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Field response of N_2O emissions, microbial communities, soil biochemical processes and winter barley growth to the addition of conventional and biodegradable microplastics

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ABSTRACT

Microplastic contamination in agroecosystems is becoming more prevalent due to the direct use of plastics in agriculture (e.g., mulch films) and via contamination of amendments (e.g., compost, biosolids application). Long-term use of agricultural plastics and microplastic pollution could lead to soil degradation and reduced crop health due to the slow degradation of conventional plastics creating legacy plastic. Biodegradable plastics are more commonly being used, both domestically and in agriculture, to minimise plastic pollution due to their biodegradable nature. However, the influence of a biodegradable plastics on soil function at the field scale is largely unknown. We investigated the effect of conventional (polyethylene) and biodegradable (PHBV) microplastics on N_2O emissions and soil biochemical processes in a field trial of winter barley. Microplastic was added to the soil at realistic levels (100 kg ha^{-1}) for both conventional and biodegradable treatments. N_2O emissions were measured throughout the growing season alongside key soil quality indicators (microbial community composition, ammonium, nitrate, moisture content, pH and EC). Overall, microplastic addition had no observable effect on crop yield, microbial communities or soil biochemical properties. Yet, we found cumulative N_2O emissions were reduced by two-thirds following conventional microplastic addition compared to the no-plastic and biodegradable microplastic treatments. We believe this response is due to the lower soil moisture levels over the winter in the conventional microplastic treatment. Overall, the response of key soil parameters to microplastic addition show fewer negative effects to those seen in high dose laboratory mesocosm experiments. Thus, it is imperative that long-term field experiments at realistic dose rates be undertaken to quantify the real risk that microplastics pose to agroecosystem health.

1. Introduction

Global use of plastics in agriculture has increased dramatically since the 1950s reaching a consumption rate of > 12.5 million tonnes of plastic per year (FAO, 2021; Sintim and Flury, 2017). While this has helped to promote food security in many countries worldwide, it has also left a widespread legacy of plastic pollution which threatens future food production and agroecosystem health (FAO, 2021; Liu et al., 2014). The main sources of plastics entering soil come from the use of plastic mulch films, sewage irrigation, sludge application and aerial deposition (Huang et al., 2020; Zhu et al., 2019). Much of this contamination is present as macro-plastic, however, this progressively fragments into smaller particles through mechanical abrasion (e.g. tillage) and UV irradiation (Liu et al., 2014). Microplastics are defined as plastic particles between 1 μm and 5 mm (Frias and Nash, 2019). Once incorporated into the soil, microplastics can influence soil properties and processes including soil structure, organic matter processing and water availability (Xu et al., 2020). In the long-term, especially with continued input, microplastic pollution could lead to soil degradation and reduced crop health (Steinmetz et al., 2016).

Studies have shown microplastic to affect soil biological properties by altering microbial community composition and abundance and

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negatively affecting macroinvertebrate populations (Büks et al., 2020; Lin et al., 2020). Other studies have shown soil plastic to affect key soil chemical and physical properties intrinsic to soil health e.g., water content, aggregate stability and nutrient content (Lehmann et al., 2021; Qi et al., 2020a, 2020b). These changes in soil properties may negatively impact plant growth (De Souza Machado et al., 2019; Rillig et al., 2019). In response to this, biodegradable plastics are becoming increasingly popular due to their short-term persistence in soil. However, the input of a large carbon (C) source to soil could affect soil properties and microorganism abundance in soils (Sintim and Flury, 2017; Zhou et al., 2021). Soil properties affected by bio-based plastics are likely to be similar to conventional plastics, although the direction and/or magnitude of effects may differ (Boots et al., 2019). Understanding how both types of plastic affect crop growth and yield as well as soil function is vital in developing guidance for more sustainable use of agricultural plastics.

The slow degradation of plastics under natural conditions ensures their legacy in the environment; for example, a study on polypropylene (PP) biodegradation in soil found only 0.4% reduction in PP weight over one year (Arkatkar et al., 2009). Thus, biodegradable plastics are more commonly being used, both domestically and in agriculture, to minimise plastic pollution due to their biodegradable nature. However, because standards of 'biodegradability' vary internationally, more research is needed to assess the degree of environmental hazard these plastics may pose (Flury and Narayan, 2021). The degradation rate of bioplastics depends on the polymer used in its manufacturing (e.g. PBAT degradation is low compared with starch; Serrano-Ruiz et al., 2021). However, the majority of bioplastics are not 100% degradable under natural conditions (Viera et al., 2020). Thus, the length of time these compounds persist in agroecosystems and the interaction with soil processes remains unknown, particularly under realistic field levels of contamination.

So far, studies on nitrous oxide (N_2O) emissions from soil with microplastics have been limited to laboratory and mesocosm studies (e.g. Ren et al., 2020; Rillig et al., 2021). These studies have found microplastic addition to reduce N_2O emissions due to a) increased aeration of the soil reducing denitrification rates (Rillig et al., 2021) and/or b) inhibition of microorganisms responsible for denitrification (Ren et al., 2020). The translation of these studies to the field is of vital importance to better understand how microplastics affect soil biochemical processes under realistic conditions. Furthermore, little research has been conducted into the effect of biodegradable plastics on greenhouse gas (GHG) emissions, yet, the input of exogenous C could lead to changes in the production and emission of GHGs (Qin et al., 2021). Increased soil C could promote microbial activity and accelerate N mineralisation, however, it might also drive N immobilisation, and thus lower N_2O emissions (Le Mer and Roger, 2001; Watts et al., 2010).

The aim of this study was to determine the effect of conventional versus biodegradable microplastics on N_2O emissions and soil biochemical properties in a field trial of winter barley. We hypothesised that 1) biodegradable microplastics will increase N_2O emissions compared with conventional plastics due to the exogenous input of labile C (Qin et al., 2021), 2) conventional microplastic addition will negatively affect crop biomass and yield by altering soil biogeochemical cycling (Urbina et al., 2020; Zhou et al., 2021), and 3) both conventional and biodegradable microplastic addition will result in shifts in soil microbial community composition which underpin the responses above (Huang et al., 2019; Zhou et al., 2021).

2. Material and methods

2.1. Experimental field site

The study site was an arable field located at the Henfaes Research Centre, Abergwyngregyn, North Wales (53°14'29"N, 04°01'15"W). The soil is classified as a freely draining Eutric Cambisol (IUSS Working Group WRB, 2015) or Typic Hapludalf (US Soil Taxonomy) with crumb structure and sandy clay loam texture. A meteorological station at the experimental site recorded air temperature and total rainfall at half-hourly intervals (Fig. 1). The site has a temperate oceanic climate regime with long term (> 10 y) mean annul temperature of 10.8 °C and annual rainfall 1066 mm y^{-1}. The site has no previous history of plastic mulching or organic waste inputs likely to contain plastics (e.g. composts, biosolids). Prior to commencing the field trial, the site had a land use history of cereal production (e.g. wheat, barley, maize) and grassland (*Lolium perenne* L.) in rotation.

