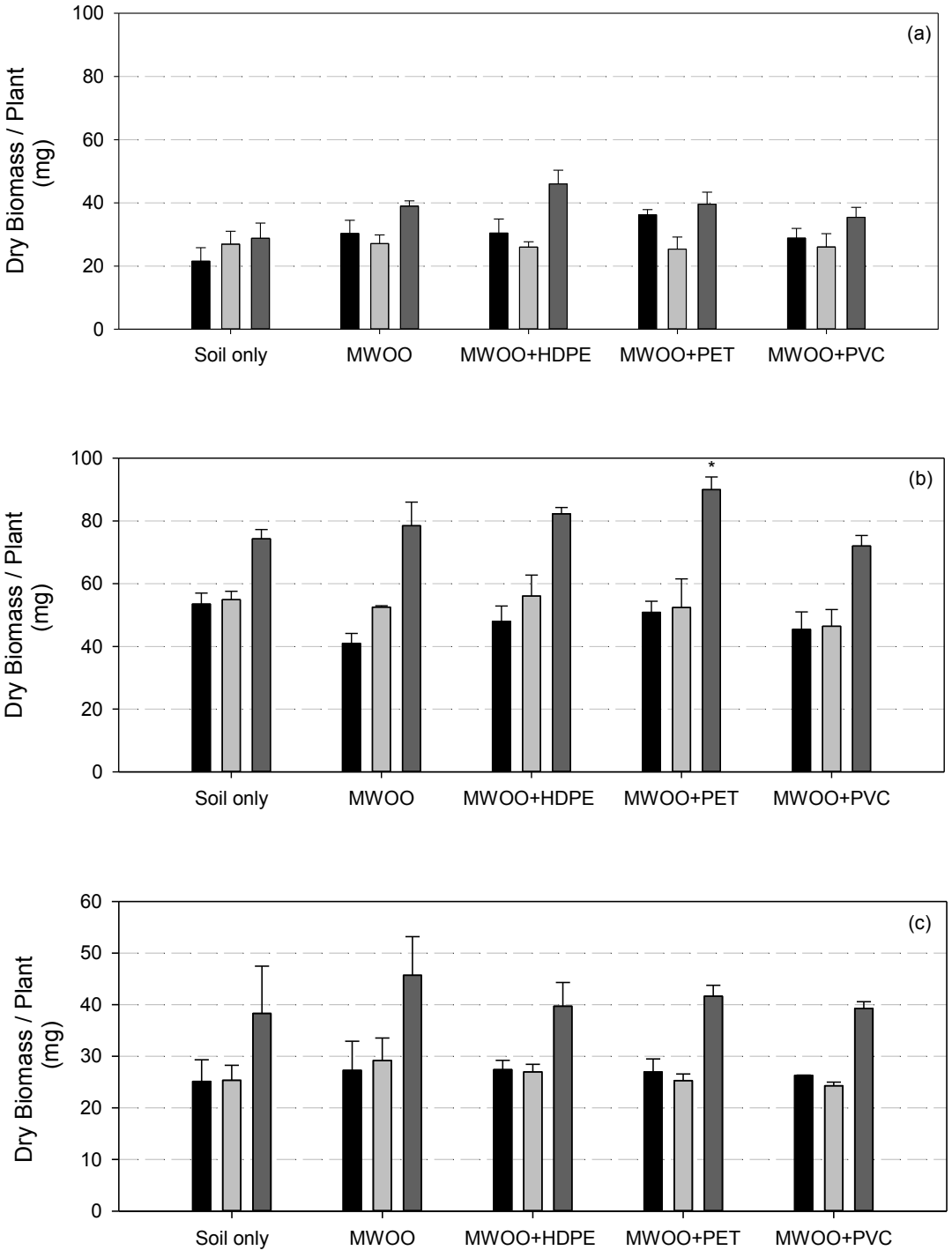


Figure 32. The mean biomass of wheat (*Triticum aestivum*) seedlings in treatments containing MWOO and microplastics spiked at 0.5% w/w in (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam after 0 (■), 3 (□) and 9 (▩) months incubation of the soil. Values represent mean ± standard deviation (n=3 samples). Asterix denotes samples significantly different (p<0.05) from MWOO control.

5 Conclusions and recommendations

Under the experimental conditions of this study the addition of MWOO containing microplastics at an equivalent rate to 10 t/ha, as well as additional dosing of three microplastics (HDPE, PET and PVC), did not lead to significant negative effects for a number of soil toxicological assessments representing a range of species and trophic levels.

Where significant effects were found, such as within the SIR and SIN assays, there were no consistent trends or concentration-response relationships observed between the rate of microplastic addition and the effect. For example, while addition of PVC to MWOO-amended Warialda Loam incubated for 9 months was found to lead to a decrease in SIR of the soil, relative to controls, SIR significantly increased in the 0.5 % w/w PVC treatment. This was also the case for PVC in Kirby Clay, where a significant decrease of SIN was observed to be independent of microplastic concentration.

With respect to other microbial endpoints no significant effect on genes related to the nitrogen cycle and microbial diversity was found following the addition of MWOO (10 t/ha) or microplastics (0.01 to 1% w/w) to soils incubated for up to 9 months. Effects relating to the length of incubation time and soilt type were, however, evident and suggested that extended incubation periods may be included in other future assessments on microplastics. This would have the additional advantage of allowing physical transformations of microplastics to proceed to a greater extent.

As with the soil microbial endpoints, there were also no negative significant effects or trends of negative effects noted for wheat seedling emergence and growth, earthworm avoidance, growth and reproduction or nematode survival and reproduction assays.

The assessment of chemical fate, for the batch sorption and soil solution analysis, suggested the major driver of changes in concentrations of organic contaminants and trace metals in pore water was likely to be related to the addition of MWOO and time of incubation, rather than addition of microplastics. Trace metal concentrations tended to decrease over the incubation period although there were a few exceptions, where Mn, Zn, Cu, As and Pb, were found to increase after 9 months of incubation.

In some instances, the addition of microplastics increased the soil pore water concentrations of trace metals, including Mn, Co, Ni, Zn, and Pb, in unincubated soils. The presence of MWOO and increasing incubation time, however, usually led to a reduction in metal concentrations. The lower trace metal concentrations in soil solutions for some treatments suggests the added microplastics were able to remove some metals into fractions, possibly through adsorption or precipitation reactions, that could not be readily released into soil solutions. In the case of Zn and Mn, where addition of microplastics, MWOO and the 9 month incubation period led to an increase in soil solution concentrations, the measured concentrations were unlikely to correspond with toxicity in the soil organisms assessed based on available literature. This conclusion is supported by the lack of toxicity observed in the various ecotoxicity endpoints in this study.

For the batch sorption analysis, there were no clear trends relating to either the addition of MWOO and microplastics, or the incubation period of the soils. For a pesticide like MCPA, batch sorption suggested that, despite the presence of microplastics and MWOO, it is likely to remain largely in soil solution where it is potentially bioavailable. Chemical analysis of the soil and pore water for 39 organic contaminants, including MCPA, was not able to measure quantifiable concentrations, further supporting the lack of observed toxicity in soil microorganism assays. Assays on higher order soil organisms, including earthworms, nematodes and wheat, also did not reveal any negative effects relating to the addition of microplastics in the soils.

This study has used a number of different chemical and soil ecotoxicological assessments, giving multiple lines of evidence to test the potential effects of microplastics in NSW agricultural soils. The soils themselves were broadly representative of a range of physicochemical and structural properties and were collected from a NSW agricultural region. Furthermore, the microplastics were added along with those already present within the supplied MWOO. This conservatively covered a number of assumptions, including the case where MWOO was added at a higher rate than is currently allowed and the organic matter degraded to leave a proportionately higher load of plastics in the soil. This scenario could also account for higher loadings of microplastics in MWOO, which may have increased due to greater consumer inputs.

Overall, this study suggests that the addition of microplastics (HDPE, PET and PVC) to MWOO at a rate of up to 1% w/w in agricultural soils with a range of physical and chemical properties will have little significant negative effects on a range of terrestrial organisms including microbes, earthworms, nematodes and plants. In some cases, addition of microplastics to MWOO-amended soil was found to have significant negative effects on microbial function, such as where PVC affected SIN and SIR in treatments incubated for 9 months. These effects did not show a concentration-response relationship, however, and were variable following ageing of the treatments. Furthermore, the highest rate of PVC added to the soil is considerably greater than what would be expected to be present in MWOO since PVC is likely to be only a minor component of the total plastics load in MWOO.

Despite these conservative assumptions, there are a number of other considerations future studies may consider. For example, the inclusion of other common soil organisms such as arthropods (e.g. slaters, collembolan, mites)would further increase the conservative nature of the assumptions relating to ecotoxicity being unlikely due to the presence of microplastics in soil. With respect to the experimental conditions, a relatively narrow range of environmental parameters such as temperature, soil moisture and light exposure were used to limit the number of variables that are likely to have influenced the chemical and ecotoxicological assessments. To more accurately reflect the fate of microplastics and MWOO, the fate of chemicals associated with microplastics and MWOO and subsequent ecotoxicological impacts would require additional treatments including a broader range of environmental conditions relevant to those expected under field conditions. Also, this study may have benefited from being less constrained with respect to the time of soil incubation. The nature of the microplastics used in this study, being highly resistant to environmental degradation, suggest that a considerably longer incubation period may be necessary to cover a greater extent of their chemical or physical degradation and subsequent interaction with organic and inorganic chemicals present in the soil mixtures. The degradation of organic matter from added MWOO would also have occurred to a greater extent, reducing the apparent protective effects it had on concentrations of trace metals and organic chemicals in soil solutions.

Further assessments of microplastics in MWOO should be considered if future regulations allow scenarios where rates of MWOO addition can be increased or applied on multiple

occasions, such that the conservative scenarios covered in this study are exceeded. Also, if the inputs of plastics within waste streams are increasing or changing in nature, then additional studies on the potential soil toxicity of these microplastics should be considered. For example, changing consumer behaviour in their use of other polymers may alter the types of microplastics in the waste stream. Compostable and biodegradable plastics, containing different polymers and/or chemical additives to change the environmental fate of the polymers, are becoming increasingly popular and market-based decisions or regulation could further increase their use rates. Biodegradable plastics contain relatively high concentrations of trace metals, especially Ce, Mn, Co and Fe, which are used to catalyse the degradation of the polymer chains and which would ultimately be present in the final MWOO product (Roy et al. 2011).

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7 Appendix A. Soil selection and characterisation

Table B 1. Summary of physicochemical properties of agricultural soils selected for the assessment.

Soil Parameters	Kirby Sand	Kirby Clay	Warialda Loam
pH (H_2O)	5.98±0.03	6.08±0.03	8.88±0.06
EC (µS/cm)	25±1	117±5	117±2
MWHC (g/100g)	36±2	75±1	45±2
Clay %	11	46	34
Silt %	10	24	13
Sand %	76	13	20
Nitrogen %	0.03±0.01	0.41±0.01	0.03±0.01
Total Organic Carbon %	0.34±0.02	4.67±0.1	0.47±0.02
Phosphorus (mg/kg)	115±8	1176±15	242±3
Extractable P (mg/kg)	4.8±0.03	146±2.5	4.4±0.13
CEC ($cmol_+/kg$)	5.5±0.07	132.5±3.72	5.2±0.09
Total Zinc (mg/kg)	14.9±1.1	77±1.1	49±0.8
Total Copper (mg/kg)	10.9±0.7	40±0.6	23±1.4
Total Nickel (mg/kg)	6±1.7	101±4.7	8±1.7
Total Lead (mg/kg)	10.1±0.4	4.1±0.2	7.4±0.2
Total Tin (mg/kg)	2.4±0.9	<1	<1

Table B 2. Summary of physicochemical properties of MWOO.

Parameter	Value
pH (H_2O)	7.1
EC ($\mu S\ cm^{-1}$)	5500
Moisture content (%)	5.3
Organic carbon (%)	16
Total nitrogen (%)	0.92
Extractable phosphorus (mg/kg)	220
CEC ($cmol_+/kg$)	17
Clay (%)	4.2
Silt (%)	5.8
Sand (%)	28
Maximum water holding capacity (%)	74

8 Appendix B. Plastic Selection and Characterisation

Table B 1. Microplastics size characterisation resulting from measurements of at least 100 particles from at least three different micrographs. As many particles were irregularly shaped, diameters were measured across each particle's smallest dimension..

Microplastics	Particle Diameter (mm) (average ± standard deviation)
HDPE	1.43±0.55
PET	1.51±0.46
PVC	1.18±0.36

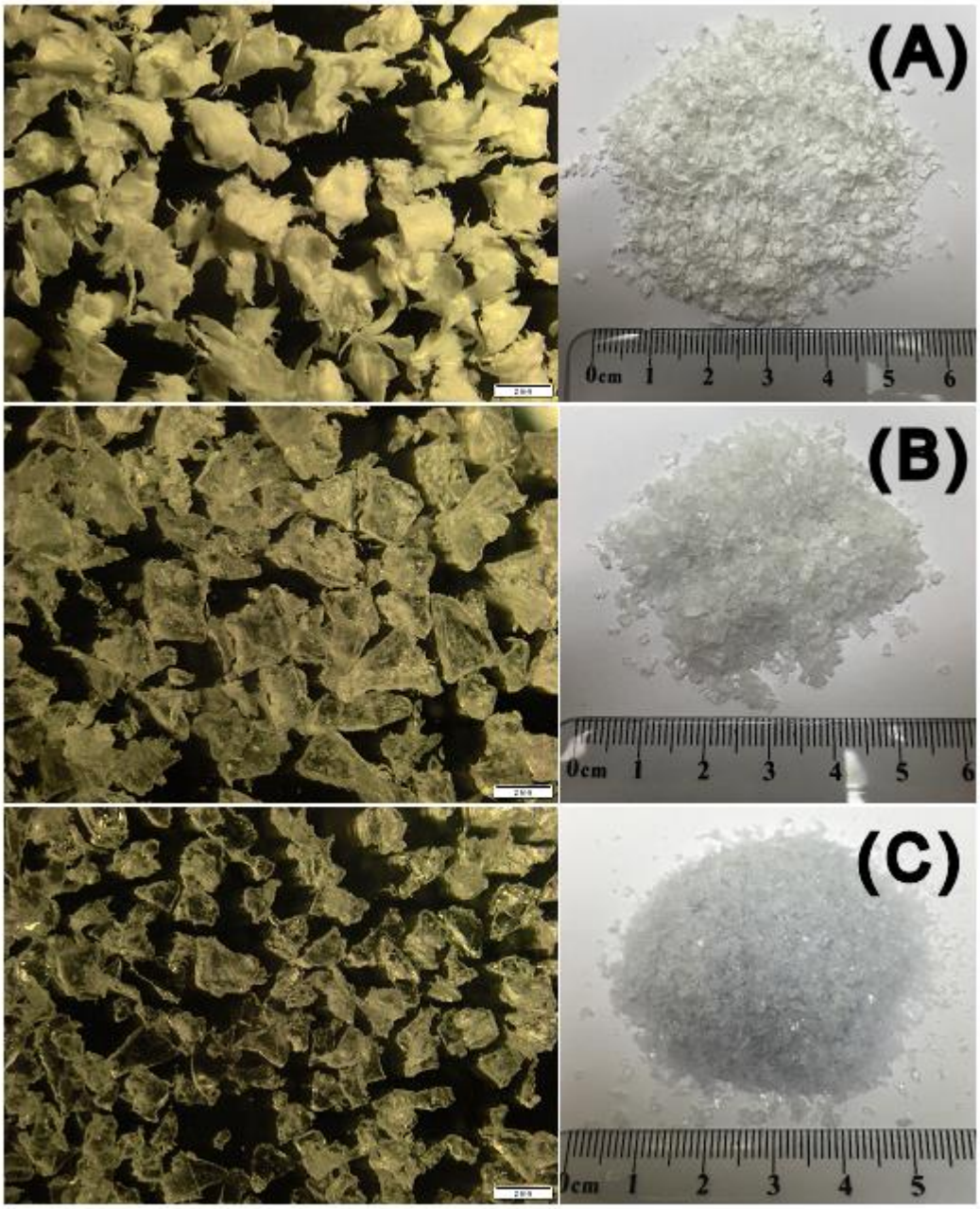


Figure B 1. Dark-field micrographs (left), generated using a Scanning Electron Microscope (SEM) model 1012 disintegrator, and photographs (right) of the HDPE bags (A), PET bottles (B) and PVC tablecloth (C) following shredding and sieving.

Table B 2. Summary of chemical analysis by ICP-OES and ICP-MS of microplastics selected for the assessment.