Experimental plots were established in a randomised block design with three treatments (conventional microplastic, biodegradable microplastic and control) and four replicates per treatment ($n = 4$) (see Fig. S1 for layout). On 16th September 2020, the soil in each 6 m × 1.2 m plot was excavated to 10 cm, placed in large plastic containers and mixed by hand with microplastics at a rate of 100 kg ha^{-1}. An application of 100 kg ha^{-1} of microplastic is the equivalent weight to ca. 1–3 years of plastic mulch film application. This equates to ca. 0.01% of the soil weight (assuming a 10 cm incorporation depth and bulk density of 1 g cm^{-3}). This dose was chosen to reflect realistic field contamination levels (Qi et al., 2020a, 2020b). Based on the typical thickness of PE mulch film used in agriculture (5–15 μm; Sun et al., 2020), this equates to a soil loading rate of 47–142 kg ha^{-1} if none of the plastic is removed from the soil at harvest and subsequently incorporated by tillage. The conventional microplastic consisted of polyethylene (PE; 40–48 μm diameter; Sigma-Aldrich, Poole UK) while the biodegradable microplastic consisted of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV; 1–15 μm diameter; TianAn Biologic Materials Ltd, Ningbo City, China). PE is a polymer chain of ethylene which is hydrophobic and ductile, the latter making it a good material for mulch films (Aggarwal and Sweeting, 2002). PHBV is a thermoplastic linear aliphatic polyester made from a copolymer of poly(3-hydroxybutyrate) and poly (3-hydroxyvalerate) which has moderate biodegradability in soil (Serrano-Ruiz et al., 2021). Neither the PE or PHBV contained any additives which would have altered properties of the microplastics. After homogenisation of the plastic within the soil, it was placed back in each plot. The control (no plastic) plots were also excavated to 10 cm and mixed, except that no plastic was added. Winter barley (*Hordeum vulgare* L. cv. L G Flynn, Limagrain UK Ltd., Market Rasen, UK) was sown on 16th October 2020 at a sowing rate of 300 seeds m^{-2}. Ammonium nitrate (NH_4NO_3) fertiliser was applied in three applications on 26th October 2020, 25th March 2021 and 19th April 2021 at rates of 20, 60 and 60 kg N ha^{-1}, respectively (AHDB, 2019). Soil P and K content was deemed sufficient from soil testing by NRM Laboratories (Cawood Scientific), UK and fertiliser for these nutrients was not recommended (AHDB, 2019).

2.2. Soil and crop measurements

Soil volumetric water content (θ) and temperature were measured using Acclima TDT Soil-Water-Temperature-BEC sensors (Acclima Inc., Meridian, ID, USA), installed horizontally at 10 cm depth in each plot. Dry bulk density (0–5 cm) was determined on intact 100 cm^3 cores following oven-drying (105 °C, 24 h) at the beginning of the experiment. Water filled pore space (WFPS) was calculated from the soil volumetric water content measured using the Acclima data. Total pore space (cm^3 cm^{-3}) in the soil was calculated from the bulk density and the assumption of a particle density of 2.65 g cm^{-3} (Rowell, 2014). Soil %WFPS was then calculated as a ratio of volumetric water content to soil porosity. Soil samples (0–20 cm) were collected weekly until June 2020 and then fortnightly using a 100 cm^3 stainless steel soil corer at 3 random locations that were combined and homogenised for each plot. Soil pH and electrical conductivity (EC) were measured in fresh soil in 1:2 (w/v) soil:H_2O extracts after shaking at 225 rev min^{-1} for 30 min. Soil inorganic N concentrations were measured in soil extracts of 1:5 (w/v) soil:0.5 M K_2SO_4. Ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were both determined colorimetrically according to Mulvaney (1996) and Miranda et al. (2001), respectively.

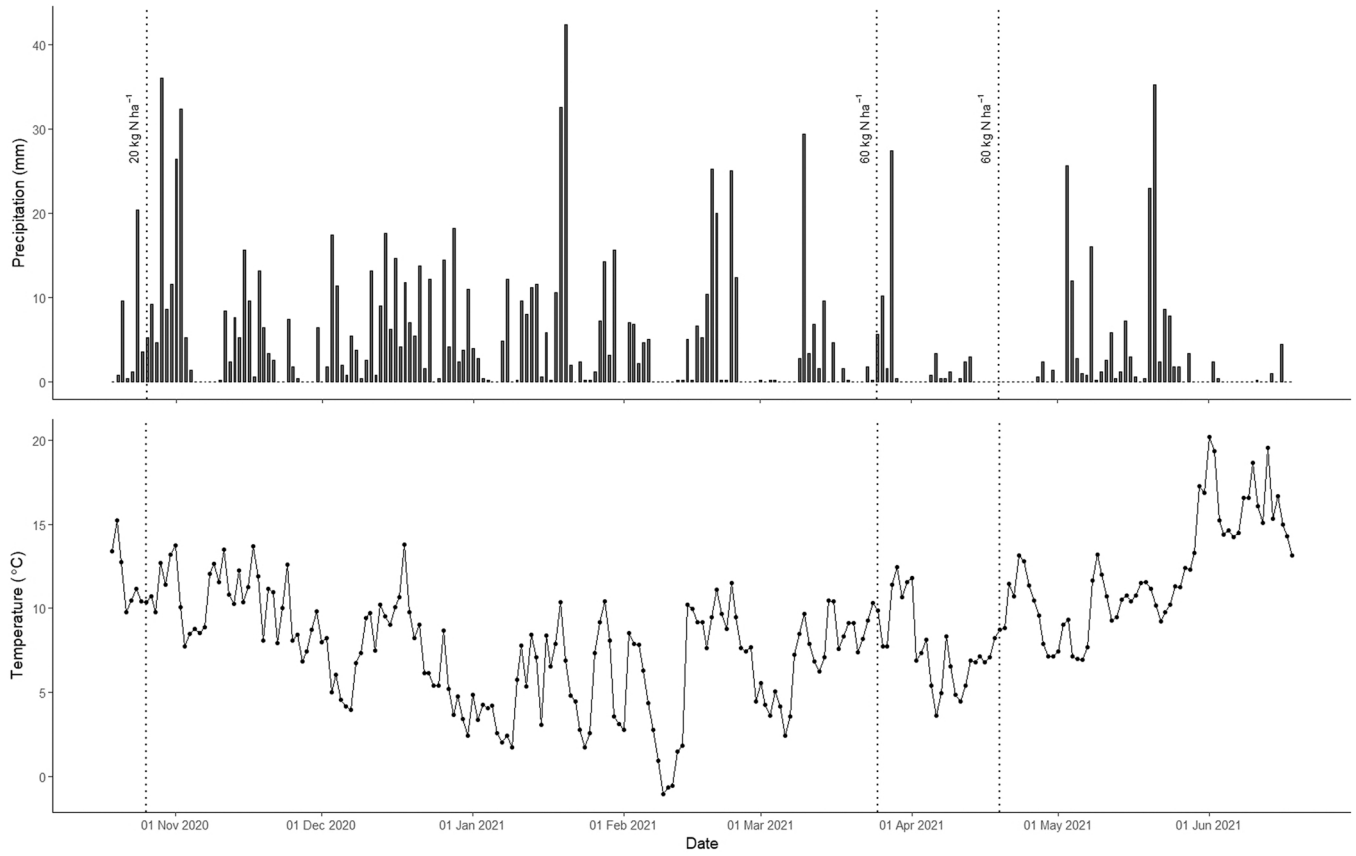

Fig. 1. Daily precipitation (mm) and air temperature (°C) over the experimental cropping season. Dotted lines show the date of fertiliser application, and the top panel is labelled with the amount of ammonium nitrate fertiliser applied.

The crop height (base of plant to the top of the stem) was measured weekly until June 2021 and then measured fortnightly on 6 randomly selected plants per plot. Leaf chlorophyll content was also measured at the same sampling times as crop height using a Soil Plant Analysis Development (SPAD) chlorophyll metre (SPAD-502 PLUS; Konica Minolta Sensing Europe B.V., Warrington, UK) on 6 randomly selected plants per plot. Pre-harvest crop yield was measured on 18th June 2020 where the plants in two rows (50 cm long) from the middle of each plot were cut 5 cm above the soil surface. The crop samples for each plot were then stored in muslin bags and left to air dry. Ears of the barley were removed counted and weighed, then, grains removed and counted. The remaining plant (straw) was weighed.

2.3. Greenhouse gas measurements

Fluxes of N_2O were monitored using an automated GHG measurement system (Queensland University of Technology, Institute for Future Environments, Brisbane, Australia). Stainless steel chamber bases (0.25 m^2 basal area) were inserted into the plots (10 cm depth) four weeks after treatment application and opaque chambers (50 cm × 50 cm × 15 cm) clipped onto the bases. A detailed description of the measurement system can be found in Marsden et al. (2018). Briefly, the system comprised 12 automated chambers linked to a Gas Chromatograph in a mobile laboratory, with headspace samples taken at T0, T15, T30 and T45 minutes. This resulted in eight gas flux measurements per 24 h period, per plot. Chamber extensions were added as the crop grew. Initially the chambers were 0.15 m in height, with an extension to 0.4 m on 16th April 2021 and then to 0.65 m on 18th May 2021. Gas measurements were stopped on 24th May 2021. Cumulative flux measurements were calculated using trapezoidal integration in Microsoft Excel.

2.4. 16S gene sequencing

16S gene sequencing was conducted on soil from each treatment. Soil samples (ca. 15 g) were collected from 0 to 10 cm prior to harvest of the plants and stored at − 80 °C before freeze-drying. Bacterial and archaeal DNA was extracted from each sample using the Zymo BIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions. One DNA extraction per sample was carried out with a high-speed bead beating for each sample. Quality and concentration of extracted DNA were assessed by gel electrophoresis and by Qubit 4.0 Fluorometer dsDNA BR Assay Kit (Life Technologies, Carlsbad, CA). Libraries of 16S rRNA gene amplicons were prepared by single PCR with double-indexed fusion primers as described previously (Fadrosh et al., 2014). Hypervariable V4 16S rRNA gene fragment was amplified using modified forward primer F515 (5′-GTGBCAGCMGCCGCG GTAA-3′) and reverse R806 prokaryotic primer (5′-GGACTACHVGG GTWTCTAAT-3′), which amplify an approximately 290 bp region. Primers were designed to contain: the Illumina adaptors and sequencing primers, a 12 bp barcode sequence, a heterogeneity spacer to mitigate the low sequence diversity amplicon issue, and 16S rRNA gene universal primers (Fadrosh et al., 2014). PCRs were performed using OneTaq DNA Polymerase (New England Biolabs, Ipswich, MA). All reactions were run with no-template negative controls. Thermocycling conditions were: initial denaturation at 95 °C for 2 min, followed by 30 cycles at 95 °C for 30 s, 50 °C for 50 s, and 72 °C for 90 s with a final elongation at 72 °C for 5 min. Amplicons were visualised in a 1.5% tris-acetate agarose gels using a GelDoc System (Bio-Rad Laboratories Inc., Hercules, CA). DNA bands of approximately 440 bp were gel-purified using QIAEX II Gel Extraction Kit (Qiagen GmbH, Hilden, Germany). The purified amplicons were then quantified using a Qubit 4.0 Fluorometer, pooled in equimolar amounts and the final pool was run on Illumina MiSeq

platform (Illumina, San Diego, CA) using 500-cycle v2 chemistry (2 × 250 bp paired-end reads) at the Centre for Environmental Biotechnology, Bangor, UK.

Raw sequencing reads were processed according to previously described protocols (Fadrosh et al., 2014; Korzhenkov et al., 2019). Briefly, the data were pre-processed to extract the barcodes from sequences, and then cleaned of primer sequences using tagcleaner. The barcodes and the sequences were re-matched again using in-house Python scripts. The resulting filtered reads were analysed using QIIME v1.3.1. First, the libraries were demultiplexed based on the different barcodes. Then, the sequences were denoised, filtered and classified on Amplicon Sequence Variants (ASVs) using dada2 pipeline integrated on QIIME2. Taxonomic assignation was performed using SILVA v138 database. The NCBI BioProject accession number is PRJNA820026.

Analysis of most abundant taxonomic groups were performed using in-house R-based scripts, selecting those groups with a relative abundance at least > 2% in any of the samples. Selection started at genus level and groups are added to the immediate upper taxonomic level when none of the samples of that group get to the 2% threshold.

2.5. Data analysis

Unless otherwise stated, all graphs and data analysis was carried out in R v4.02. (R Core Team, 2018). Kruskal-Wallis (for non-parametric data) and one-way analysis of variance (ANOVA) (for parametric data) were used to determine whether experimental variables (N_2O flux, soil ammonium and nitrate content, pH, EC, SPAD and plant height) differed with plastic treatment. Data are expressed on a soil dry weight basis unless otherwise specified. Differences were compared over four time points: 1) the two weeks following the first fertiliser application, 2) the two weeks following the second fertiliser application, 3) the two weeks following the third fertiliser application, and 4) the last day/sampling point. One-way ANOVAs were performed on the most abundant groups from 16S gene sequencing to determine whether relative abundance differed between plastic treatment for each group. Normality was checked visually using qqplot plots and heterogeneity using residual plots. Relative abundance at the family level of taxa in different treatments was graphed using phyloseq (McMurdie and Holmes, 2013). Non-metric multidimensional scaling (NMDS) analysis and permanova test of the 16S gene sequencing data at family level was performed on Shaman (Volant et al., 2020). Microbial diversity plots were also created on Shaman.