Soil Parameters	HDPE	PET	PVC
Calcium (mg/kg)	34200	<250	<250
Potassium (mg/kg)	<250	<250	<250
Magnesium (mg/kg)	176	<250	<250
Sodium (mg/kg)	189	187	207
Sulphur (mg/kg)	245	<100	<100
Phosphorus (mg/kg)	575	531	712
Aluminium (mg/kg)	184	<50	<50
Arsenic (mg/kg)	<0.4	<0.4	<0.4
Cadmium (mg/kg)	<0.1	<0.1	<0.1
Cobalt (mg/kg)	0.3	<0.3	0.3
Chromium (mg/kg)	2.4	<1	<1
Copper (mg/kg)	<3	<3	<3
Iron (mg/kg)	20	7	16
Manganese (mg/kg)	<1	<1	<1
Molybdenum (mg/kg)	<0.3	<0.3	<0.3
Nickel (mg/kg)	<3	<3	<3
Lead (mg/kg)	0.8	0.3	0.3
Antimony (mg/kg)	<1	230	<1
Tin (mg/kg)	<1	<1	<1
Zinc mg/kg)	286	<3	152

Total concentrations of the selected metals in the plastics were determined using a strong acid microwave digestion procedure followed by analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES, Thermo iCAP 6000) and/or inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700). Approximately 0.1 g (3 replicates) of each plastic was weighed into 50 mL perfluoroalkoxy resin (PFA) digestion vessels with 9 mL concentrated nitric acid and 1 mL hydrogen peroxide added. The samples were cold digested for 12 h, sealed and microwave digested (CEN MARS 5) using the following time and temperature program: 15 min ramp to 100 ^{o}C, 15 min ramp to 150 ^{o}C, 15 min ramp to 200 ^{o}C and 90 min hold at 200 ^{o}C. After digestion, samples (10 mL) were transferred to 50 mL centrifuges tubes, diluted to 50 mL using ultrapure deionised water (Milli-Q, Millipore) and filtered through 0.45 µm syringe filters (Sartorius) before analysis. The filtered samples were analysed for selected metals using inductively coupled plasma-optical emission spectrometry (ICP-OES) and/or inductively coupled plasma-mass spectrometry (ICP-MS). Two certified reference materials (ERM-EC680m and ERM-EC681) were used to examine the accuracy of the digestion and analysis procedures (Table B3).

Table B 3. Certified and measured selected trace metal concentrations in low-density polyethylene certified reference materials (CRM).

Element	Low concentration CRM		High concentration CRM	
	ERM-EC680m		ERM-EC681m	
	Certified	Measured	Certified	Measured
	mg/kg			
As	4.7±0.4	4.2±0.32	17.0±1.2	16.7±1.4
Cd	20.8±0.9	18.9±1.0	146±5	152±5.9
Cr	9.6±0.5	9.5±0.6	45.1±1.9	46.7±3.2
Pb	11.3±0.4	10.8±0.6	69.7±2.5	68.3±2.0
Sb	9.6±0.7	8.5±0.8	86±7	78.6±4.3
Sn	20.7±1.6	18.9±0.5	99±6	ND
Pb	194±12	180±13	1170±40	1095±24

For analysis of organic chemicals, plastics (~1 g) were weighed into glass along with 5 mL of a solvent mixture of 50:50 methanol and acetone and ultrasonicated for 20 min at 50 ^{o}C. The tubes were then vigorously shaken by vortexing for 30 s, centrifuged for 30 min at 650 *g* and

the supernatant was removed. This extraction procedure was repeated a further two times, once more with methanol:acetone and once with dichloromethane. The solvent extracts were combined into a glass tube. The solvent extracts were then gently blown to dryness under a stream of N_2 gas and reconstituted in 1 mL dichloromethane for GC-MS analysis. Due to the complex nature of polymers, a non-target analysis of the plastics was undertaken. Samples were samples were injected into an Agilent 6890 gas chromatograph (GC) coupled with a 5973 mass spectrometer (MS), with separation of analytes performed using an Agilent HP-5MS column. The MS was set to full scan mode, covering a mass range of m/z 50-500 and data from the GC-MS were analysed using the NIST/EPA/NIH mass spectral library (2002) within Agilent MSD Chemstation software (version D.02.00.275). Mass spectral data of all significant peaks (defined as approximately >3 times the signal to noise ratio of the baseline) were compared with mass spectral data stored in the NIST library, which contains mass spectra for more than 147,000 organic compounds. A range of organic compounds were found in the plastics relating to the manufacture of polymers, including plasticisers (e.g. glycerine), preservatives (e.g. 2,4-di-*t*-butylphenol) and lubricants (e.g. stearic acid), although none of the target compounds (Table F1) were detected.

Since phthalates, especially DEHP, are detected in MWOO in mg/kg concentrations, plastic and MWOO samples were also sent to the National Measurement Institute (NMI) for phthalate analysis. This revealed a broad phthalate peak in the PVC tablecloth (Figure B2) but no phthalates were detected in the MWOO (Figure B3).

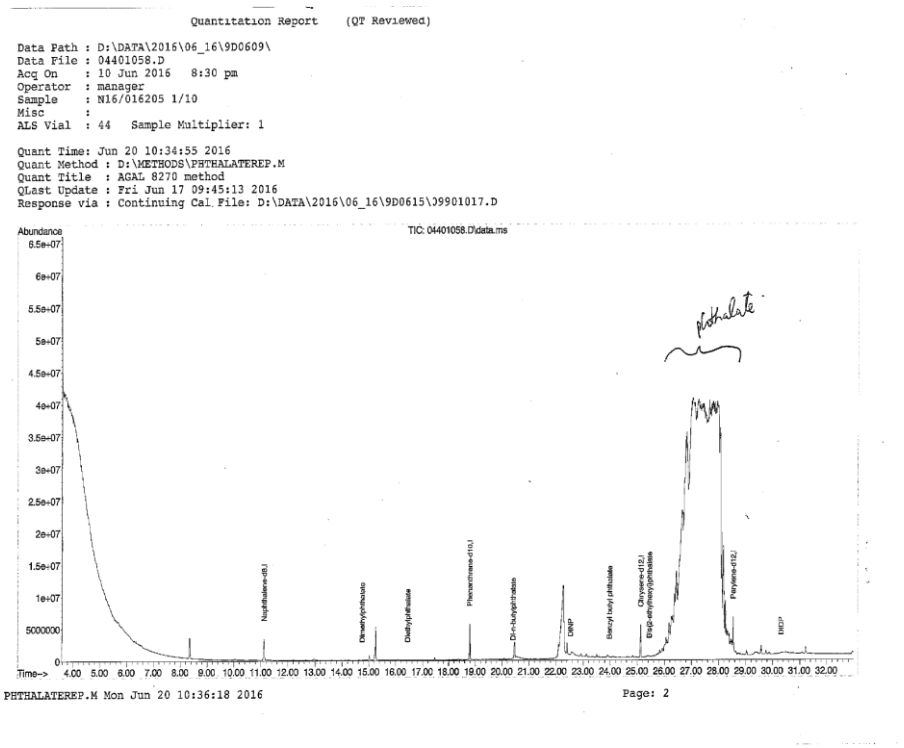


Figure B 2. A GC-MS chromatogram of a solvent extract of PVC tablecloth showing a substantial and broad phthalate peak from 26 to 29 minutes.

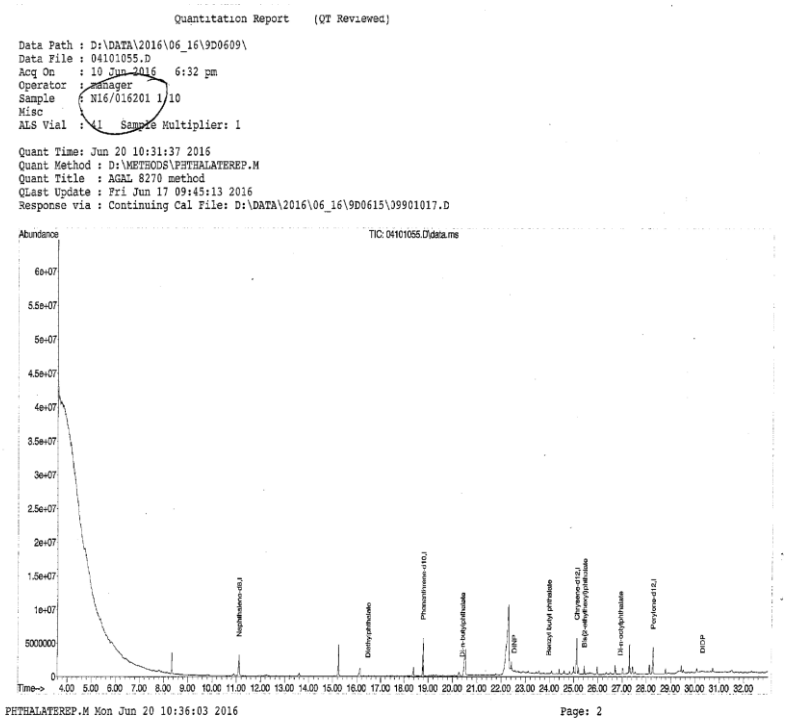


Figure B 3. A GC-MS chromatogram of a solvent extract of MWOO showing an absence of phthalate peaks.

9 Appendix C. Batch sorption - effect of time on batch sorption solution pH

Due to the use of 0.01 M $CaCl_2$ in the batch sorption experiments, the measured solution pH for the respective soils was less than that measured in the initial characterisation of the soil (Table A1). These pH values are lower than those found in the soil solutions due to an increase in solution H^+ concentrations due to displacement by the additional Ca^{2+}.

Table C 1. Average (± standard deviation) pH for each soil, in a 1:5 0.01 M $CaCl_2$ (w/v) solution for each incubation period (0, 3 and 9 months).

Soil	0 months		3 months		9 months	
	Blank	Spiked	Blank	Spiked	Blank	Spiked
Kirby Clay	5.09 ±0.04	5.00±0.06	5.62±0.14	5.38±0.11	5.19±0.1	5.07±0.16
Kirby Sand	4.64±0.31	4.47±0.27	5.25±0.54	4.98±0.34	5.07±0.31	4.93±0.26
Warialda Loam	7.01±0.10	6.95±0.10	7.36±0.34	7.35±0.07	7.45±0.06	7.37±0.11

10 Appendix D. Soil solution - effect of MWOO and incubation time on pH and EC

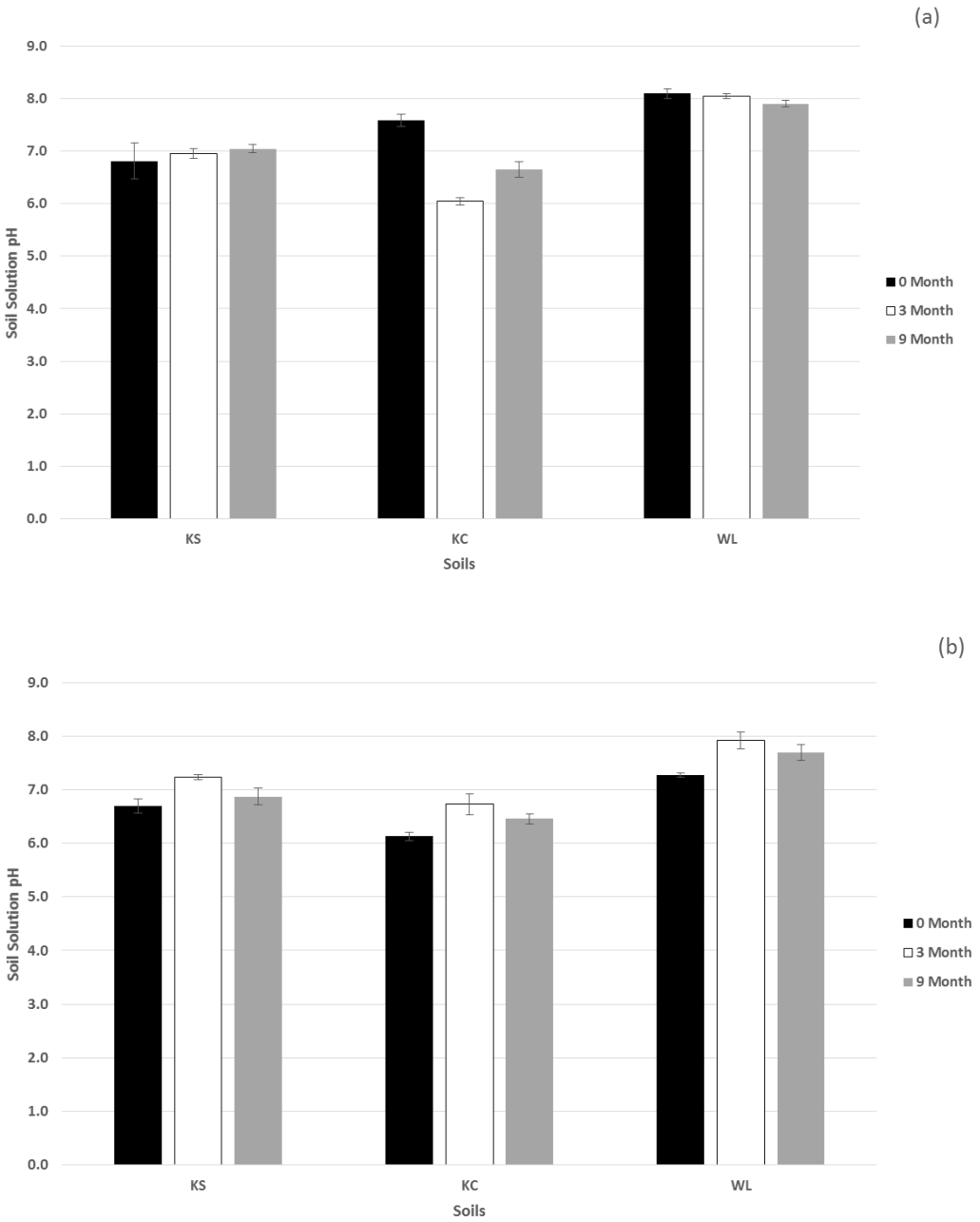
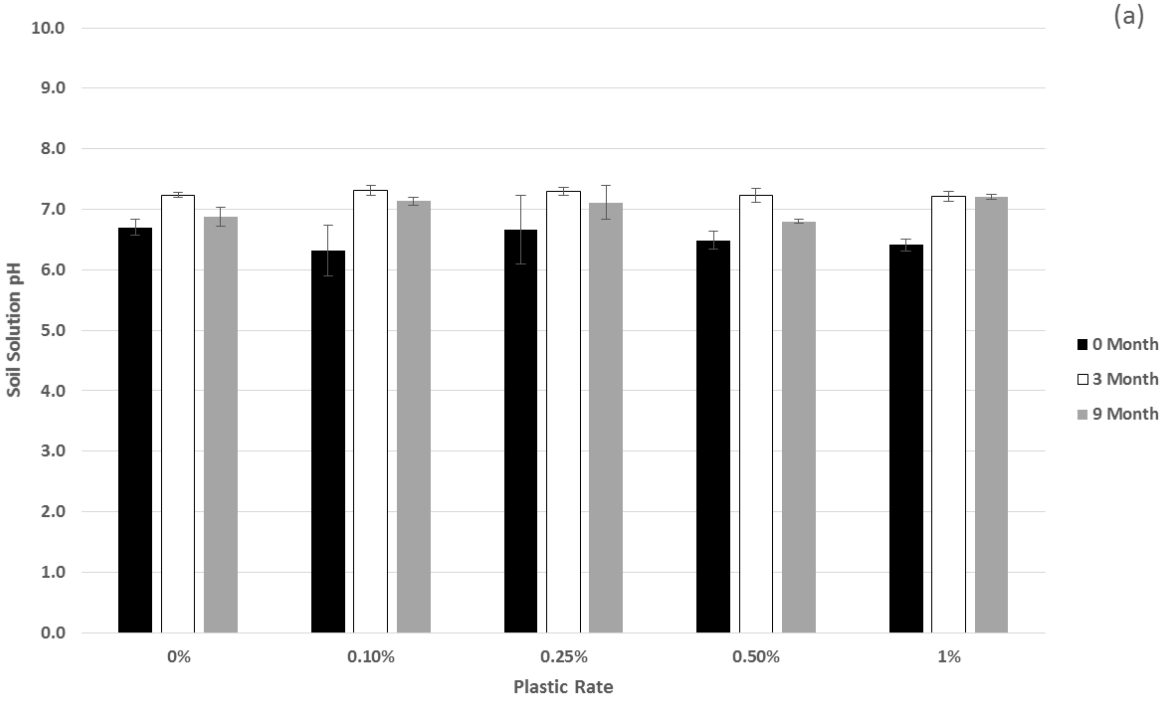
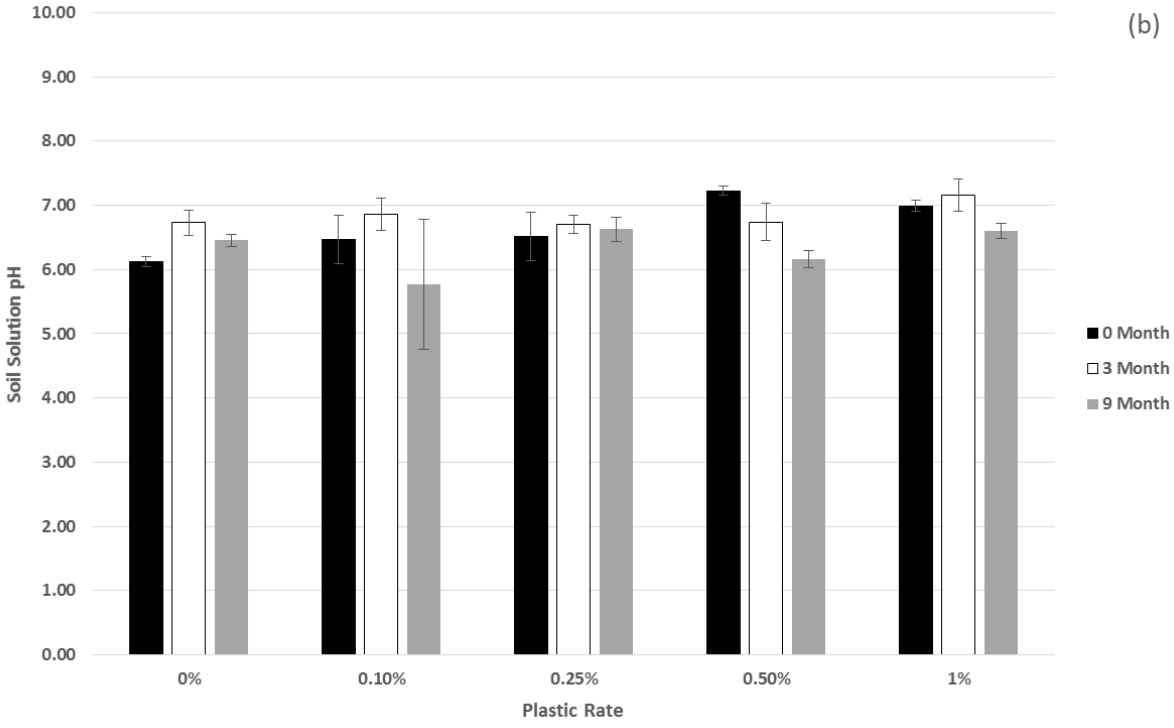


Figure D 1. Soil solution pH after 0, 3 and 9 months incubation in (a) soil and (b) MWOO-amended soil controls for Kirby Sand (KS), Kirby Clay (KC) and Warialda Loam (WL).