3. Results

3.1. Soil properties

Ammonium and nitrate concentrations remained low in the soil, except after the N fertiliser applications where all treatments had similar responses of increased soil ammonium and nitrate (Fig. 2a–b, Table 1). Soil pH and EC remained constant except after the fertiliser applications where pH decreased, and EC increased for all treatments (Fig. 2c–d, Table 1). WFPS was consistently lower in the conventional microplastic treatment whilst the biodegradable microplastic treatment was

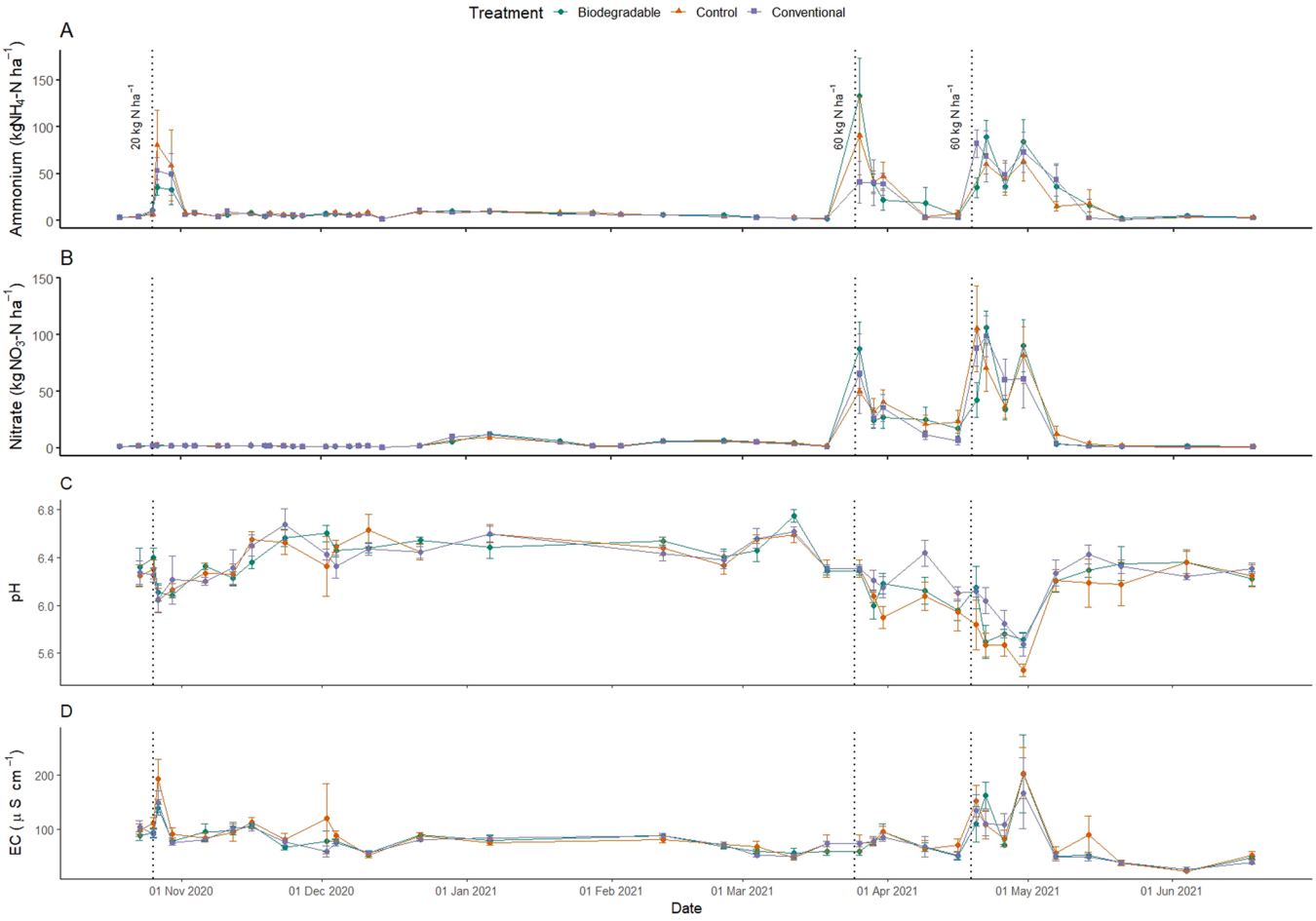

Fig. 2. Soil chemical properties over the growing season with no plastic addition (control), conventional and biodegradable microplastic addition (mean ± S.E., $n = 4$). A) Ammonium content (kg NH_4-N ha^{-1}), B) nitrate content (kg NO_3-N ha^{-1}), C) pH and D) electrical conductivity (EC) (µS cm^{-1}). Dotted lines show the date of fertiliser application, and the top panel is labelled with the amount of ammonium nitrate fertiliser applied.

Table 1
Summary table for Kruskal-Wallis/one-way ANOVA test to determine difference between microplastic treatments for each variable for the two-week period following fertiliser application and the last sample day. Bold *p* values indicate significant difference between plastic treatments ($p < 0.05$).

	1st application			2nd application			3rd application			End		
	chi^2	*df*	*p*	chi^2	*df*	*P*	chi^2	*df*	*p*	chi^2	*df*	*p*
Daily N_2O flux	347	2	**< 0.001**	66	2	**< 0.001**	57	2	**< 0.001**	1386	2	**< 0.001**
Cumulative N_2O flux	402	2	**< 0.001**	5760	2	**< 0.001**	348	2	**< 0.001**	5981	2	**< 0.001**
Ammonium	3.07	2	0.22	1.56	2	0.46	0.15	2	0.93	0.81	2	0.67
Nitrate	3.95	2	0.14	1.23	2	0.54	0.35	2	0.84	0.27	2	0.87
pH[a]	0.30	2	0.74	3.62	2	**0.04**	4.0	2	**0.03**	0.42	2	0.70
EC[a]	1.06	2	0.36	0.05	2	0.95	0.03	2	0.97	1.66	2	0.24
Soil moisture	592	2	**< 0.001**	329	2	**< 0.001**	15	2	**< 0.001**	77	2	**< 0.001**
Plant height				0.10	2	0.91	0.06	2	0.97	0.46	2	0.79
SPAD[a]				1.16	2	0.32	6.78	2	**0.003**	0.48	2	0.64

[a] One-way ANOVA test. Chi^2 is F value for these tests.

consistently the highest (Fig. 3b, Table 1).

3.2. N_2O emissions

N_2O emissions were variable over the cropping cycle with two distinct peaks after the first (ca. 180 μg N_2O-N m^{-2} h^{-1} in the control) and second (ca. 60 μg N_2O-N m^{-2} h^{-1} in the control) fertiliser application for each treatment (Fig. 3a, Table 1). The N_2O peak after the third fertiliser application was delayed by over a week. Average cumulative N_2O flux, with the conventional plastic treatment, was approximately one-third of the cumulative biodegradable and control fluxes (Fig. 4, Table 1). Biodegradable and control cumulative fluxes followed a similar trend over the growing season.

3.3. Bacteria and archaeal diversity

In total, 2796 bacterial ASVs, were identified across all 16S DNA gene reads. Most abundant group analysis showed minimal, insignificant differences between treatments for the relative abundance of taxa (Fig. 5). NMDS analysis did not show any significant clustering within treatments (Fig. S2b, permanova test: $p = 0.27$). Alpha and Shannon diversities did not show any significant differences between experimental treatments (Fig. S2c).

3.4. Crop measurements

Plant heights showed no difference between treatments after fertiliser applications and at the end of the experiment (Fig. 6, Table 1). Leaf

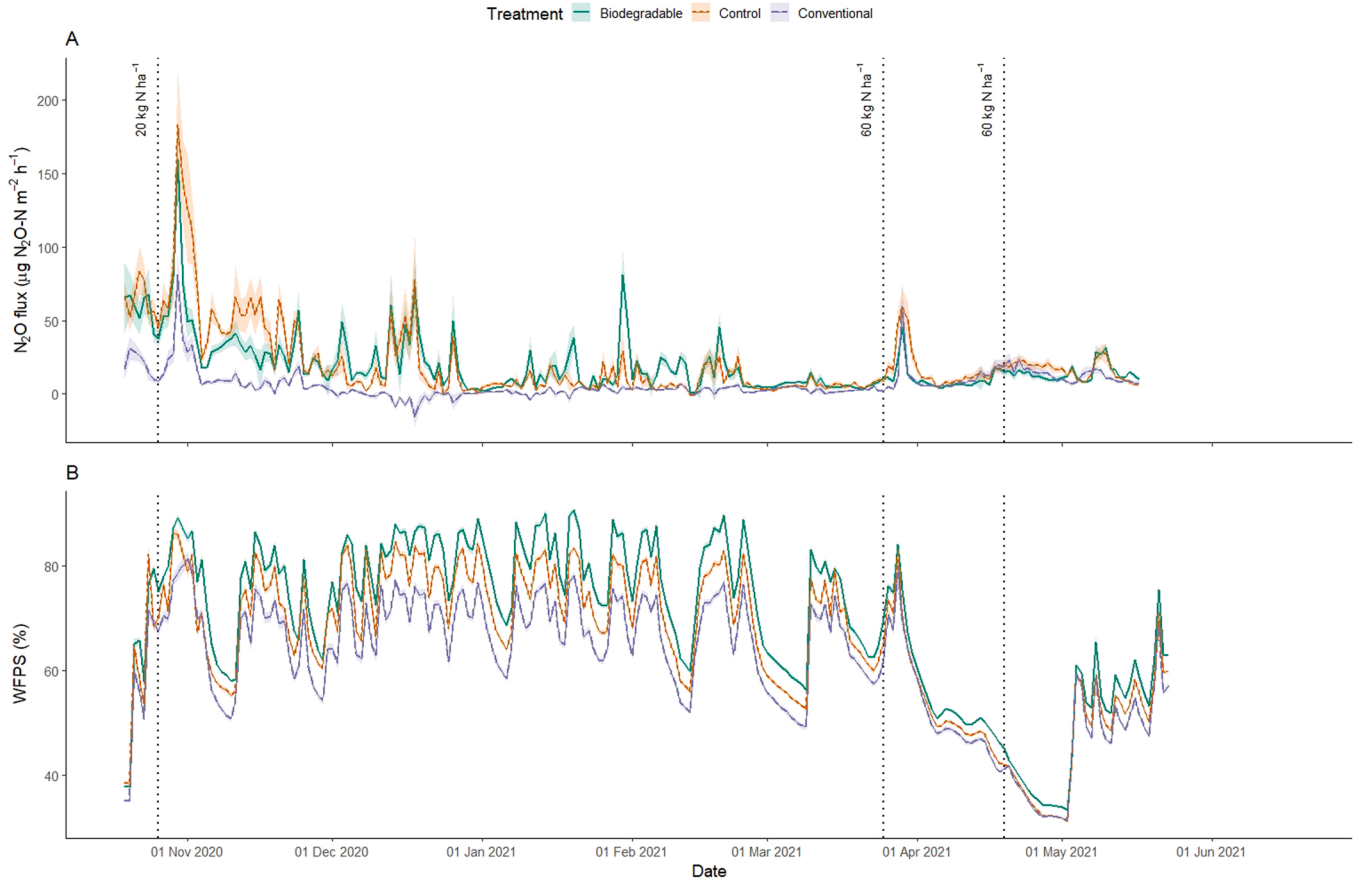

Fig. 3. A) Daily mean N_2O flux (μg N_2O-N m^{-2} h^{-1}) and B) daily mean water filled pore space (WFPS) (%) in soil over the growing season for no plastic addition (control), conventional and biodegradable microplastic addition (mean ± S.E., $n = 4$). Dotted lines show the date of fertiliser application, and the top panel is labelled with the amount of ammonium nitrate fertiliser applied.