(a)


10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Soil Solution pH
0%
0.10%
0.25%
0.50%
1%
Plastic Rate
0 Month
3 Month
9 Month

(b)


10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Soil Solution pH
0%
0.10%
0.25%
0.50%
1%
Plastic Rate
0 Month
3 Month
9 Month

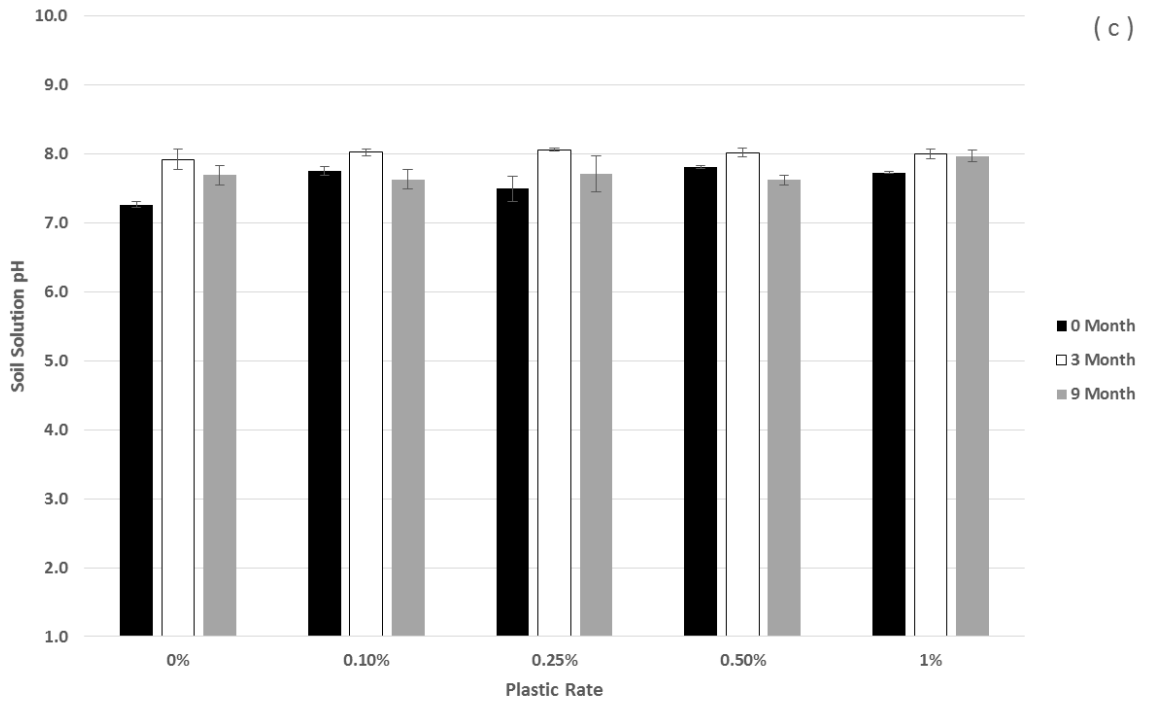


Figure D 2. Example of the influence of increasing amounts (rates) of HDPE on soil solutions pH after 0, 3 and 9 months incubation in MWOO-amended (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam soil controls.

Table D 1. Soil solution pH from soil and MWOO-amended soil treatments with added microplastics following incubation for 0, 3 and 9 months.

Soil	Rate	Microplastic	Incubation Period (months)			Soil	Rate	Microplastic	Incubation Period (months)		
			0	3	9				0	3	9
Kirby Sand	0%	HDPE	6.81±0.35	6.95±0.09	7.04±0.08	Kirby Sand +MWOO	0%	HDPE	6.7±0.13	7.24±0.04	6.87±0.16
	0.1%		6.9±0.39	6.91±0.15	7.06±0.17		0.1%		6.31±0.42	7.31±0.09	7.13±0.07
	0.25%		6.84±0.15	7.04±0.28	6.96±0.1		0.25%		6.67±0.57	7.3±0.06	7.11±0.28
	0.5%		6.64±0.21	7.01±0.21	7.15±0.46		0.5%		6.49±0.14	7.23±0.12	6.8±0.03
	1%		6.84±0.16	7.12±0.1	6.58±0.26		1%		6.41±0.1	7.21±0.08	7.21±0.04
Kirby Clay	0%	HDPE	7.58±0.12	6.04±0.07	6.65±0.15	Kirby Clay +MWOO	0%	HDPE	6.13±0.08	6.73±0.2	6.46±0.09
	0.1%		6.56±0.05	6.95±0.2	6.34±0.18		0.1%		6.47±0.38	6.86±0.26	5.77±1.01
	0.25%		6.94±0.56	7±0.09	6.44±0.14		0.25%		6.52±0.38	6.71±0.14	6.63±0.19
	0.5%		6.67±0.18	6.81±0.18	6.23±0.4		0.5%		7.23±0.08	6.74±0.3	6.16±0.14
	1%		7.38±0.2	6.9±0.16	6.23±0.37		1%		7±0.09	7.15±0.25	6.6±0.12
Warialda Loam	0%	HDPE	8.09±0.09	8.04±0.04	7.9±0.06	Warialda Loam +MWOO	0%	HDPE	7.27±0.04	7.92±0.15	7.69±0.14

	0.1%		7.88±0.07	7.98±0.16	7.93±0.09		0.1%		7.75±0.06	8.02±0.05	7.63±0.14
	0.25%		8.1±0.04	8.05±0.06	8.09±0.18		0.25%		7.49±0.19	8.06±0.02	7.71±0.26
	0.5%		8.06±0.05	8.08±0.01	8.01±0.02		0.5%		7.81±0.02	8.02±0.07	7.62±0.07
	1%		8.13±0.12	8.06±0.03	7.85±0.19		1%		7.73±0.01	8±0.07	7.97±0.09
Kirby Sand	0%	PET	6.81±0.35	6.95±0.09	7.04±0.08	Kirby Sand +MWOO	0%	PET	6.7±0.13	7.24±0.04	6.87±0.16
	0.1%		6.35±0.09	7.06±0.24	7.27±0.61		0.1%		6.38±0.15	7.18±0.06	7.17±0.06
	0.25%		6.29±0.18	6.88±0.26	7.13±0.19		0.25%		6.51±0.14	7.24±0.09	7.19±0.47
	0.5%		6.68±0.13	7.08±0.27	6.67±0.65		0.5%		5.91±0.06	7.46±0.12	6.8±0.12
	1%		6.78±0.11	7.06±0.16	6.28±0.18		1%		5.87±0.06	7.12±0.1	7.41±0.45
Kirby Clay	0%	PET	7.58±0.12	6.04±0.07	6.65±0.15	Kirby Clay +MWOO	0%	PET	6.13±0.08	6.73±0.2	6.46±0.09
	0.1%		6.41±0.19	6.47±0.21	6.55±0.15		0.1%		7.26±0.16	7.04±0.28	6.87±0.06
	0.25%		6.53±0.09	6.71±0.16	6.58±0.29		0.25%		6.58±0.23	6.65±0.09	6±0.26
	0.5%		6.67±0.23	6.45±0.45	6.36±0.07		0.5%		7.03±0.35	6.81±0.14	6.39±0.25
	1%		6.71±0.1	6.94±0.14	6.66±0.29		1%		6.85±0.08	7.01±0.22	6.64±0.02

Warialda Loam	0%	PET	8.09±0.09	8.04±0.04	7.9±0.06	Warialda Loam +MWOO	0%	PET	7.27±0.04	7.92±0.15	7.69±0.14
	0.1%		7.98±0.08	8.09±0.03	7.98±0.14		0.1%		7.75±0.02	7.88±0.17	7.92±0.07
	0.25%		7.88±0.16	8.04±0.02	8.09±0.09		0.25%		7.35±0.1	7.99±0.07	7.59±0.08
	0.5%		7.95±0.05	7.95±0.08	8.08±0.25		0.5%		7.82±0.08	7.96±0.06	7.66±0.07
	1%		7.93±0.03	8.07±0.02	7.97±0.16		1%		7.91±0.06	7.97±0.08	7.77±0.2
Kirby Sand	0%	PVC	6.81±0.35	6.95±0.09	7.04±0.08	Kirby Sand +MWOO	0%	PVC	6.7±0.13	7.24±0.04	6.87±0.16
	0.01%		5.95±0.18	7.3±0.13	6.97±0.11		0.01%		6.17±0.07	7.29±0.14	7.29±0.11
	0.1%		5.94±0.1	7.07±0.07	7.22±0.09		0.1%		6.86±0.5	7.31±0.12	7.15±0.06
	0.25%		5.84±0.18	6.95±0.21	6.97±0.07		0.25%		5.89±0.06	7±0.11	7.22±0.13
	0.5%		5.98±0.23	7±0.04	6.98±0.27		0.5%		5.89±0.12	7.25±0.21	6.82±0.24
	1%		6.62±0.25	7.11±0.09	7.16±0.18		1%		6.53±0.34	7.4±0.17	7.35±0.25
Kirby Clay	0%	PVC	7.58±0.12	6.04±0.07	6.65±0.15	Kirby Clay +MWOO	0%	PVC	6.13±0.08	6.73±0.2	6.46±0.09
	0.01%		6.47±0.15	7.02±0.13	6.54±0.26		0.01%		7.13±0.5	7.21±0.58	6.86±0.25
	0.1%		6.91±0.06	7.06±0.19	6.57±0.04		0.1%		6.69±0.21	6.47±0.15	6.87±0.08

	0.25%		6.69±0.16	6.99±0.15	6.8±0.19		0.25%		6.8±0.13	7.02±0.14	6.75±0.12
	0.5%		6.52±0.24	6.61±0.29	6.78±0.26		0.5%		7.15±0.35	6.77±0.15	6.47±0.26
	1%		6.51±0.23	7.32±0.31	6.43±0.09		1%		7.07±0.33	7.33±0.21	7.29±0.17
Warialda Loam	0%	PVC	8.09±0.09	8.04±0.04	7.9±0.06	Warialda Loam +MWOO	0%	PVC	7.27±0.04	7.92±0.15	7.69±0.14
	0.01%		8.08±0.04	7.13±0.12	8±0.1		0.01%		7.98±0.04	7.97±0.07	7.9±0.05
	0.1%		8.08±0.03	7.95±0.06	7.94±0.07		0.1%		7.41±0.32	7.99±0.07	7.93±0.03
	0.25%		7.86±0.35	8±0.05	8.06±0.09		0.25%		7.85±0.1	7.97±0.07	7.91±0.09
	0.5%		7.98±0.04	7.72±0.56	8.02±0.04		0.5%		7.82±0.1	7.98±0.05	7.64±0.04
	1%		8.05±0.06	8.03±0.14	8.04±0.08		1%		7.83±0.09	7.98±0.11	8.01±0.05

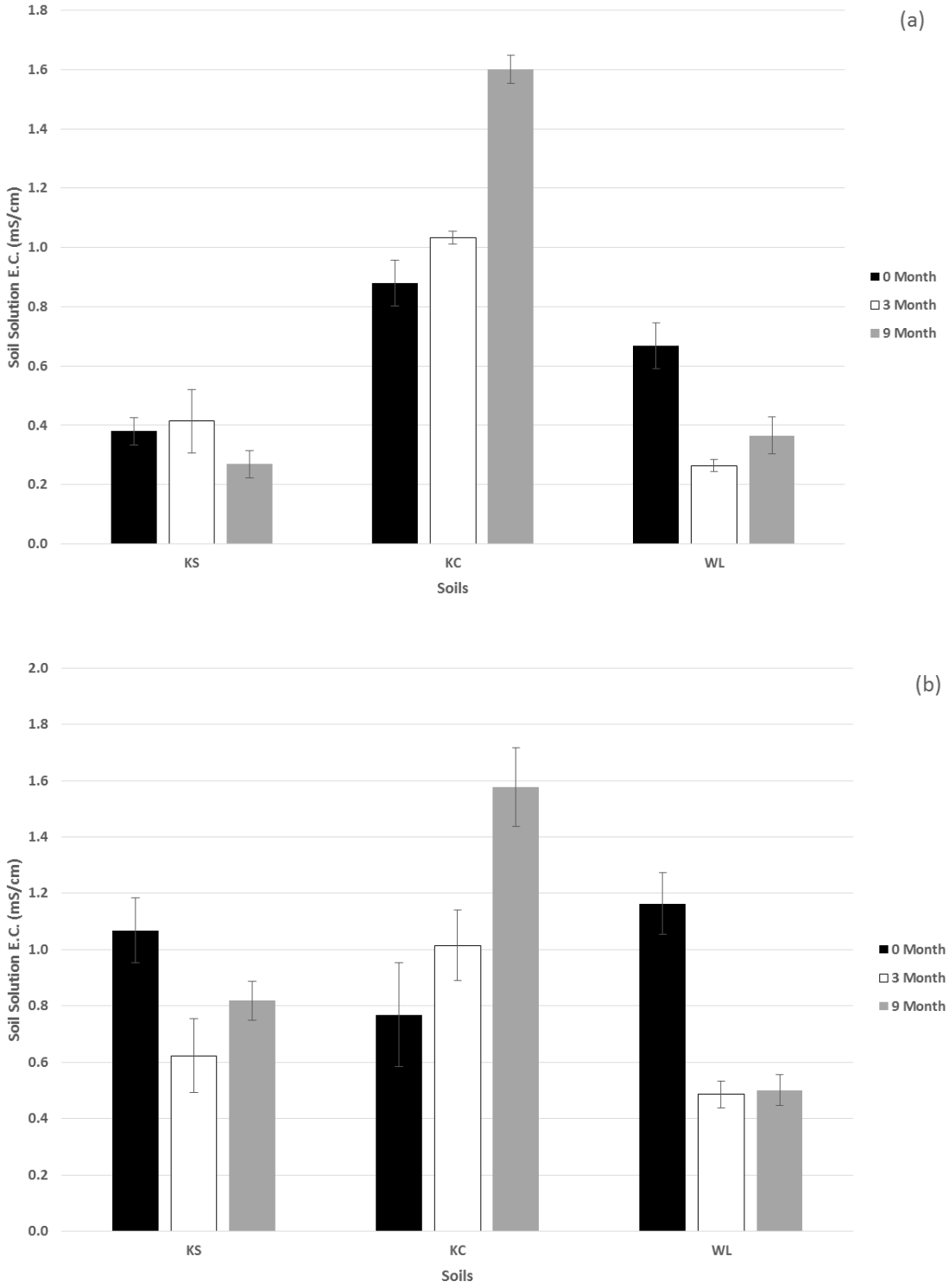
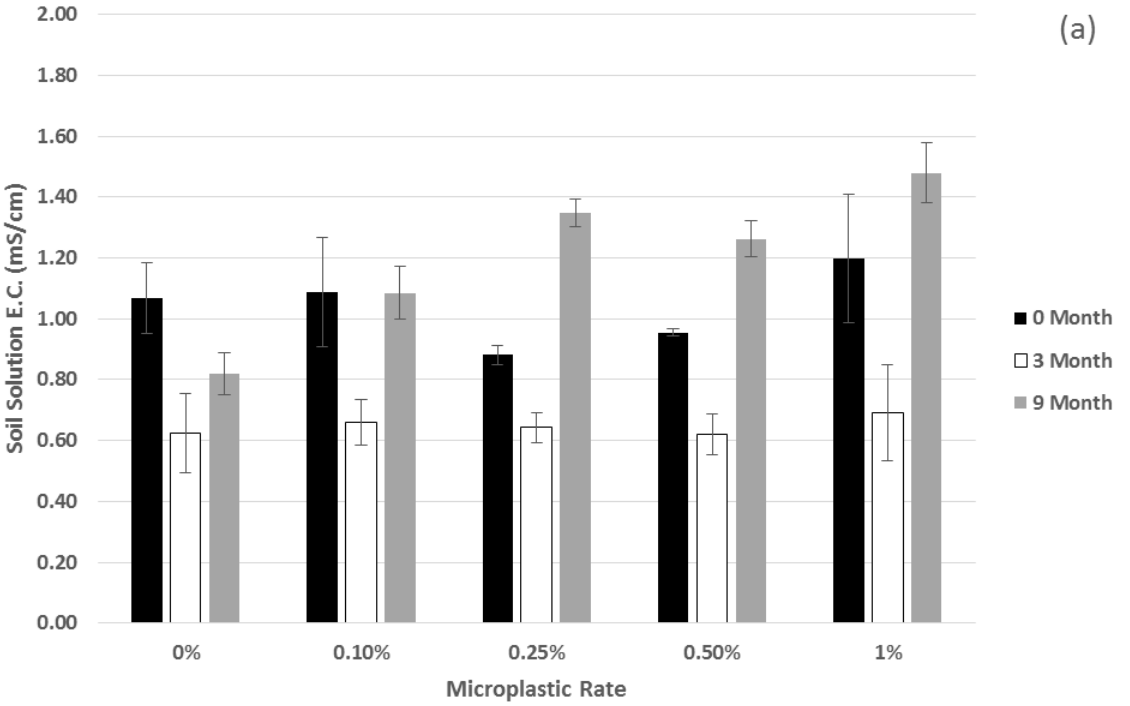
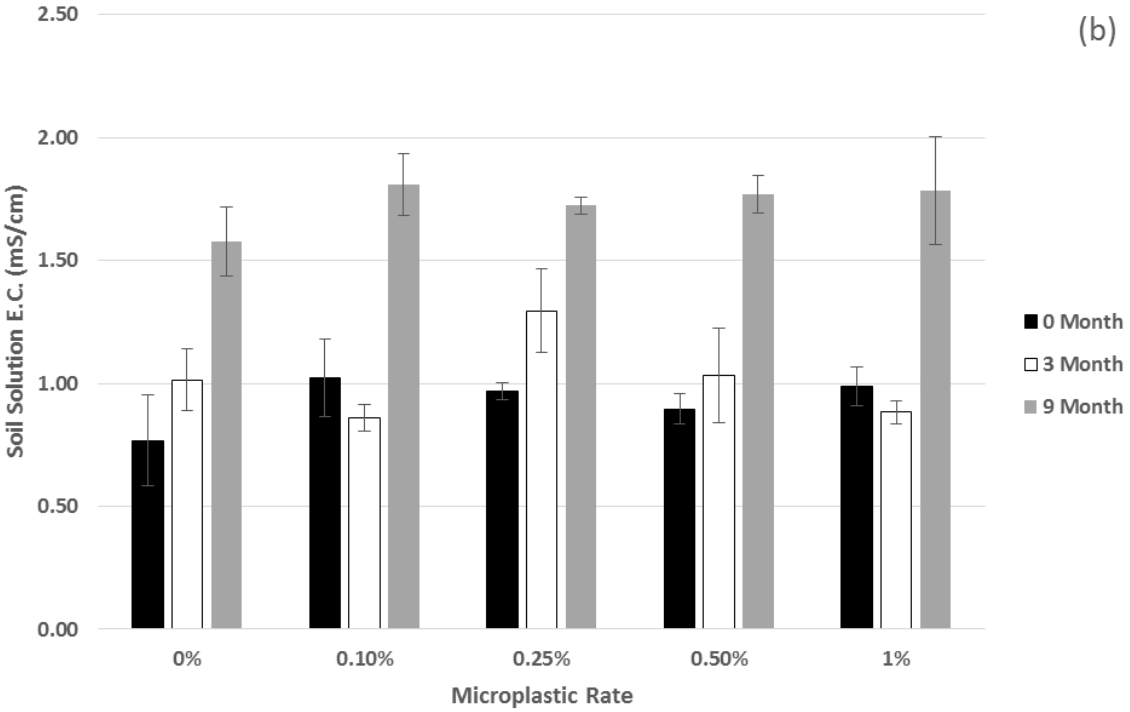


Figure D 3. Soil solution EC after 0, 3 and 9 months incubation in (a) soil and (b) MWOO-amended soil controls for Kirby Sand (KS), Kirby Clay (KC) and Warialda Loam (WL).


(a)
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Soil Solution E.C. (mS/cm)
0%
0.10%
0.25%
0.50%
1%
Microplastic Rate
0 Month
3 Month
9 Month


(b)
2.50
2.00
1.50
1.00
0.50
0.00
Soil Solution E.C. (mS/cm)
0%
0.10%
0.25%
0.50%
1%
Microplastic Rate
0 Month
3 Month
9 Month

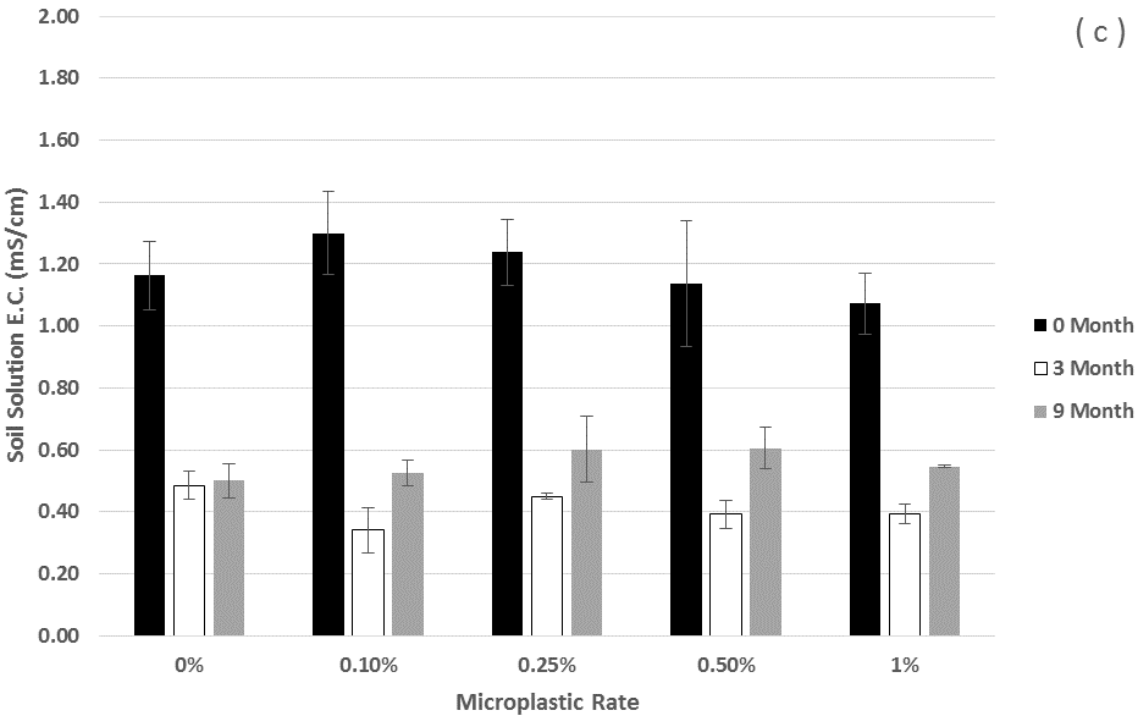


Figure D 4. Example of the influence of increasing amounts (rates) of HDPE on soil solution EC after 0, 3 and 9 months incubation in MWOO-amended (a) Kirby Sand, (b) Kirby Clay and (c) Warialda Loam soil controls.

Table D 2. Soil solution EC (mS/cm) from soil and MWOO-amended soil treatments with added microplastics following incubation for 0, 3 and 9 months

Soil	Rate	Microplastic	Incubation Period (months)			Soil	Rate	Microplastic	Incubation Period (months)		
			0	3	9				0	3	9
Kirby Sand	0%	HDPE	0.381±0.046	0.414±0.106	0.269±0.047	Kirby Sand +MWOO	0%	HDPE	1.07±0.116	0.624±0.131	0.819±0.069
	0.1%		0.361±0.012	1.1±0.059	0.96±0.187		0.1%		1.09±0.18	0.661±0.075	1.09±0.087
	0.25%		0.474±0.055	0.585±0.106	0.828±0.064		0.25%		0.881±0.031	0.642±0.048	1.35±0.046
	0.5%		0.463±0.061	0.745±0.194	0.881±0.098		0.5%		0.955±0.013	0.619±0.068	1.26±0.06
	1%		0.449±0.098	0.636±0.054	0.985±0.117		1%		1.2±0.211	0.691±0.158	1.48±0.098
Kirby Clay	0%	HDPE	0.88±0.078	1.03±0.022	1.6±0.047	Kirby Clay +MWOO	0%	HDPE	0.769±0.184	1.02±0.126	1.58±0.139
	0.1%		0.663±0.046	0.726±0.104	1.74±0.163		0.1%		1.02±0.158	0.859±0.054	1.81±0.126
	0.25%		0.55±0.048	0.813±0.148	1.9±0.09		0.25%		0.969±0.036	1.29±0.17	1.72±0.034
	0.5%		0.638±0.102	0.766±0.061	1.83±0.074		0.5%		0.896±0.061	1.03±0.193	1.77±0.076
	1%		0.61±0.041	0.571±0.117	1.91±0.125		1%		0.988±0.081	0.883±0.048	1.79±0.221
Warialda Loam	0%	HDPE	0.669±0.077	0.264±0.02	0.365±0.062	Warialda Loam +MWOO	0%	HDPE	1.16±0.11	0.486±0.047	0.501±0.055

	0.1%		0.491±0.035	0.252±0.012	0.328±0.064		0.1%		1.3±0.134	0.341±0.073	0.526±0.042
	0.25%		0.453±0.045	0.231±0.034	0.312±0.019		0.25%		1.24±0.106	0.449±0.01	0.602±0.107
	0.5%		0.544±0.006	0.208±0.033	0.312±0.013		0.5%		1.14±0.203	0.392±0.046	0.606±0.067
	1%		0.481±0.012	0.209±0.009	0.397±0.028		1%		1.07±0.097	0.393±0.03	0.546±0.004
Kirby Sand	0%	PET	0.381±0.046	0.414±0.105	0.269±0.047	Kirby Sand +MWOO	0%	PET	1.07±0.116	0.624±0.131	0.819±0.069
	0.1%		0.26±0.016	0.605±0.132	0.888±0.066		0.1%		1.06±0.186	0.526±0.122	0.865±0.063
	0.25%		0.29±0.012	0.668±0.201	0.962±0.089		0.25%		1.25±0.166	0.527±0.018	1.35±0.027
	0.5%		0.299±0.007	0.726±0.122	0.875±0.025		0.5%		0.956±0.071	0.513±0.045	0.998±0.143
	1%		0.269±0.04	0.992±0.067	1.04±0.096		1%		1.22±0.164	0.57±0.132	1.02±0.133
Kirby Clay	0%	PET	0.88±0.078	1.03±0.022	1.6±0.047	Kirby Clay +MWOO	0%	PET	0.769±0.184	1.02±0.126	1.58±0.139
	0.1%		0.631±0.123	0.706±0.264	1.58±0.044		0.1%		0.973±0.061	0.825±0.172	1.63±0.134
	0.25%		0.627±0.024	0.751±0.023	1.86±0.154		0.25%		1.5±0.075	0.984±0.189	1.81±0.013
	0.5%		0.713±0.037	0.688±0.132	1.84±0.231		0.5%		1±0.106	0.726±0.063	1.76±0.071
	1%		0.659±0.042	0.608±0.042	1.69±0.093		1%		1.02±0.065	0.831±0.033	1.33±0.133

Warialda Loam	0%	PET	0.669±0.077	0.264±0.02	0.365±0.062	Warialda Loam +MWOO	0%	PET	1.16±0.11	0.486±0.047	0.501±0.055
	0.1%		0.471±0.009	0.205±0.037	0.331±0.034		0.1%		1.3±0.064	0.411±0.008	0.45±0.042
	0.25%		0.477±0.008	0.22±0.02	0.345±0.018		0.25%		1.28±0.355	0.469±0.022	0.538±0.071
	0.5%		0.485±0.042	0.242±0.028	0.331±0.02		0.5%		1.24±0.127	0.369±0.045	0.765±0.104
	1%		0.462±0.014	0.221±0.025	0.338±0.039		1%		1.28±0.111	0.41±0.04	0.588±0.069
Kirby Sand	0%	PVC	0.381±0.046	0.414±0.106	0.269±0.047	Kirby Sand +MWOO	0%	PVC	1.07±0.116	0.624±0.131	0.819±0.069
	0.01%		0.557±0.097	0.6±0.254	0.831±0.042		0.01%		1.04±0.122	0.717±0.157	1.13±0.147
	0.1%		0.442±0.064	0.656±0.095	0.875±0.073		0.1%		1.08±0.113	0.528±0.024	0.952±0.097
	0.25%		0.43±0.063	0.756±0.286	0.823±0.027		0.25%		1.12±0.21	0.588±0.043	1.07±0.153
	0.5%		0.392±0.025	0.615±0.031	0.82±0.094		0.5%		1.1±0.246	0.623±0.169	1.05±0.111
	1%		0.264±0.023	0.572±0.149	0.605±0.141		1%		1.22±0.077	0.646±0.067	1.02±0.189
Kirby Clay	0%	PVC	0.88±0.078	1.03±0.022	1.6±0.047	Kirby Clay +MWOO	0%	PVC	0.769±0.184	1.02±0.126	1.58±0.139
	0.01%		0.748±0.046	0.586±0.18	1.43±0.127		0.01%		0.927±0.154	0.861±0.065	1.05±0.225
	0.1%		0.761±0.03	0.623±0.079	1.58±0.159		0.1%		0.941±0.195	1.11±0.156	1.58±0.108

	0.25%		0.672±0.03	0.59±0.096	1.68±0.212		0.25%		0.998±0.165	1.08±0.132	1.69±0.078
	0.5%		0.642±0.028	0.691±0.053	1.52±0.249		0.5%		1.13±0.105	0.828±0.107	1.56±0.214
	1%		0.551±0.01	0.524±0.107	1.64±0.096		1%		0.933±0.138	0.884±0.17	1.47±0.046
Warialda Loam	0%	PVC	0.669±0.077	0.264±0.02	0.365±0.062	Warialda Loam +MWOO	0%	PVC	1.16±0.11	0.486±0.047	0.501±0.055
	0.01%		0.519±0.027	0.231±0.04	0.327±0.044		0.01%		1.19±0.061	0.367±0.041	0.459±0.128
	0.1%		0.523±0.009	0.21±0.016	0.401±0.051		0.1%		1.09±0.368	0.426±0.014	0.493±0.043
	0.25%		0.523±0.045	0.178±0.023	0.271±0.044		0.25%		1.21±0.096	0.428±0.048	0.514±0.095
	0.5%		0.515±0.025	0.224±0.011	0.347±0.036		0.5%		1.18±0.052	0.436±0.014	0.496±0.062
	1%		0.506±0.018	0.191±0.007	0.295±0.041		1%		1.24±0.048	0.379±0.038	0.432±0.021

11 Appendix E. Soil solution - effect of MWOO and incubation time on trace metal concentrations

Table E 1. Soil solution concentrations of Fe, Mn, Co and Ni from soil and MWOO-amended soil treatments following incubation for 0, 3 and 9 months.

Soil Treatments	Iron (Fe) mg/L			Manganese (Mn) µg/L			Cobalt (Co) µg/L			Nickel (Ni) µg/L		
	Incubation period (months)											
	0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	1.8±0.16	1.1±0.40	0.67±0.31	210±16	140±15	190±7.6	31±2.2	7.8±0.60	4.7±0.84	3.9±0.75	3.6±1.1	6.1±0.96
Kirby Sand +MWOO	0.39±0.06	0.45±0.06	0.11±0.11	1012±36	71±5.0	38±6.1	101±8.2	4.5±0.38	4.9±0.30	15±1.7	6.9±0.30	6.5±1.1
Kirby Clay	0.11±0.01	0.05±0.01	<0.05	558±10	66±6.1	122±3.2	12±2.1	1.3±0.21	1.1±0.10	21±1.4	26±0.36	59±5.1
Kirby Clay +MWOO	0.13±0.05	<0.05	<0.05	2101±89	39±3.1	8.6±13	31±1.6	1.3±0.15	1.1±0.23	27±2.9	25±1.6	55±8.0
Warialda Loam	<0.05	0.05±0.02	<0.05	32±2.3	<2	<2	1.0±0.15	<0.3	<0.3	2.2±0.35	1.0±0.15	0.93±0.12

Warialda Loam +MWOO	0.11±0.01	<0.05	<0.05	313±28	<2	<2	13±1.6	0.47±0.15	<0.3	6.1±0.70	5.3±0.71	3.2±1.1

Table E 2. Soil solution concentrations of Cu, Zn, As and Mo from soil and MWOO-amended soil treatments following incubation for 0, 3 and 9 months.

Soil Treatments	Copper (Cu) µg/L			Zinc (Zn) µg/L			Arsenic (As) µg/L			Molybdenum (Mo) µg/L		
	Incubation period (months)											
	0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	25±2.3	15±1.5	13±0.81	33±5.5	45±8.4	196±30	21±3.0	8.2±0.66	13±2.4	0.90±0.17	<0.6	<0.6
Kirby Sand +MWOO	102±8.5	27±9.6	8.3±0.58	102±4.2	30±4.0	414±69	10±0.55	12±0.76	11±1.0	0.77±0.15	<0.6	<0.6
Kirby Clay	40±1.4	5.7±0.58	8.0±1.7	28±2.4	19±4.0	96±10	3.2±0.32	<0.6	1.9±0.50	<0.6	<0.6	<0.6
Kirby Clay +MWOO	42±1.9	22±5.3	5.2±0.30	12±1.4	18±6.7	86±4.2	3.2±0.20	<0.6	1.3±0.21	<0.6	<0.6	<0.6
Warialda Loam	8.9±0.57	13±1.7	<0.6	3.0±0.42	12±2.6	11±0.58	1.8±0.32	<0.6	<0.6	2.2±0.21	1.0±0.10	<0.6
Warialda Loam +MWOO	102±8.7	30±5.0	3.9±0.35	11±0.69	14±3.0	16±5.3	2.1±0.50	<0.6	1.5±0.42	1.8±0.23	2.0±1.7	<0.6

Table E 3. Soil solution concentrations of Cd, Cr, Sn and Pb from soil and MWOO-amended soil treatments following incubation for 0, 3 and 9 months.