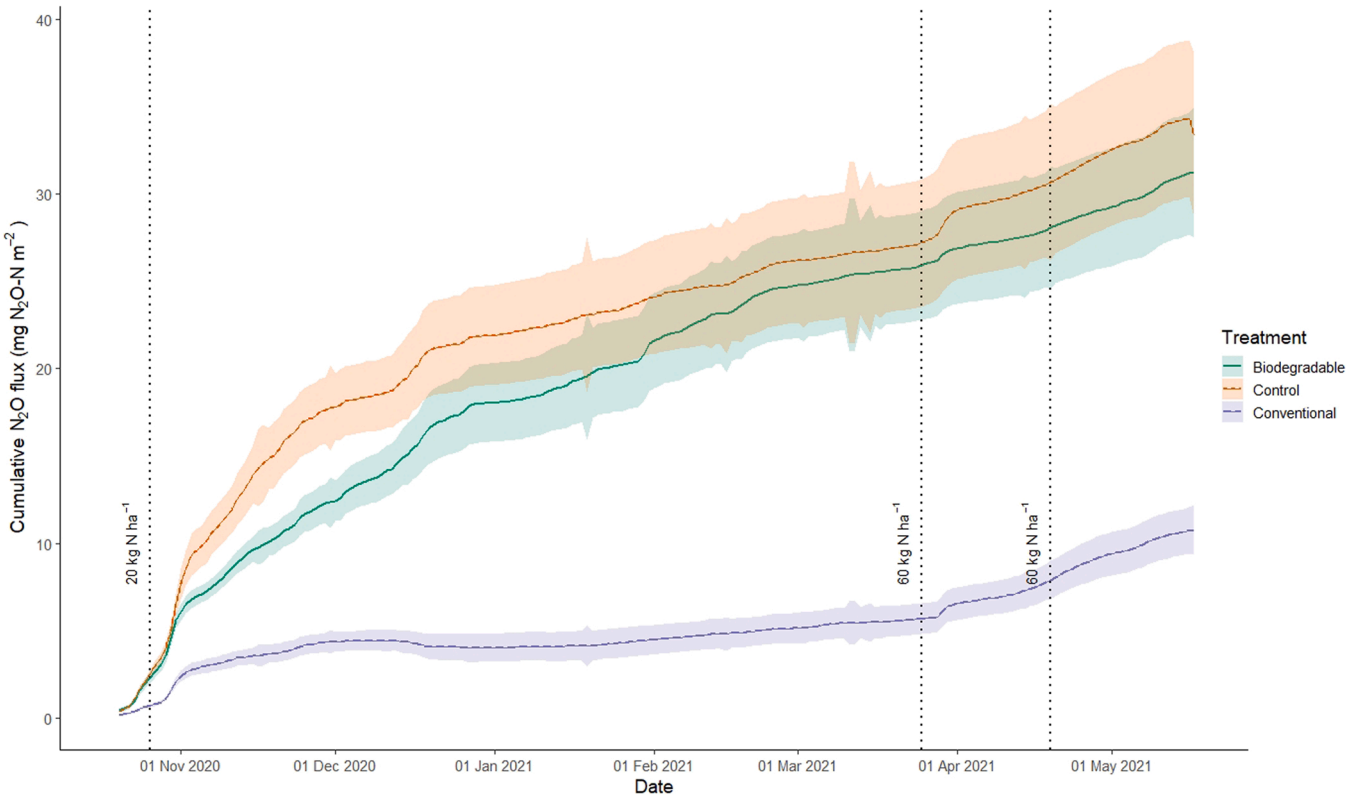

Fig. 4. Cumulative N_2O flux (mg N_2O-N m^{-2}) over the growing season for no plastic addition (control), conventional and biodegradable microplastic addition (mean ± S.E., $n = 4$). Dotted lines show the date of fertiliser application and are labelled with the rate of ammonium nitrate fertiliser applied.

chlorophyll content (SPAD) remained similar between treatments except after the second fertiliser application where the biodegradable plastic treatment had higher SPAD readings (Fig. 6, Table 1). SPAD readings decreased across all treatments from 21st May 2021 as the crop and leaf senescence began.

For each of the harvest measurements taken (straw biomass, 1000 grain weight, ear weigh and grains per ear), there were no observational or significant differences between treatments (Table 2).

4. Discussion

4.1. Effect of microplastic on N_2O emissions

Understanding how microplastic addition affects N_2O emissions (along with other GHGs) is key to understanding their effect on agro-ecosystems as a whole. We found emissions from conventional microplastic addition to be two-thirds lower than no plastic addition or biodegradable plastic addition. Rillig et al. (2021) found lower N_2O emissions in soils with microplastic addition due to changes in soil structure caused by the microplastic. This effect was also seen in soil cores sampled from an agricultural field by Ren et al. (2020) where the addition of PE microplastics reduced N_2O emissions by 7 times relative to the control. We observed a similar result with our conventional microplastic treatment, but we believe this was more likely due to a reduced water filled pore space (WFPS) allowing for more aerated pockets and thus reduced N_2O emissions produced via denitrification (Bollmann and Conrad, 1998). The WFPS in the conventional microplastic treatment was only > 80% on one occasion, otherwise, WFPS remained between 50% and 75%. In contrast, the biodegradable and control treatments had several peaks in WFPS > 80%. Conducting a denitrification assay would provide further evidence of reduced denitrification in the conventional microplastic treatment. Changes in nitrification between treatments could also affect N_2O emissions, however, there is not enough evidence in our study (from soil mineral N contents) to suggest this is a likely driver. At the same experimental site, another field study looking at the effect of different concentrations of PE microplastic on N_2O emissions found no difference in N_2O emissions between the control and 100 kg ha^{-1} plastic treatment (Brown et al., 2021). The wheat crop was sown in spring and harvested in summer in contrast to our study that used winter barley sown in October 2020 and harvested in June 2021. The largest differences between the conventional PE microplastic and the control N_2O emissions were during the winter months (October–March) where rainfall was higher and more consistent. From March onwards, there were longer periods of no/little rainfall mirroring reduced N_2O emissions in the control and biodegradable treatments. Soil moisture is a vital factor in influencing N_2O emissions with high soil moisture levels generally enhancing N_2O emissions (Liu et al., 2007). Thus, the influence of conventional PE microplastic on soil moisture could be an important mechanism for determining N_2O emissions from soil with plastic addition.

The lower WFPS in the conventional plastic treatment could be due to conventional PE microplastic being more hydrophobic than the bio-based PHBV, suggesting water was repelled from the plastic particles allowing more oxygen in between the microplastic and soil micro-aggregates limiting denitrification and thus N_2O emissions (De Souza Machado et al., 2019; De Souza MacHado et al., 2018). However, evidence on the differing hydrophobic nature of between different plastic types, especially conventional and biodegradable, is lacking. Another suggestion is PHBV was approximately three-times larger than PE microplastic which could have altered the soil properties particularly physical properties and soil moisture (Wang et al., 2022), but again specific evidence is lacking. More in depth experiments are needed to determine how different plastics are affecting soil water properties. Overall, we can determine that the biodegradable microplastic does not have an effect on soil N_2O emissions as PE microplastic because control and biodegradable treatments were similar.