Soil Treatments	Cadmium (Cd) µg/L			Chromium (Cr) µg/L			Tin (Sn) µg/L			Lead (Pb) µg/L		
	Incubation period (months)											
	0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	<0.3	<0.3	<0.3	10±0.58	<2	<2	<0.6	<0.6	<0.6	2.4±0.47	1.8±0.75	4.2±1.5
Kirby Sand +MWOO	1.3±0.06	<0.3	<0.3	11±0.58	<2	<2	<0.6	<0.6	<0.6	2.2±0.92	1.9±0.46	2.2±0.45
Kirby Clay	<0.3	<0.3	<0.3	11±0.51	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.57±0.15
Kirby Clay +MWOO	0.70±0.10	<0.3	<0.3	10±0.61	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
Warialda Loam	<0.3	<0.3	<0.3	10±0.61	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.70±0.10
Warialda Loam +MWOO	1.3±0.26	<0.3	<0.3	9.3±1.5	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3

Table E 4. Soil solution concentrations of Fe, Mn, Co and Ni from soil and MWOO-amended soil treatments with HDPE added at various rates (0-1% w/w) following incubation for 0, 3 and 9 months.

Soil Treatment	Microplastic Rate	Iron (Fe) mg/L			Manganese (Mn) µg/L			Cobalt (Co) µg/L			Nickel (Ni) µg/L		
		Incubation period (months)											
		0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	0%	1.8±0.16	1.1±0.40	0.67±0.31	210±16	140±15	190±7.6	31±2.2	7.8±0.60	4.7±0.84	3.9±0.75	3.6±1.1	6.1±0.96
	0.1%	0.90±0.12	0.07±0.04	<0.05	311±34	800±38	152±14	52±0.15	33±0.44	5.1±1.4	11±1.0	6.2±0.67	5.3±0.64
	0.25%	0.86±0.21	<0.05	<0.05	448±46	627±114	109±11	45±1.7	21±5.0	8.0±0.5	10±0.89	3.9±0.53	7.4±2.2
	0.5%	1.0±0.49	0.08±0.06	<0.05	470±74	491±65	138±27	45±3.8	20±0.23	10±0.8	<0.6	2.9±1.5	6.1±2.2
	1%	1.2±0.17	0.10±0.01	<0.05	473±38	175±47	315±86	30±6.8	5.3±1.2	30±9.5	<0.6	1.9±0.20	15±3.9
Kirby Sand +MWOO	0%	0.39±0.06	0.45±0.06	0.11±0.01	1012±36	71±5.0	38±6.1	101±8	4.5±0.38	4.9±0.30	15±1.7	6.9±0.30	6.5±1.1
	0.1%	0.30±0.01	0.33±0.02	0.22±0.11	1712±75	62±6.0	49±5.7	116±13	4.7±0.20	4.5±0.47	44±4.4	7.1±0.20	7.5±1.0
	0.25%	0.43±0.07	0.15±0.03	<0.05	1394±30	52±5.6	48±6.8	97±9.1	1.5±0.36	5.2±0.47	30±4.6	3.9±0.66	7.4±0.53
	0.5%	0.33±0.10	0.49±0.15	0.08±0.02	1057±42	56±7.9	50±6.6	107±13	1.2±0.10	5.0±0.61	32±0.32	3.5±0.44	7.3±1.3
	1%	0.38±0.05	0.34±0.03	<0.05	1089±49	28±2.1	62±12	103±5.9	1.6±0.12	4.6±0.31	38±1.5	4.4±1.0	7.7±0.57

Kirby Clay	0%	0.11±0.01	0.05±0.01	<0.05	558±10	66±6.1	122±3.2	12±2.1	1.3±0.21	1.1±0.10	21±1.4	26±0.36	59±5.1
	0.1%	0.06±0.003	<0.05	<0.05	1652±85	44±1.2	231±47	31±1.3	1.2±0.26	1.1±0.10	90±4.2	21±4.4	57±6.0
	0.25%	0.11±0.004	<0.05	<0.05	1474±37	26±7.4	248±42	23±1.4	0.90±0.61	2.2±0.3	90±2.3	17±2.7	67±1.1
	0.5%	0.08±0.007	<0.05	<0.05	1266±91	34±3.1	303±64	19±2.6	0.93±0.31	2.7±0.7	70±7.7	21±5.7	72±6.4
	1%	0.06±0.006	0.10±0.07	<0.05	1041±96	31±7.6	228±19	15±0.36	0.77±0.38	2.4±0.7	63±1.7	18±4.4	87±7.7
Kirby Clay +MWOO	0%	0.13±0.05	<0.05	0.025±0.005	2101±89	39±3.1	86±13	31±1.6	1.3±0.15	1.1±0.23	27±2.9	25±1.6	55±8.0
	0.1%	<0.05	<0.05	<0.05	2639±63	52±2.5	95±11	58±1.4	1.5±0.25	1.4±0.26	174±7.8	22±2.6	59±8.2
	0.25%	0.10±0.01	<0.05	<0.05	2547±84	46±3.2	108±7.5	39±3.7	1.7±0.17	1.4±0.40	135±5.7	21±6.1	55±10
	0.5%	0.07±0.01	<0.05	<0.05	2103±57	47±2.3	123±6.5	31±1.1	1.3±0.12	1.4±0.15	68±5.0	21±1.6	71±6.8
	1%	<0.05	<0.05	<0.05	2272±52	45±2.0	123±12	33±3.0	1.6±0.25	1.4±0.06	118±8.7	23±2.6	66±5.0
Warialda Loam	0%	<0.05	0.05±0.02	<0.05	32±2.3	<2	<2	1.0±0.15	<0.3	<0.3	2.2±0.35	1.0±0.15	0.93±0.12
	0.1%	<0.05	<0.05	<0.05	83±8.4	<2	<2	1.6±0.40	<0.3	<0.3	<0.6	2.0±0.32	2.4±0.85
	0.25%	<0.05	<0.05	<0.05	47±4.2	<2	3.2±1.0	1.3±0.12	<0.3	<0.3	<0.6	1.2±0.51	<0.6
	0.5%	0.07±0.009	<0.05	<0.05	29±4.5	<2	<2	1.1±0.06	<0.3	<0.3	<0.6	1.8±0.35	<0.6
	1%	0.06±0.01	<0.05	<0.05	39±2.3	<2	<2	1.2±0.06	<0.3	<0.3	<0.6	0.80±0.10	<0.6

Warialda Loam +MWOO	0%	0.11±0.01	<0.05	0.01±0.02	313±28	<2	<2	13±1.6	0.47±0.15	<0.3	6.1±0.70	5.3±0.71	3.2±1.1
	0.1%	<0.05	<0.05	<0.05	247±11	<2	<2	15±2.9	<0.3	<0.3	32±5.1	4.1±0.15	3.2±0.83
	0.25%	<0.05	<0.05	<0.05	224±28	<2	<2	13±3.0	<0.3	<0.3	25±4.5	3.9±0.5	3.2±1.1
	0.5%	<0.05	<0.05	<0.05	155±7.9	<2	<2	12±1.9	<0.3	<0.3	21±0.91	4.4±0.81	3.4±0.90
	1%	<0.05	<0.05	<0.05	141±3.5	<2	<2	8.0±1.9	<0.3	<0.3	26±7.2	4.5±0.75	3.0±0.25

Table E 5. Soil solution concentrations of Cu, Zn, As and Mo from soil and MWOO-amended soil treatments with HDPE added at various rates (0-1% w/w) following incubation for 0, 3 and 9 months.

Soil Treatment	Microplastic Rate	Copper (Cu) µg/L			Zinc (Zn) µg/L			Arsenic (As) µg/L			Molybdenum (Mo) µg/L		
		Incubation period (months)											
		0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	0%	25±2.3	15±1.5	13±0.81	33±5.5	45±8.4	196±30	21±3.0	8.2±0.66	13±2.4	0.90±0.17	<0.6	<0.6
	0.1%	27±0.58	3.0±0.50	3.7±0.64	32±7.0	54±2.5	487±13	19±1.7	1.7±0.25	1.1±0.26	<0.6	<0.6	<0.6
	0.25%	28±0.58	2.8±0.26	3.5±0.92	27±2.6	25±2.0	469±10	18±1.7	2.3±0.40	<0.6	<0.6	<0.6	<0.6
	0.5%	27±2.1	2.5±0.83	3.3±0.58	27±2.5	23±6.1	1047±43	19±2.3	2.1±0.60	<0.6	<0.6	<0.6	<0.6
	1%	25±3.5	2.9±0.15	5.9±1.0	24±2.9	25±2.5	1341±165	18±0.85	2.2±0.30	<0.6	<0.6	<0.6	<0.6
Kirby Sand +MWOO	0%	102±8.5	27±9.6	8.3±0.58	102±4.2	30±4.0	414±69	10±0.55	12±0.76	11±1.0	0.77±0.15	<0.6	<0.6
	0.1%	126±7.0	13±0.50	5.3±0.64	136±9.0	28±6.8	254±9	15±5.8	6.6±0.95	4.9±0.79	1.6±0.17	<0.6	<0.6
	0.25%	101±9.1	5.9±0.85	6.4±0.47	127±8.5	18±2.8	237±25	16±2.7	5.6±0.75	4.1±0.46	1.1±0.10	<0.6	<0.6
	0.5%	111±5.4	5.1±0.36	6.8±0.29	125±4.5	15±2.1	248±14	11±2.0	5.4±0.45	4.8±0.61	1.1±0.40	<0.6	<0.6
	1%	105±9.6	5.3±0.29	7.4±0.55	122±16	14±4.7	259±4.9	10±0.57	4.0±0.47	4.2±0.75	1.0±0.10	<0.6	<0.6

Kirby Clay	0%	40±1.4	5.7±0.58	8.0±1.7	28±2.4	19±4.0	96±10	3.2±0.32	<0.6	1.9±0.50	<0.6	<0.6	<0.6
	0.1%	31±1.5	6.7±1.2	3.9±0.17	20±1.6	20±6.7	161±13	2.1±0.10	<0.6	1.0±0.21	<0.6	<0.6	<0.6
	0.25%	33±1.4	4.8±0.40	4.3±0.58	20±2.0	16±4.4	159±14	2.2±0.20	<0.6	<0.6	<0.6	<0.6	<0.6
	0.5%	33±1.0	4.9±0.64	4.6±1.0	9.0±1.0	13±2.3	235±23	2.2±0.26	<0.6	<0.6	0.70±0.10	<0.6	<0.6
	1%	31±5.7	4.5±1.8	5.0±1.0	9.3±0.61	12±1.2	276±57	2.3±0.10	<0.6	<0.6	0.90±0.10	<0.6	<0.6
Kirby Clay +MWOO	0%	42±1.9	22±5.3	5.2±0.30	12±1.4	18±6.7	86±4.2	3.2±0.20	<0.6	1.3±0.21	<0.6	<0.6	<0.6
	0.1%	54±7.5	20±3.3	3.3±0.52	36±6.0	27±3.2	82±6.1	2.8±0.70	<0.6	<0.6	1.1±0.15	<0.6	<0.6
	0.25%	52±5.1	20±3.8	3.3±0.58	31±4.5	22±6.1	72±8.2	2.9±0.26	<0.6	<0.6	0.930.06	<0.6	<0.6
	0.5%	52±5.5	21±4.1	3.9±0.61	31±2.1	16±4.2	76±6.7	2.7±0.72	<0.6	<0.6	1.2±0.15	<0.6	<0.6
	1%	48±6.7	20±4.2	4.2±0.35	34±1.2	18±1.5	76±13	2.8±0.59	<0.6	<0.6	1.0±0.10	<0.6	<0.6
Warialda Loam	0%	8.9±0.57	13±1.7	<0.6	3.0±0.42	12±2.6	11±0.58	1.8±0.32	<0.6	<0.6	2.2±0.21	1.0±0.10	<0.6
	0.1%	11±0.40	4.4±1.3	4.3±0.58	3.7±1.2	13±1.2	9.3±0.68	1.8±0.10	<0.6	<0.6	1.8±0.15	1.2±0.21	<0.6
	0.25%	9.6±0.55	2.8±0.68	4.5±0.40	3.7±0.59	5.2±0.87	9.2±1.0	1.9±0.15	<0.6	<0.6	2.1±0.15	1.2±0.17	<0.6
	0.5%	9.8±0.29	2.3±0.64	2.7±0.58	4.2±0.29	3.3±0.58	7.8±1.8	1.8±0.06	<0.6	<0.6	2.4±0.06	1.3±0.17	<0.6
	1%	10±1.1	2.2±0.29	2.7±1.2	3.3±0.58	3.3±0.58	8.7±1.2	1.8±0.06	<0.6	<0.6	2.2±0.20	1.4±0.06	<0.6

Warialda Loam +MWOO	0%	102±8.7	30±5.0	3.9±0.35	11±0.69	14±3.0	16±5.3	2.1±0.50	<0.6	1.5±0.42	1.8±0.23	2.0±0.17	<0.6
	0.1%	32±2.0	2.9±0.90	3.3±0.58	8.1±2.0	5.0±2.0	6.7±1.5	4.4±0.21	<0.6	<0.6	2.5±0.26	2.0±0.69	<0.6
	0.25%	32±3.1	3.7±0.58	3.5±0.81	8.3±2.1	4.3±0.58	7.2±1.9	3.8±0.31	<0.6	<0.6	2.5±0.36	2.2±0.35	<0.6
	0.5%	30±2.4	4.2±0.93	3.8±0.30	8.8±0.29	7.9±0.81	7.4±1.2	3.3±0.15	<0.6	<0.6	2.5±0.17	1.8±0.91	<0.6
	1%	27±4.5	4.9±0.36	3.4±0.64	8.0±0.93	8.0±1.1	8.9±1.1	3.6±1.2	<0.6	<0.6	2.2±0.15	1.6±0.10	<0.6

Table E 6. Soil solution concentrations of Cd, Cr, Sn and Pb from soil and MWOO-amended soil treatments with HDPE added at various rates (0-1% w/w) following incubation for 0, 3 and 9 months.

Soil Treatment	Microplastic Rate	Cadmium (Cd) µg/L			Chromium (Cr) µg/L			Tin (Sn) µg/L			Lead (Pb) µg/L		
		Incubation period (months)											
		0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	0%	<0.3	<0.3	<0.3	10±0.58	<2	<2	<0.6	<0.6	<0.6	2.4±0.47	1.8±0.75	4.2±1.5
	0.1%	0.40±0.10	<0.3	<0.3	8.0±1.0	<2	<2	<0.6	<0.6	<0.6	13±0.50	<0.3	22±1.1
	0.25%	0.50±0.17	<0.3	<0.3	9.3±0.58	<2	<2	<0.6	<0.6	<0.6	12±1.4	<0.3	33±3.9
	0.5%	0.40±0.10	<0.3	<0.3	9.0±1.0	<2	<2	<0.6	<0.6	<0.6	11±2.4	<0.3	27±3.2
	1%	0.40±0.10	<0.3	<0.3	9.2±1.0	<2	<2	<0.6	<0.6	<0.6	10±1.3	<0.3	29±3.4
Kirby Sand +MWOO	0%	1.3±0.06	<0.3	<0.3	11±0.58	<2	<2	<0.6	<0.6	<0.6	2.2±0.35	1.9±0.46	2.2±0.45
	0.1%	<0.3	<0.3	<0.3	8.7±0.75	<2	<2	<0.6	<0.6	<0.6	3.0±0.29	1.4±0.10	5.2±0.72
	0.25%	<0.3	<0.3	<0.3	8.8±0.29	<2	<2	<0.6	<0.6	<0.6	3.1±0.78	1.2±0.07	6.2±1.3
	0.5%	<0.3	<0.3	<0.3	10±1.0	<2	<2	<0.6	<0.6	<0.6	3.4±0.21	1.4±0.06	5.1±1.1
	1%	<0.3	<0.3	<0.3	9.6±0.55	<2	<2	<0.6	<0.6	<0.6	3.3±0.55	1.2±0.15	5.5±0.60

Kirby Clay	0%	<0.3	<0.3	<0.3	11±0.51	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.57±0.15
	0.1%	<0.3	<0.3	<0.3	7.7±0.15	<2	<2	<0.6	<0.6	<0.6	3.0±0.80	<0.3	1.8±0.91
	0.25%	<0.3	<0.3	<0.3	7.1±1.4	<2	<2	<0.6	<0.6	<0.6	2.4±0.42	<0.3	1.1±0.20
	0.5%	<0.3	<0.3	<0.3	7.2±1.0	<2	<2	<0.6	<0.6	<0.6	2.5±0.26	<0.3	1.9±0.80
	1%	<0.3	<0.3	<0.3	7.6±0.35	<2	<2	<0.6	<0.6	<0.6	2.4±0.75	<0.3	1.3±0.12
Kirby Clay +MWOO	0%	0.70±0.10	<0.3	<0.3	10±0.61	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.1%	<0.3	<0.3	<0.3	8.5±1.0	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.1±0.25
	0.25%	<0.3	<0.3	<0.3	8.3±0.58	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.2±0.15
	0.5%	<0.3	<0.3	<0.3	9.0±1.0	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.1±0.29
	1%	<0.3	<0.3	<0.3	10±0.85	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.3±0.15
Warialda Loam	0%	<0.3	<0.3	<0.3	10±0.61	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.70±0.10
	0.1%	<0.3	<0.3	<0.3	4.7±0.58	<2	<2	<0.6	<0.6	<0.6	1.0±0.15	<0.3	1.5±0.35
	0.25%	<0.3	<0.3	<0.3	5.6±0.55	<2	<2	<0.6	<0.6	<0.6	0.80±0.36	<0.3	1.6±0.25
	0.5%	<0.3	<0.3	<0.3	6.1±0.67	<2	<2	<0.6	<0.6	<0.6	0.77±0.12	<0.3	1.4±0.25
	1%	<0.3	<0.3	<0.3	5.7±0.58	<2	<2	<0.6	<0.6	<0.6	0.83±0.32	<0.3	1.5±0.31

Warialda Loam +MWOO	0%	1.3±0.26	<0.3	<0.3	9.3±1.5	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.1%	<0.3	<0.3	<0.3	8.4±0.65	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.3±0.20
	0.25%	<0.3	<0.3	<0.3	8.6±0.55	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.1±0.15
	0.5%	<0.3	<0.3	<0.3	8.7±0.58	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.3±0.20
	1%	<0.3	<0.3	<0.3	7.9±1.0	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.4±0.12

Table E 7. Soil solution concentrations of Fe, Mn, Co and Ni from soil and MWOO-amended soil treatments with PET added at various rates (0-1% w/w) following incubation for 0, 3 and 9 months.

Soil Treatment	Microplastic Rate	Iron (Fe) mg/L			Manganese (Mn) µg/L			Cobalt (Co) µg/L			Nickel (Ni) µg/L		
		Incubation period (months)											
		0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	0%	1.8±0.16	1.1±0.4	0.67±0.31	210±16	140±15	190±7.6	31±2.2	7.8±0.6	4.7±0.84	3.9±0.75	3.6±1.1	6.1±0.96
	0.1%	1.4±0.49	<0.05	<0.05	153±7.8	236±11	323±32	22±1.9	10±0.85	36±9.4	2.7±0.76	3±0.68	8.6±0.7
	0.25%	2.3±0.69	<0.05	<0.05	181±17	340±31	353±47	27±1.2	12±1.8	25±5.8	2.8±0.2	2.6±0.31	7.8±0.68
	0.5%	1.1±0.27	0.11±0.03	<0.05	174±24	526±65	344±61	29±3.1	19±3.7	34±7.2	3.2±0.3	3.6±0.38	12±1.2
	1%	2±0.32	0.06±0.03	<0.05	168±71	507±39	782±29	28±3.1	22±3.4	73±10	3.3±1.2	4.2±0.36	16±2.3
Kirby Sand +MWOO	0%	0.39±0.06	0.45±0.06	0.11±0.01	1012±36	71±5	38±6.1	101±8.2	4.5±0.38	4.9±0.3	15±1.7	6.9±0.3	6.5±1.1
	0.1%	0.3±0.08	0.35±0.08	0.46±0.09	1489±23	43±5.5	59±8.5	113±4.3	2.1±0.55	4±0.47	33±7.8	5.8±1.2	6.9±1.8
	0.25%	0.3±0.05	0.22±0.04	<0.05	1384±16	49±8.7	68±14	113±4	2.4±0.67	2.7±0.21	26±1.8	6.5±1.2	8.4±1
	0.5%	0.38±0.02	0.4±0.08	0.15±0.17	1559±46	54±1.5	51±17	141±4.9	2.8±0.23	3.2±0.36	25±2.5	7±0.21	7.8±1.9
	1%	0.4±0.13	0.4±0.12	<0.05	2202±90	44±14	58±2.1	181±6.4	2.4±0.06	2.5±0.31	25±2.8	6.4±0.5	6.1±1.7

Kirby Clay	0%	0.11±0.01	0.05±0.01	0.05±0.005	558±10	66±6.1	122±3.2	12±2.1	1.3±0.21	1.1±0.1	21±1.4	26±0.36	59±5.1
	0.1%	0.14±0.02	<0.05	<0.05	1602±48	33±6	120±18	25±1.9	0.8±0.1	1.3±0.15	78±2.4	21±1.5	40±7.2
	0.25%	0.2±0.06	<0.05	<0.05	1612±62	30±6.7	155±18	24±0.87	1±0.18	1.7±0.25	74±6.9	21±1.2	59±7.9
	0.5%	0.13±0.03	<0.05	<0.05	1595±155	18±5.6	274±40	27±2.2	0.8±0.26	2.1±0.2	93±5.4	22±4	71±7.5
	1%	0.13±0.01	<0.05	<0.05	1778±85	20±2.6	320±60	27±1.1	0.9±0.56	2.8±0.61	89±4.1	22±6.9	72±5.8
Kirby Clay +MWOO	0%	0.13±0.05	<0.05	0.03±0.01	2101±89	39±3.1	86±13	31±1.6	1.3±0.15	1.1±0.23	27±2.9	25±1.6	55±8
	0.1%	0.08±0.01	<0.05	<0.05	2173±35	46±3.1	63±11	35±0.91	0.9±0.2	1±0.23	103±1.8	21±1.6	27±5.3
	0.25%	0.07±0.01	<0.05	<0.05	2590±60	50±3.5	127±7.4	36±2.8	1.2±0.15	1.4±0.17	106±4.7	27±2.5	56±2.8
	0.5%	0.12±0.03	<0.05	<0.05	2365±52	46±7.6	137±17	39±4.3	1.2±0.1	1.4±0.15	102±12	24±0.12	67±7.7
	1%	0.06±0.01	<0.05	<0.05	2756±119	52±8.1	117±6.4	42±5	0.73±0.06	1.1±0.36	99±1.4	20±1.2	70±3.5
Warialda Loam	0%	<0.05	0.05±0.02	0.01±0.005	32±2.3	<2	<2	1±0.15	<0.3	<0.3	2.2±0.35	1±0.15	0.93±0.12
	0.1%	0.12±0.01	<0.05	<0.05	44±3.3	<2	<2	0.63±0.06	<0.3	<0.3	<0.6	1±0.31	<0.6
	0.25%	0.1±0.03	<0.05	<0.05	41±4.4	<2	<2	0.87±0.21	<0.3	<0.3	<0.6	1±0.12	<0.6
	0.5%	<0.05	<0.05	<0.05	53±7.2	<2	<2	1.2±0.26	<0.3	<0.3	<0.6	1±0.25	<0.6
	1%	<0.05	<0.05	<0.05	49±6.1	<2	<2	1.2±0.21	<0.3	<0.3	<0.6	1.1±0.12	1.8±0.45

Warialda Loam +MWOO	0%	0.11±0.01	<0.05	0.01±0.02	313±28	<2	<2	13±1.6	0.47±0.15	<0.3	6.1±0.7	5.3±0.71	3.2±1.1
	0.1%	<0.05	<0.05	<0.05	176±10	<2	<2	10±2.1	<0.3	<0.3	34±4.6	4.5±0.79	3.3±0.51
	0.25%	<0.05	<0.05	<0.05	184±9.7	<2	<2	10±0.9	<0.3	<0.3	24±4	4.3±0.55	3.3±0.17
	0.5%	<0.05	<0.05	<0.05	187±8.6	<2	<2	8.7±0.75	<0.3	<0.3	25±3.5	5.2±1.1	3±0.4
	1%	<0.05	<0.05	<0.05	191±7.6	<2	<2	9.2±1.1	<0.3	<0.3	27±3.9	5.2±0.32	3.2±0.06

Table E 8. Soil solution concentrations of Cu, Zn, As and Mo from soil and MWOO-amended soil treatments with PET added at various rates (0-1% w/w) following incubation for 0, 3 and 9 months.

Soil Treatment	Microplastic Rate	Copper (Cu) µg/L			Zinc (Zn) µg/L			Arsenic (As) µg/L			Molybdenum (Mo) µg/L		
		Incubation period (months)											
		0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	0%	25±2.3	15±1.5	13±0.81	33±5.5	45±8.4	196±30	21±3	8.2±0.66	13±2.4	0.9±0.17	<0.6	<0.6
	0.1%	24±2.1	2.6±0.38	2.6±0.42	21±2.6	30±1.2	476±51	14±0.65	2.6±0.46	2.4±0.3	<0.6	<0.6	<0.6
	0.25%	27±4.4	2.3±0.58	2.8±0.76	23±1	31±4.5	449±27	18±2.6	2.3±0.38	<0.6	<0.6	<0.6	<0.6
	0.5%	33±3.5	<0.6±	3.3±0.46	25±3.2	27±6.6	617±57	23±1.5	2.5±0.35	<0.6	<0.6	<0.6	<0.6
	1%	30±3.5	<0.6±	3.3±0.58	27±2.5	28±5.1	670±56	22±2.1	2.1±0.47	<0.6	<0.6	<0.6	<0.6
Kirby Sand +MWOO	0%	102±8.5	27±9.6	8.3±0.58	102±4.2	30±4	414±69	10±0.55	12±0.76	11±1	0.77±0.15	<0.6	<0.6
	0.1%	114±17	19±2.7	6±1.7	133±7.1	28±3.1	217±69	13±1.4	8.6±0.25	4.7±0.8	<0.6	<0.6	<0.6
	0.25%	108±2.6	9.7±0.58	6.3±1.5	123±7.8	11±1.05	227±31	13±0.78	8.1±1.6	4.6±1.2	<0.6	<0.6	<0.6
	0.5%	108±7.7	8.1±1.9	7.7±2.9	120±9	3.4±0.17	267±54	12±1.4	7.3±0.53	4.3±0.44	<0.6	<0.6	<0.6
	1%	114±7.1	5.8±1.5	6.7±1.5	112±5.6	2.6±0.35	264±44	13±1.2	5.7±1.2	5±1.1	<0.6	<0.6	<0.6

Kirby Clay	0%	40±1.4	5.7±0.58	8±1.7	28±2.4	19±4	96±10	3.2±0.32	<0.6	1.9±0.5	<0.6	<0.6	<0.6
	0.1%	30±2	6±1	3±1	11±1.2	17±1.4	71±5	2±0.25	<0.6	1±0.25	<0.6	<0.6	<0.6
	0.25%	29±0.61	5.2±0.82	3±0.6	11±1	16±0.7	74±6.8	2.6±0.26	<0.6	1±0.21	<0.6	<0.6	<0.6
	0.5%	38±4.2	5.2±1.1	4±1	15±2	15±1.5	154±4	2.5±0.1	<0.6	<0.6	<0.6	<0.6	<0.6
	1%	40±1.2	4.4±0.59	5±1.7	16±1.5	17±1.3	281±27	2.5±0.1	<0.6	<0.6	<0.6	<0.6	<0.6
Kirby Clay +MWOO	0%	42±1.9	22±5.3	5.2±0.3	12±1.4	18±6.7	86±4.2	3.2±0.2	<0.6	1.3±0.21	<0.6	<0.6	<0.6
	0.1%	39±3.1	5.7±1.3	5±1	13±2	31±7.2	59±19	3.4±0.35	<0.6	1±0.17	<0.6	<0.6	<0.6
	0.25%	36±3.8	6.4±2.3	4.3±0.58	13±0.82	18±3.1	111±10	3.7±0.78	<0.6	<0.6	<0.6	<0.6	<0.6
	0.5%	36±3.4	6.8±1.9	4.7±0.58	14±1.7	16±1.5	155±32	2.9±0.66	<0.6	<0.6	<0.6	<0.6	<0.6
	1%	37±3	5.7±0.58	4±0.5	14±2.2	15±3.2	142±11	2.3±0.15	<0.6	<0.6	<0.6	<0.6	<0.6
Warialda Loam	0%	8.9±0.57	13±1.7	<0.6	3±0.42	12±2.6	11±0.58	1.8±0.32	<0.6	<0.6	2.2±0.21	1±0.1	<0.6
	0.1%	11±1	4.7±1.5	<0.6	<1	4.3±0.58	4.9±3.2	2±0.29	<0.6	1.8±0.35	1.6±0.12	1.1±0.2	<0.6
	0.25%	10±0.58	3.8±0.76	<0.6	<1	4.2±0.76	7.6±1.1	1.8±0.35	<0.6	1.1±0.15	1.6±0.26	1.2±0.21	<0.6
	0.5%	11±1	3.6±0.75	<0.6	3.3±0.61	4.5±0.76	8±1	1.3±0.25	<0.6	<0.6	1.6±0.12	1.1±0.06	<0.6
	1%	10±0.58	4.1±1.4	3.7±0.58	4.7±0.64	4.8±1	7.7±0.72	1.5±0.1	<0.6	<0.6	1.5±0.3	1.1±0.21	<0.6

Warialda Loam +MWOO	0%	102±8.7	30±5	3.9±0.35	11±0.69	14±3	16±5.3	2.1±0.5	<0.6	1.5±0.42	1.8±0.23	2±0.17	<0.6±
	0.1%	93±4.9	4.7±0.58	3.3±0.58	11±1.5	4±1	4.3±0.58	3.3±0.21	<0.6	<0.6	2.5±0.3	2.5±0.4	1.4±0.32
	0.25%	91±6.7	4.4±0.69	4.1±1.1	9.3±1.1	4.3±0.58	5±1	2.7±0.36	<0.6	<0.6	2.3±0.26	2±0.17	1.2±0.17
	0.5%	88±7.4	4.2±0.87	3.3±0.58	9.5±1	4.3±0.58	11±3.1	3±0.4	<0.6	<0.6	2.3±0.4	1.7±0.2	2.5±0.9
	1%	85±4.2	4.7±1.1	3.3±0.58	9.2±1	4.8±0.29	9.2±1.1	2.9±0.4	<0.6	<0.6	2.2±0.25	1.5±0.15	1.9±0.53

Table E 9. Soil solution concentrations of Cd, Cr, Sn and Pb from soil and MWOO-amended soil treatments with PET added at various rates (0-1% w/w) following incubation for 0, 3 and 9 months.

Soil Treatment	Microplastic Rate	Cadmium (Cd) µg/L			Chromium (Cr) µg/L			Tin (Sn) µg/L			Lead (Pb) µg/L		
		Incubation period (months)											
		0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	0%	<0.3	<0.3	<0.3	10±0.58	<2	<2	<0.6	<0.6	<0.6	2.4±0.47	1.8±0.75	4.2±1.5
	0.1%	<0.3	<0.3	<0.3	8.8±0.29	<2	<2	<0.6	<0.6	<0.6	21±0.83	<0.3	13±0.7
	0.25%	<0.3	<0.3	<0.3	8.7±0.42	<2	<2	<0.6	<0.6	<0.6	24±1	<0.3	12±0.55
	0.5%	<0.3	<0.3	<0.3	9.3±0.67	<2	<2	<0.6	<0.6	<0.6	25±1.9	<0.3	13±1.8
	1%	<0.3	<0.3	<0.3	8.9±0.32	<2	<2	<0.6	<0.6	<0.6	23±1.4	<0.3	14±1.2
Kirby Sand +MWOO	0%	1.3±0.06	<0.3	<0.3	11±0.58	<2	<2	<0.6	<0.6	<0.6	2.2±0.92	1.9±0.46	2.2±0.45
	0.1%	<0.3	<0.3	<0.3	9.5±0.5	<2	<2	<0.6	<0.6	<0.6	36±3.8	1.7±0.17	6±0.72
	0.25%	<0.3	<0.3	<0.3	9.9±0.35	<2	<2	<0.6	<0.6	<0.6	27±3.9	1.6±0.21	5.6±2.1
	0.5%	<0.3	<0.3	<0.3	11±1.3	<2	<2	<0.6	<0.6	<0.6	26±3.1	1.7±0.06	6.5±0.89
	1%	<0.3	<0.3	<0.3	11±2.5	<2	<2	<0.6	<0.6	<0.6	26±2.1	1.6±0.3	4.2±1.8

Kirby Clay	0%	<0.3	<0.3	<0.3	11±0.51	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.57±0.15
	0.1%	<0.3	<0.3	<0.3	8.4±0.45	<2	<2	<0.6	<0.6	<0.6	1.4±0.4	<0.3	1.7±0.47
	0.25%	<0.3	<0.3	<0.3	8.3±0.58	<2	<2	<0.6	<0.6	<0.6	1.2±0.1	<0.3	1±0.4
	0.5%	<0.3	<0.3	<0.3	8.1±1	<2	<2	<0.6	<0.6	<0.6	1±0.15	<0.3	1.1±0.21
	1%	<0.3	<0.3	<0.3	8.5±0.57	<2	<2	<0.6	<0.6	<0.6	0.92±0.08	<0.3	1.3±0.06
Kirby Clay +MWOO	0%	0.7±0.1	<0.3	<0.3	10±0.61	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.1%	<0.3	<0.3	<0.3	9.7±0.58	<2	<2	<0.6	<0.6	<0.6	2.3±0.21	<0.3	1.3±0.4
	0.25%	<0.3	<0.3	<0.3	9.5±0.6	<2	<2	<0.6	<0.6	<0.6	2.2±0.12	<0.3	0.7±0.2
	0.5%	<0.3	<0.3	<0.3	9.8±0.35	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.67±0.387
	1%	<0.3	<0.3	<0.3	9.6±0.66	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.1±0.15
Warialda Loam	0%	<0.3	<0.3	<0.3	10±0.61	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.7±0.1
	0.1%	<0.3	<0.3	<0.3	8.4±0.75	<2	<2	<0.6	<0.6	<0.6	1.2±0.23	<0.3	1.6±0.36
	0.25%	<0.3	<0.3	<0.3	6.9±0.91	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.53±0.15
	0.5%	<0.3	<0.3	<0.3	4.8±0.26	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	1%	<0.3	<0.3	<0.3	4.5±0.68	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3

Warialda Loam +MWOO	0%	1.3±0.26	<0.3	<0.3	9.3±1.5	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.1%	<0.3	<0.3	<0.3	10±0.32	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.25%	<0.3	<0.3	<0.3	9.2±1.4	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.5%	<0.3	<0.3	<0.3	9.6±1	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	1%	<0.3	<0.3	<0.3	9±1	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	1.4±0.58

Table E 10. Soil solution concentrations of Fe, Mn, Co and Ni from soil and MWOO-amended soil treatments with PVC added at various rates (0-1% w/w) following incubation for 0, 3 and 9 months.