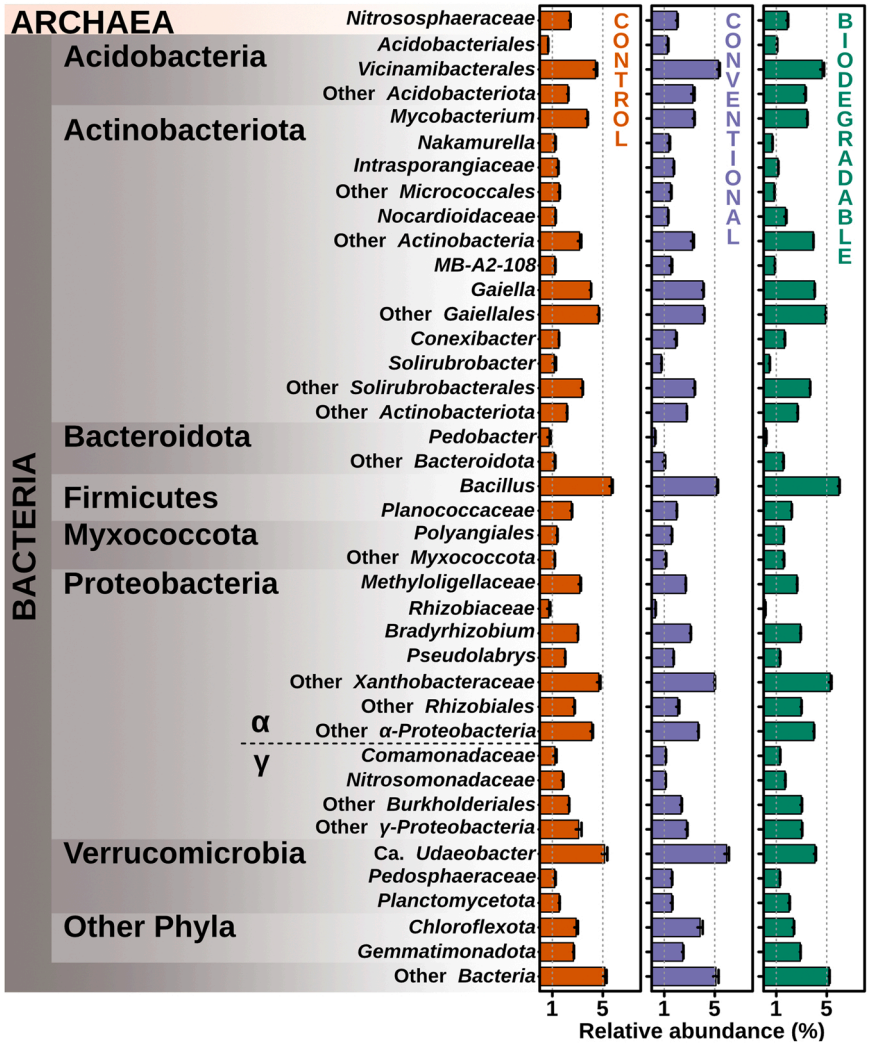

Fig. 5. Most abundant group analysis with a relative abundance at least > 2% in any of the samples. One-way ANOVA was used to determine significant differences between plastic addition treatment for each taxon ($n = 4$). All taxa showed no significant differences with treatment ($p > 0.05$).

Our study showed the largest peaks in N_2O emission after the first and second ammonium nitrate fertiliser application in the control treatment. Whilst Rillig et al. (2021) found the differences between N_2O emissions with and without microplastic addition are less pronounced after urea fertilisation. Urea fertiliser typically causes less N_2O emissions (Cardenas et al., 2019; Smith et al., 2012) and more NH_3 emissions compared with ammonium nitrate based fertiliser (Chambers and Dampney, 2009; Cowan et al., 2019). The N_2O peak from the third fertiliser application was delayed by over a week and is likely due to the drier conditions experienced at this time (Figs. 1 and 6). The soil ammonium and nitrate peaks after the third application were more pronounced than the second application due to reduced leaching below the sampling depth during this drier period or greater N uptake by the crop during the stem elongation growth stage.

4.2. *Effect of microplastic on soil bacterial and archaeal diversity*

Despite the differences in N_2O emissions between the conventional microplastic addition and the control, we found no effect of conventional or biodegradable microplastic addition on bacterial and archaeal diversity. There have been few studies on the soil biodegradation of PHBV but evidence from Zaidi et al. (2019) suggest natural degradation is slow after finding no difference after 112 days buried in the soil. Our study ran for 275 days, however, over the winter period when soil temperatures were below 5 °C it is unlikely much plastic degradation took place in these months (Rudnik and Briassoulis, 2011). The majority of studies undertaken to date on microorganism-plastic interactions have been based on laboratory incubations, typically at very high loading rates. Several laboratory studies have found changes in soil microbial composition and diversity as a result of conventional and biodegradable microplastic addition (Fei et al., 2020; Qi et al., 2020a, 2020b; Zhou et al., 2021). Our application of 100 kg ha^{-1} of microplastic equates to ca. 0.01% of the soil weight. In contrast, plastic addition rates within laboratory incubations typically ranges between 0.1% and 10% (Fei et al., 2020; Y. Qi et al., 2020; Zhou et al., 2021). De Souza MacHado et al. (2018) found low concentrations of microplastic addition (0.1%) had a greater effect on soil physical properties compared to soil microbial properties. Thus, higher concentrations of microplastic could be needed in order to see major effects on soil biological properties and community composition. Brown et al. (2021) also found little variation in microbial diversity between no plastic addition and 100 kg ha^{-1} of PE microplastic. Therefore, it is likely the 100 kg ha^{-1} of plastic applied for one cropping season does not alter microbial community composition significantly. Our results also suggest that microplastics did not greatly alter root exudation and turnover as no change was seen in the rhizosphere microbial community. A caveat of our experiment, however, is that we only assessed the bacterial and archaeal components of the community and at the end of the growing season. Further work is