Soil Treatment	Microplastic Rate	Iron (Fe) mg/L			Manganese (Mn) µg/L			Cobalt (Co) µg/L			Nickel (Ni) µg/L		
		Incubation period (months)											
		0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	0%	1.8±0.16	1.1±0.4	0.67±0.31	210±16	140±15	190±7.6	31±2.2	7.8±0.6	4.7±0.84	3.9±0.75	3.6±1.1	6.1±0.96
	0.01%	1.2±0.33	0.17±0.11	<0.05	552±76	103±7.4	256±29	53±4.6	5.2±1.9	25±13	5.7±0.61	2.1±0.56	11±1.9
	0.1%	1.3±0.24	0.08±0.02	<0.05	539±51	111±12	221±53	47±2.3	16±3.5	17±0.6	5.3±0.26	2.6±0.45	7.3±1.2
	0.25%	1.3±0.39	0.17±0.09	<0.05	556±36	215±32	281±38	51±2.6	13±2.1	26±5	5.3±0.12	4.1±0.9	7.6±0.9
	0.5%	1.2±0.27	0.15±0.03	<0.05	440±50	197±24	286±30	43±5.2	8.3±1.3	26±16	4.5±0.15	2.8±0.87	8.2±1.5
	1%	1.8±0.57	0.2±0.05	<0.05	139±17	195±39	261±52	20±2.4	10±0.85	12±5.1	4.6±0.66	1.5±0.15	7.8±0.5
Kirby Sand +MWOO	0%	0.39±0.06	0.45±0.06	0.11±0.01	1012±36	71±5	38±6.1	101±8.2	4.5±0.38	4.9±0.3	15±1.7	6.9±0.3	6.5±1.1
	0.01%	0.31±0.05	0.09±0.02	0.13±0.05	1245±75	110±21	112±13	125±3.4	4.7±0.87	3±0.56	35±1.3	6.9±1.2	7.9±0.4
	0.1%	0.32±0.08	0.42±0.07	<0.05±	1186±84	115±7.5	136±16	122±4.4	7.4±0.86	3±0.78	36±2.8	6±1.8	6.5±1.1
	0.25%	0.33±0.1	<0.05	0.18±0.1	1233±97	121±11	129±17	120±3.2	5.7±0.8	4.3±0.85	33±3.2	5.3±0.64	7.8±0.4

	0.5%	0.33±0.01	0.38±0.03	0.26±0.15	1271±21	116±8.2	140±6.1	117±7.4	5.7±2.1	4.5±0.87	33±2	8.1±1.3	7.8±1
	1%	0.33±0.06	0.52±0.16	0.14±0.03	1238±114	117±14	158±20	118±3	4.1±0.72	3.2±0.59	33±2.3	7.7±1.4	7.2±1.6
Kirby Clay	0%	0.11±0.01	0.05±0.01	0.05±0.005	558±10	66±6.1	122±3.2	12±2.1	1.3±0.21	1.1±0.1	21±1.2	26±0.36	59±5.1
	0.01%	0.08±0.03	<0.05	<0.05	2568±228	18±1.5	106±11	42±6.3	0.7±0.3	2±0.06	95±4.4	16±1.7	44±2.2
	0.1%	0.07±0.02	0.05±0.02	<0.05	2106±120	30±1.2	153±13	29±5.4	0.8±0.4	1.9±0.06	85±2.9	18±2.9	53±3.4
	0.25%	0.06±0.02	<0.05	<0.05	2011±173	34±7.8	170±33	30±4.2	0.9±0.44	1.5±0.38	87±3.1	18±1.9	41±8
	0.5%	0.08±0.04	<0.05	<0.05	1961±77	26±5.5	165±22	35±1.6	1±0.57	1.2±0.35	84±3.4	23±3.4	37±4.8
	1%	0.17±0.03	0.14±0.07	<0.05	1429±54	23±2	172±35	23±1.7	0.63±0.32	1.5±0.06	71±0.64	25±4.1	39±1
Kirby Clay +MWOO	0%	0.13±0.05	<0.05	<0.05	2101±89	39±3.1	86±13	31±1.6	1.3±0.15	1.1±0.23	27±2.9	25±1.6	55±8
	0.01%	0.07±0.02	<0.05	<0.05	2123±117	26±5.9	124±12	33±2.5	1±0.21	1.2±0.15	91±7.1	20±1.9	21±6.7
	0.1%	0.07±0.01	<0.05	<0.05	2133±188	23±1.2	116±13	31±4	1±0.1	1.2±0.1	89±2.5	21±0.71	31±8.2
	0.25%	0.07±0.01	<0.05	<0.05	2061±86	33±6.7	131±18	33±2.9	1±0.29	1.4±0.25	90±7.3	21±5.7	38±6.2
	0.5%	0.1±0.004	<0.05	<0.05	2058±162	53±6.7	131±14	32±2.2	1.4±0.12	1±0.31	73±1.9	26±1.7	55±8.9
	1%	0.06±0.004	<0.05	<0.05	1959±53	51±4.5	128±6.1	30±0.55	1.4±0.38	1±0.31	75±4	21±1.8	59±8.2
Warialda Loam	0%	<0.05	0.05±0.02	<0.05	32±2.3	<2	<2	1±0.15	<0.3	<0.3	2.2±0.35	1±0.15	0.93±0.12

	0.01%	0.08±0.01	<0.05	<0.05	59±1.7	<2	<2	0.73±0.12	<0.3	<0.3	1.2±0.15	0.83±0.21	1.3±0.25
	0.1%	0.07±0.01	0.06±0.003	<0.05	65±2	<2	<2	0.83±0.06	<0.3	<0.3	<0.6	1.1±0.4	1±0.13
	0.25%	0.05±0.02	<0.05	<0.05	60±4.6	<2	<2	0.93±0.06	<0.3	<0.3	<0.6	1.2±0.35	0.93±0.32
	0.5%	<0.05	<0.05	<0.05	62±3	<2	<2	0.8±0.17	<0.3	<0.3	<0.6	1.1±0.2	0.77±0.12
	1%	0.09±0.01	0.08±0.02	<0.05	48±9.3	<2	<2	0.77±0.12	<0.3	<0.3	<0.6	1.1±0.26	0.86±0.15
Warialda Loam +MWOO	0%	0.11±0.01	<0.05	<0.05	313±28	<2	<2	13±1.6	0.47±0.15	<0.3	6.1±0.7	5.3±0.71	3.2±1.1
	0.01%	<0.05	<0.05	<0.05	127±4.9	<2	<2	11±0.38	<0.3	<0.3	26±2.3	3.6±0.3	3.4±0.35
	0.1%	<0.05	<0.05	<0.05	127±6.1	<2	<2	10±0.75	<0.3	<0.3	24±1.2	4.4±1.3	3.6±0.12
	0.25%	<0.05	<0.05	<0.05	126±4.3	<2	<2	9.4±0.56	<0.3	<0.3	24±2.4	4.7±0.3	2.9±0.12
	0.5%	<0.05	<0.05	<0.05	116±7.1	<2	<2	9.4±0.5	<0.3	<0.3	24±0.57	3.8±0.52	3.3±0.93
	1%	<0.05	<0.05	<0.05	116±5.7	<2	<2	9.1±0.6	<0.3	<0.3	22±1.3	4.3±1	2.8±0.21

Table E 11. Soil solution concentrations of Cu, Zn, As and Mo from soil and MWOO-amended soil treatments with PVC added at various rates (0-1% w/w) following incubation for 0, 3 and 9 months.

Soil Treatment	Microplastic Rate	Copper (Cu) µg/L			Zinc (Zn) µg/L			Arsenic (As) µg/L			Molybdenum (Mo) µg/L		
		Incubation period (months)											
		0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	0%	25±2.3	15±1.5	13±0.81	33±5.5	45±8.4	196±30	21±3	8.2±0.66	13±2.4	0.9±0.17	<0.6	<0.6
	0.01%	25±1	29±7.8	2.3±0.58	50±1	79±8.7	1425±26	21±1.9	3.1±0.32	2±0.15	<0.6	<0.6	<0.6
	0.1%	23±2	18±3.1	2.8±0.29	50±2.2	77±12	351±94	23±2.3	3±0.65	2.5±0.38	<0.6	<0.6	<0.6
	0.25%	22±1	12±1.5	2.4±0.55	51±2.7	51±9.3	328±37	21±1.3	2.2±0.56	2.4±0.61	<0.6	<0.6	<0.6
	0.5%	21±0.81	2.9±1.1	2.8±0.76	38±5	21±6.1	393±55	20±0.42	2.3±0.5	2±0.32	<0.6	<0.6	<0.6
	1%	20±1.4	3.3±0.52	2.9±0.81	16±2.3	11±1.8	263±17	13±1.1	2.3±0.35	2.8±0.51	<0.6	<0.6	<0.6
Kirby Sand +MWOO	0%	102±8.5	27±9.6	8.3±0.58	102±4.2	30±4	414±69	10±0.55	12±0.76	11±1	0.77±0.15	<0.6	<0.6
	0.01%	88±6.9	15±1.5	5.3±1.2	123±6.5	64±8.6	489±104	16±1.7	9.6±0.75	4.9±0.62	<0.6	<0.6	<0.6
	0.1%	70±4.7	17±1.7	7.2±1.1	118±5.1	74±8.9	517±117	14±1.6	9.2±1.2	5.1±0.55	<0.6	<0.6	<0.6
	0.25%	72±2.1	17±2.6	6.5±1.8	118±8.1	19±5.1	417±49	14±1.6	8.5±0.67	5.7±1.1	<0.6	<0.6	<0.6

	0.5%	72±4.2	17±2.5	11±0.98	111±5.1	21±3	362±59	10±0.65	9±0.42	4.8±0.72	0.8±0.1	<0.6	<0.6
	1%	74±7.6	16±4.6	8.5±2.3	113±3	22±5.1	204±87	11±0.51	9.3±0.68	4.8±1.5	1.1±0.06	<0.6	<0.6
Kirby Clay	0%	40±1.4	5.7±0.58	8±1.7	28±2.4	19±4	96±10	3.2±0.32	<0.6	1.9±0.5	<0.6	<0.6	<0.6
	0.01%	29±1	6.7±0.87	4.6±0.79	9.8±1	11±3.2	44±10	2.3±0.72	<0.6	<0.6	<0.6	<0.6	<0.6
	0.1%	27±2	5.8±0.35	4±0.81	8.1±1.2	11±3.5	64±9.1	1.7±0.2	<0.6	<0.6	<0.6	<0.6	<0.6
	0.25%	27±1.2	5.1±1.2	3.1±0.12	8.8±0.68	11±2.1	56±13	1.9±0.31	<0.6	1±0.2	<0.6	<0.6	<0.6
	0.5%	27±1.9	5.1±0.9	2.9±0.28	8.6±0.3	12±2	54±5.7	2.1±0.32	<0.6	0.96±0.15	<0.6	<0.6	<0.6
	1%	25±1.5	4.9±0.23	3.3±0.42	7.6±0.55	10±1.2	63±6.8	1.8±0.21	<0.6	1±0.23	<0.6	<0.6	<0.6
Kirby Clay +MWOO	0%	42±1.9	22±5.3	5.2±0.3	12±1.4	18±6.7	86±4.2	3.2±0.2	<0.6	1.3±0.21	<0.6	<0.6	<0.6
	0.01%	34±1.6	6.8±2	4.5±0.5	12±1.2	20±1.1	31±4.7	3±0.71	<0.6	<0.6	<0.6	<0.6	<0.6
	0.1%	36±4.6	5.2±1	4.2±0.4	13±2.3	17±1.5	33±3.8	3±0.44	<0.6	<0.6	<0.6	<0.6	<0.6
	0.25%	35±2.6	6.3±0.89	4.3±0.58	11±1	15±2.8	47±5.5	3.1±0.26	<0.6	<0.6	<0.6	<0.6	<0.6
	0.5%	33±1.5	6.9±1.2	4.7±0.58	11±0.85	14±1.5	55±10	2.7±0.72	<0.6	<0.6	<0.6	<0.6	<0.6
	1%	36±5.5	6.6±1	4.8±0.29	11±0.74	12±2.1	46±8.3	2.6±0.46	<0.6	<0.6	<0.6	<0.6	<0.6
Warialda Loam	0%	8.9±0.57	13±1.7	<0.6	3±0.42	12±2.6	11±0.58	1.8±0.32	<0.6	<0.6	2.2±0.21	1±0.1	<0.6

	0.01%	9.3±0.58	12±2.3	2.6±0.43	<1	5.3±0.75	8.7±1.6	3.1±0.58	<0.6	<0.6	2±0.1	1.2±0.1	1±0.15
	0.1%	9±1	7.1±1.9	<0.6	<1	5.5±1.3	4.3±0.55	1.9±0.32	<0.6	<0.6	2±0.06	1.3±0.23	0.93±0.12
	0.25%	9.6±0.55	7±1.8	<0.6	<1	4.2±0.29	4.8±0.53	1.8±0.21	<0.6	<0.6	2.1±0.1	1.3±0.35	1.1±0.31
	0.5%	9.3±0.58	7.5±3.9	<0.6	<1	3.3±0.58	4.8±0.76	1.5±0.4	<0.6	1.3±0.26	2±0.12	1.3±0.26	1.1±0.06
	1%	9.1±0.72	3.1±0.86	<0.6	<1	3.8±0.76	3.9±0.81	1.9±0.23	<0.6	1.3±0.31	1.9±0.15	1.4±0.25	1.2±0.2
Warialda Loam +MWOO	0%	102±8.7	30±5	3.9±0.35	11±0.69	14±3	16±5.3	2.1±0.5	<0.6	1.5±0.42	1.8±0.23	2±0.17	<0.6
	0.01%	73±3.6	4.3±0.52	3.7±0.58	7.8±1	5.7±1.2	4.5±0.9	2.6±0.32	<0.6	<0.6	2.6±0.17	2.1±0.4	1.2±0.12
	0.1%	64±2	4.7±0.59	3.3±0.58	8.6±0.67	5.7±1.5	5.6±1.2	2.3±0.49	<0.6	<0.6	2.4±0.21	2.1±0.21	1.3±0.15
	0.25%	64±6.3	5±0.15	3.2±0.29	8.3±0.79	5.6±0.64	7±1	2.5±0.35	<0.6	<0.6	2.5±0.31	2.1±0.31	1.3±0.15
	0.5%	62±2.5	4.6±0.89	2.7±0.58	8.8±1.4	5.3±0.61	7.1±0.72	2.4±0.55	<0.6	<0.6	2.3±0.45	1.9±0.29	1.3±0.45
	1%	64±2.2	5.1±1.3	3.5±0.87	8.5±0.5	4.9±1	5.2±1	2.4±0.23	<0.6	<0.6	2.6±0.35	2.4±0.46	1.2±0.31

Table E 12. Soil solution concentrations of Cd, Cr, Sn and Pb from soil and MWOO-amended soil treatments with PVC added at various rates (0-1% w/w) following incubation for 0, 3 and 9 months.

Soil Treatment	Microplastic Rate	Cadmium (Cd) µg/L			Chromium (Cr) µg/L			Tin (Sn) µg/L			Lead (Pb) µg/L		
		Incubation period (months)											
		0	3	9	0	3	9	0	3	9	0	3	9
Kirby Sand	0%	<0.3	<0.3	<0.3	10±0.58	<2	<2	<0.6	<0.6	<0.6	2.4±0.47	1.8±0.75	4.2±1.5
	0.01%	<0.3	<0.3	<0.3	10±0.76	<2	<2	<0.6	<0.6	<0.6	132±9.1	0.63±0.15	5±1.3
	0.1%	<0.3	<0.3	<0.3	11±1	<2	<2	<0.6	<0.6	<0.6	83±3.5	0.6±0.1	4.3±1.4
	0.25%	<0.3	<0.3	<0.3	12±0.58	<2	<2	<0.6	<0.6	<0.6	80±8.7	<0.3	<0.3
	0.5%	<0.3	<0.3	<0.3	11±0.68	<2	<2	<0.6	<0.6	<0.6	55±5.2	<0.3	<0.3
	1%	<0.3	<0.3	<0.3	11±0.58	<2	<2	<0.6	<0.6	<0.6	22±3.9	<0.3	<0.3
Kirby Sand +MWOO	0%	1.3±0.06	<0.3	<0.3	11±0.58	<2	<2	<0.6	<0.6	<0.6	2.2±0.92	1.9±0.46	2.2±0.45
	0.01%	<0.3	<0.3	<0.3	13±1	<2	<2	<0.6	<0.6	<0.6	21±1.1	1.6±0.45	2.6±1.2
	0.1%	<0.3	<0.3	<0.3	12±1	<2	<2	<0.6	<0.6	<0.6	20±0.36	1.3±0.1	2.9±0.35
	0.25%	<0.3	<0.3	<0.3	11±1	<2	<2	<0.6	<0.6	<0.6	19±0.4	1.1±0.4	2.9±0.51

	0.5%	<0.3	<0.3	<0.3	10±1.7	<2	<2	<0.6	<0.6	<0.6	19±0.35	1.5±0.4	3.1±0.6
	1%	<0.3	<0.3	<0.3	9.7±0.61	<2	<2	<0.6	<0.6	<0.6	19±0.4	1.2±0.6	3.1±0.49
Kirby Clay	0%	<0.3	<0.3	<0.3	11±0.51	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.57±0.15
	0.01%	<0.3	<0.3	<0.3	9.3±0.61	<2	<2	<0.6	<0.6	<0.6	3.2±0.25	<0.3	<0.3
	0.1%	<0.3	<0.3	<0.3	11±1.5	<2	<2	<0.6	<0.6	<0.6	3.1±0.26	<0.3	<0.3
	0.25%	<0.3	<0.3	<0.3	10±1.2	<2	<2	<0.6	<0.6	<0.6	2.4±0.31	<0.3	<0.3
	0.5%	<0.3	<0.3	<0.3	9.6±0.55	<2	<2	<0.6	<0.6	<0.6	1.6±0.31	<0.3	<0.3
	1%	<0.3	<0.3	<0.3	10±0.58	<2	<2	<0.6	<0.6	<0.6	1.7±0.15	<0.3	<0.3
Kirby Clay +MWOO	0%	0.7±0.1	<0.3	<0.3	9.7±0.61	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.01%	<0.3	<0.3	<0.3	10±0.64	<2	<2	<0.6	<0.6	<0.6	2.4±0.15	<0.3	0.9±0.1
	0.1%	<0.3	<0.3	<0.3	10±1.2	<2	<2	<0.6	<0.6	<0.6	2.2±0.15	<0.3	0.6±0.1
	0.25%	<0.3	<0.3	<0.3	9.7±0.57	<2	<2	<0.6	<0.6	<0.6	2.1±0.21	<0.3	0.6±0.26
	0.5%	<0.3	<0.3	<0.3	9.6±0.53	<2	<2	<0.6	<0.6	<0.6	1.9±0.25	<0.3	<0.3
	1%	<0.3	<0.3	<0.3	9.7±0.7	<2	<2	<0.6	<0.6	<0.6	1.9±0.1	<0.3	<0.3
Warialda Loam	0%	<0.3	<0.3	<0.3	10±0.61	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	0.7±0.1

	0.01%	<0.3	<0.3	<0.3	9.4±0.7	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.1%	<0.3	<0.3	<0.3	9.7±1.5	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.25%	<0.3	<0.3	<0.3	9.4±0.5	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.5%	<0.3	<0.3	<0.3	9.3±1.1	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	1%	<0.3	<0.3	<0.3	9.7±1.5	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
Warialda Loam +MWOO	0%	1.3±0.26	<0.3	<0.3	9.3±1.5	<2	<2	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3
	0.01%	<0.3	<0.3	<0.3	8.3±0.58	<2	<2	<0.6	<0.6	<0.6	2.2±0.21	<0.3	0.67±0.25
	0.1%	<0.3	<0.3	<0.3	8.8±0.72	<2	<2	<0.6	<0.6	<0.6	2.3±0.49	<0.3	0.47±0.06
	0.25%	<0.3	<0.3	<0.3	9.5±0.62	<2	<2	<0.6	<0.6	<0.6	2.2±0.15	<0.3	<0.3
	0.5%	<0.3	<0.3	<0.3	9.6±1	<2	<2	<0.6	<0.6	<0.6	2±0.15	<0.3	<0.3
	1%	<0.3	<0.3	<0.3	9.7±0.61	<2	<2	<0.6	<0.6	<0.6	2.1±0.26	<0.3	<0.3

12 Appendix F. Recoveries and quantification limits of trace organic analytes

Table F 1. Overview of percentage recoveries in various matrices and limits of quantification (LOQ) for 39 organic analytes. LOQ values are in parts per billion (μg/L or μg/kg), determined from n=6 samples. % recovery values are mean ± standard deviation (n=6).

Compound	Pore water %recovery	Kirby Sand %recovery	Kirby Clay %recovery	Warialda Loam %recovery	LOQ[a] (ppb)
Plastic additives/by-products					
BPA	71±21	161±15	87±19	161±22	2
DEHP	89±3	97±8	92±16	84±8	45
Dioctyl phthalate	77±7	103±41	66±37	67±25	32
Dibutylphthalate	83±11	98±10	83±11	85±7	41
Pesticides and by-products					
2,4-D	104±4	57±4	30±15	12±5	5
MCPA	134±20	55±30	62±18	7±2	8
3,5-Dichlorobenzoic acid	82±6	62±10	52±14	79±9	22
Chlorfenvinphos	126±18	157±15	138±44	180±49	8
Clothianidin	135±14	127±31	119±9	131±13	2
Permethrin	-[b]	-	-	-	10
Ametryn	120±9	78±33	51±21	105±18	58
Tebuthiuron	106±15	104±9	97±13	100±15	29
Chlorpyrifos	10±3	5±2	2±0.1	6±1	49

Fipronil	70±30	53±25	8±4	64±18	2
Imidacloprid	115±10	104±22	95±3	114±30	9
Bifenthrin	-	-	-	-	10
Difenconazole	43±12	19±10	5±2	45±7	3
Metalaxyl	117±5	120±16	121±10	118±5	2
Prochloraz	66±13	20±11	11±4	70±8	3
Pyraclostrobin	67±26	34±10	10±3	39±4	1
Pyrimethanil	80±7	49±23	24±10	90±24	1
Trifloxystrobin	42±4	22±6	8±2	30±4	1
Carbaryl	105±1	89±12	90±8	99±6	2
Cypermethrin	-	-	-	-	10
Diazinon	63±17	93±20	46±10	74±21	4
Indoxacarb	22±7	15±6	3±1	13±2	12
Pirimicarb	133±16	108±19	117±13	143±24	4
Atrazine	107±17	83±26	52±22	116±11	1
Diuron	112±9	104±10	80±10	108±7	0.8
Simazine	105±8	94±18	74±21	111±7	1
Thiabendazole	99±11	36±10	17±5	88±3	1
Prometryn	80±13	45±36	12±7	101±8	2
Acifluorfen	131±15	203±48	164±47	231±92	37
Pharmaceuticals and personal care					
Lidocaine	146±35	99±15	88±7	127±11	1
Tramadol	124±12	50±19	51±10	53±3	1

DEET	56±15	75±16	70±9	86±17	3
Other					
Benzotriazole	94±7	106±16	87±9	94±7	2
4-Nonylphenol-mono-ethoxylate	28±13	33±11	33±16	47±1	36
4-Nonylphenol-di-ethoxylate	27±8	9±3	3±1	17±1	44

[a]limit of quantification determined following the methodology of USEPA (1997) Guidelines establishing test procedures for the analysis of pollutants (App. B, Part 136, Definition and procedures for the determination of the method detection limit): U.S. Code of Federal Regulations, Title 40, revised July 1, 1997, p. 265–267.

[b]not able to be determined

13 Appendix G. Summary of ecotoxicological assay statistical analyses

Table G 1. Summary of significant differences detected for 16s rRNA, *amoA, nirK and nifH* quantification by qPCR, based on the microplastic added at a dosing rate of 0.5% w/w with the addition of MWOO. Values are *p*-values ($p < 0.05$) determined by one-way ANOVA. Treatments that had values significantly less than those of controls (negative effects) are shaded black. Controls were MWOO-amended soil.

.Soil	Incubation period (months)	HDPE				PET				PVC			
		16s rRNA	*amoA*	*nirK*	*nifH*	16s rRNA	*amoA*	*nirK*	*nifH*	16s rRNA	*amoA*	*nirK*	*nifH*
Kirby Sand	0	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	-
Kirby Clay	0	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
	9												0.022
Warialda Loam	0	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	0.03	-	-	-	-	-	-	-	-	-	-

Table G 2. Summary of significant differences detected for SIR assays, based on the dosing rate of the plastics added on a weight/weight (w/w) basis, with (MWOO) and without (SOIL) the addition of MWOO. Values are *p*-values ($p<0.05$) determined by one-way ANOVA. Treatments that had values significantly less than those of controls (negative effects) are shaded black. Controls were soil only and MWOO-amended soil, respectively.

Soil	Incubation period (months)	HDPE				PET				PVC				
SOIL		0.1%	0.25%	0.5%	1% w/w	0.1%	0.25%	0.5%	1%	0.01 %	0.1%	0.25%	0.5%	1%
KS[b]	0	-[a]	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	0.004	0.004	-	0.006	0.007	0.021	0.004	-	-	-	0.022	0.004
KC[c]	0	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	0.023	-	0.011	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	-	-
WL[d]	0	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	0.039	-	-	-	-	-	-	-	-	-	-	-
MWOO[e]														
KS	0	-	-	-	-	-	-	-	-	-	-	-	-	<0.001
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	-	-
KC	0	-	-	-	-	0.006	-	-	0.038	-	-	0.004	-	0.04
	3	0.003	0.002	-	0.001	<0.001	0.001	-	0.005	0.011	<0.001	<0.001	-	0.001
	9	-	-	-	-	.018	-	-	0.004	0.005	-	-	-	0.049
WL	0	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	0.009	0.041	-	-	0.012	<0.001	0.026	-	0.016

[a]no significant difference found; [b]Kirby Sand; [c]Kirby Clay; [d]Warialda Loam; [e]MWOO-amended soil

Table G 3. Summary of significant differences detected for SIN assays, based on the dosing rate of the plastics added on a weight/weight (w/w) basis, with (MWOO) and without (SOIL) the addition of MWOO. Values are *p*-values ($p<0.05$) determined by one-way ANOVA. Treatments that had values significantly less than those of controls (negative effects) are shaded black. Controls were soil only and MWOO-amended soil, respectively.

Soil	Incubation period (months)	HDPE				PET					PVC			
SOIL		0.1%	0.25%	0.5%	1% w/w	0.1%	0.25%	0.5%	1%	0.01 %	0.1%	0.25%	0.5%	1%
KS[b]	0	0.025[a]	-	-	-	-	-	-	-	-	-	0.014	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	-	-
KC[c]	0	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	0.035	-	-	-	-	-	0.036	-	-	0.014	0.022	0.002	-
WL[d]	0	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	0.043	0.049	-	0.004	-	-	-
MWOO[e]														
KS	0	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	<0.001	0.036	-	-	-	-	-	-	-	-	-	-
KC	0	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	0.001	0.006	-	0.034	0.008	-	0.04	0.016	0.002	0.021	0.008	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	-	-
WL	0	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	<0.001	-	-	-
	9	-	-	-	<0.001	-	-	-	-	-	-	-	-	-

[a]no significant difference found; [b]Kirby Sand; [c]Kirby Clay; [d]Warialda Loam; [e]MWOO-amended soil

Table G 4. Summary of significant differences detected for mortality and reproduction of nematodes (*Caenorhabditis elegans*), based on the microplastic added at a dosing rate of 0.5% w/w with the addition of MWOO. Values are *p*-values ($p<0.05$) determined by one-way ANOVA. Treatments that had values significantly less than those of controls (negative effects) are shaded black. Controls were MWOO-amended soil.

Soil	Incubation period (months)	HDPE		PET		PVC	
		Mortality	Reproduction	Mortality	Reproduction	Mortality	Reproduction
Kirby Sand	0	-	-	-	-	-	-
	3	-	-	-	-	-	-
	9	-	-	-	-	-	-
Kirby Clay	0	-	-	-	-	-	-
	3	-	-	-	-	-	-
	9	-	-	-	-	-	-
Warialda Loam	0	-	-	-	-	-	-
	3	-	-	-	-	-	-
	9	-	0.023	-	-	-	0.007

Table G 5. Summary of significant differences detected for avoidance, growth and reproduction of earthworms (*Eisenia fetida*), based on the microplastic added at a dosing rate of 0.5% w/w with the addition of MWOO. Values are *p*-values ($p<0.05$) determined by one-way ANOVA. Treatments that had values significantly less than those of controls (negative effects) are shaded black. Controls were MWOO-amended soil.

Soil	Incubation period (months)	HDPE			PET			PVC		
		Avoidance	Growth	Reproduction	Avoidance	Growth	Reproduction	Avoidance	Growth	Reproduction
Kirby Sand	0	<0.001	-	-	<0.001	-	-	0.002	-	-
	3	-	-	-	-	-	-	-	-	-
	9	-	-	-	0.017	-	-	0.031	-	-
Kirby Clay	0	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-
Warialda Loam	0	-	-	-	0.044	-	-	-	-	-
	3	-	-	0.026	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-

Table G 6. Summary of significant differences detected for germination and growth of wheat (*Triticum aestivum*) seedlings, based on the microplastic added at a dosing rate of 0.5% w/w with the addition of MWOO. Values are *p*-values ($p<0.05$) determined by one-way ANOVA. Treatments that had values significantly less than those of controls (negative effects) are shaded black. Controls were MWOO-amended soil.

Soil	Incubation period (months)	HDPE		PET		PVC	
		Germination	Growth	Germination	Growth	Germination	Growth
Kirby Sand	0	-	-	-	-	-	-
	3	<0.001	-	<0.001	-	-	-
	9	-	-	-	-	-	-
Kirby Clay	0	-	-	-	-	-	-
	3	-	-	-	-	-	-
	9	-	-	-	0.003	-	-
Warialda Loam	0	-	-	-	-	-	-
	3	-	-	-	-	-	-
	9	-	-	-	-	-	-

14 Appendix H. TRFLP fluorescence chromatograms

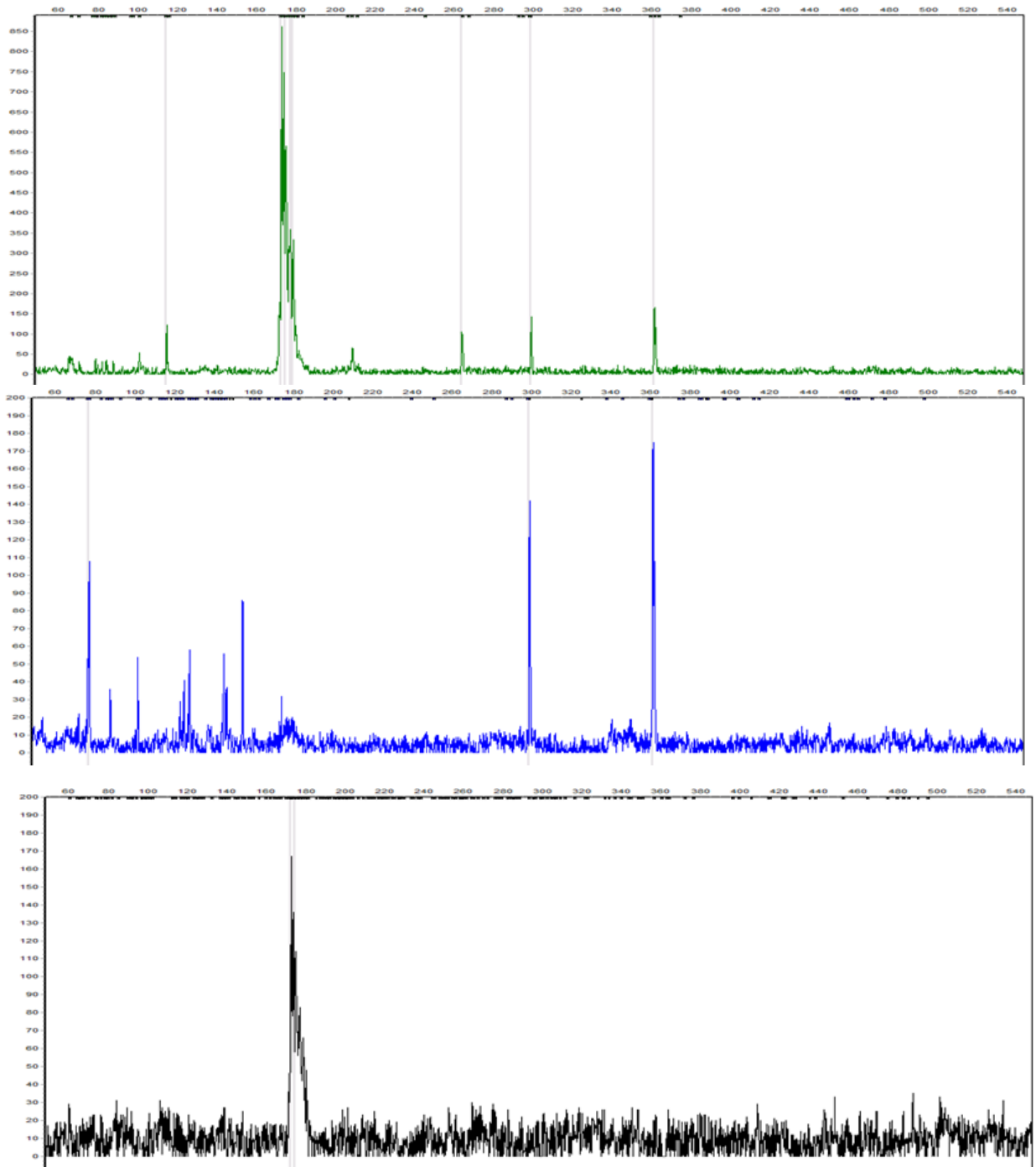


Figure H 1. An example of a TRFLP fluorescence chromatogram, in this case MWOO-amended unincubated (0 months) Kirby Clay controls, for bacterial (green), fungal (blue) and archaeal (black) communities. The x-axis represents the TRF size, while the y-axis (fluorescence intensity) relates to the number of TRFs of a particular size. Note the fluorescence intensity bacteria>fungi>archaea.

FURTHER INFORMATION

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