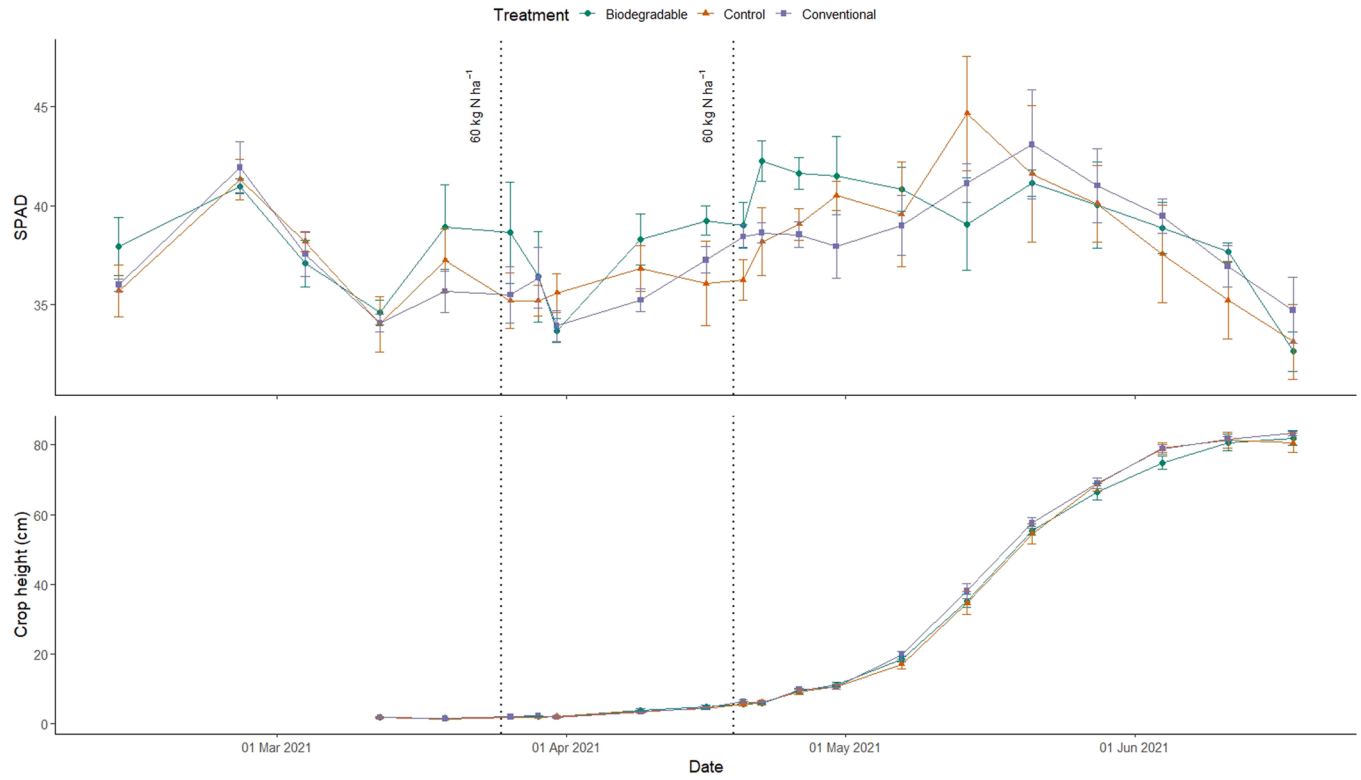

Fig. 6. Leaf chlorophyll content (SPAD) (top) and crop height (cm) (bottom) over the growing season for no plastic addition (control), conventional and biodegradable microplastic addition (mean ± S.E., $n = 4$). Dotted lines show the date of fertiliser application, and the top panel is labelled with the rate of ammonium nitrate fertiliser applied.

Table 2
Summary table of pre-harvest measurements (on a dry weight basis) for each microplastic treatment ($n = 4$) (mean ± S.E.). Different letters indicate significant differences between treatments for each variable.

Treatment	Straw weight (kg ha^{-1})	1000 grain weight (g)	Ear weight (g)	Grains per ear
Control	5150 ± 522^{a}	23.8 ± 3.1^{a}	47.0 ± 12.0^{a}	26.6 ± 1.0^{a}
Synthetic	4338 ± 349^{a}	27.5 ± 1.4^{a}	48.5 ± 4.00^{a}	26.9 ± 1.5^{a}
Biodegradable	5488 ± 577^{a}	28.8 ± 1.3^{a}	54.5 ± 10.9^{a}	26.5 ± 1.0^{a}

therefore needed to characterise changes in the fungi and mesofauna communities. If microplastics affect higher trophic levels (e.g. earthworms, collembola, mites) over longer timescales then this is likely to profoundly affect the soil microbial community both directly (e.g. via excretion of their gut microbiome) and indirectly (e.g. by a reduction in aeration and loss of soil structure) (Ding et al., 2021; Jiang et al., 2020).

4.3. *Effect of microplastic on crop yields*

Pre-harvest metrics showed no differences in plant performance between either microplastic treatment or the control. Once again, few studies have investigated the effect of microplastic on crop yield and heath especially in a field experiment. Laboratory incubations at soil addition rates ranging between 0.1% and 2% (w/w) have reported negative effects of a range conventional microplastics on plant quality indicators and biomass (De Souza Machado et al., 2019; Lozano and Rillig, 2020; Zang et al., 2020). However, it could be argued that the amounts used in these mesocosms experiments are far greater than are naturally found in agricultural soils with long term plastic mulching. We deliberately chose a microplastic dose which better reflects natural pollution levels. The difference between the negative effects seen in laboratory studies and our field experiment may also be due to the complete homogenisation of microplastic in laboratory studies and the inability of plants to avoid microplastics in a containerised situation. In our experiment, microplastics were added and homogenised to a depth 10 cm, yet 50% of the roots are typically found in the top 20 cm of soil, effectively avoiding the contamination layer (Fan et al., 2016). If limited downwards movement of microplastic through the soil profile occurred, then the amount of microplastic directly affecting the rhizosphere would be much less in the field. Further, our experiment was much longer in duration than many other trials. We conclude, therefore that in the short-term (< 1 y), microplastic pollution is unlikely to affect crop yield. Based on experiments performed in the laboratory with the same soil which showed strong inhibitory effects of microplastics on plant performance (Zang et al., 2020), we recommend that care should therefore be taken when extrapolating very high dose laboratory experiments to actual field conditions, thus ensuring that the risks are seen in proportion to the real-world problem. Long-term field trials of both single and continued application are still needed, however, to determine microplastic legacy effects on agroecosystem health.

An additional caveat of our experiment is that we only used pure plastics of a tightly defined size range. It is possible that additives contained in commercial plastics (e.g. phthalates, nanoparticulate metals) and co-contaminants bound to the plastics (e.g. pesticides) found under different agronomic management regimes might negatively impact on plant and soil health. In addition, it has also been shown that plant roots can take up microplastic particles, potentially leading to negative impacts on growth and metabolism. However, most of these studies have been undertaken in soil-less laboratory culture on small seedlings at unnaturally high plastic concentrations. The response also appears to be dependent on the size and type of plastic particle studied (Azeem et al., 2021; Li et al., 2019). Based on the size of pores in the plant cell wall of crop plants (2–10 nm; Carpita et al., 1979; Fleischer et al., 1999), we

conclude that the microplastic particles used in our experiment would be too large to directly enter root cells. This is supported by Taylor et al. (2020) who showed that while nano- and micro-plastics may be associated with the surface of roots, direct uptake of these plastics does not readily occur. Although we did not measure root biomass directly, based on published experiments in the same soil, if a large microplastic-root effect had occurred this would have been evident in foliar growth and nutrient content (Zang et al., 2020).

5. Conclusion

One application of conventional (PE) microplastic over one cropping season reduced N_2O emissions. Based on this study, it is likely that conventional microplastic will continue to reduce N_2O emissions, depending on soil moisture conditions, as it does not readily degrade. However, this ultimately depends on interactions between microplastic and soil constituents and whether the plastic particles develop a hydrophilic biofilm. It is therefore vital to conduct more long-term field trials to determine more realistic responses of microplastic addition to greenhouse gas emissions and soil biochemical processes. Further, we ascribe the differences in results obtained from previous laboratory-based experiments (which frequently show strong negative effects on plant and soil health) to our field-based experiment to the more realistic concentrations of plastic used and the greater plant-to-soil volume. Even after 20 years of plastic mulch incorporation, and assuming no degradation or loss, most plastic will be present as macro-plastic. We therefore conclude that high dose laboratory mesocosm experiments are failing to reflect reality and are significantly overstating the risk of microplastics to agroecosystem health. Going forward, it is imperative that we understand the benefits and disadvantages of either mulch films or organic resources recycled to land (which may contain plastics), to make informed decisions on the continued use of plastic in agriculture.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2022.108023.

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