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CONTAMINACIÓN CON MICROPLÁSTICOS: EVALUACIÓN DE SUS EFECTOS SOBRE EL SISTEMA SUELO-PLANTA

MICROPLASTIC POLLUTION: EVALUATION ON SOIL-PLANT SYSTEM RESPONSE

Tesis para optar al grado de Doctor en Ciencias de la Agronomía

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RESUMEN

Los microplásticos (MPs) son plásticos de un tamaño entre 0.1 µm y 5 mm, estos pueden ser fabricados intencionadamente pequeños o generarse por la fragmentación de plásticos más grandes. Su creciente presencia en suelos representa un desafío ambiental, afectando las propiedades físicas, químicas y biológicas del suelo. Al realizar una de las primeras revisiones sistemáticas de la literatura se evidenció que los MPs alteran el ciclo del nitrógeno (N) en varias áreas; actúando como sustrato para microorganismos, alterando las comunidades microbianas/enzimas, afectando la fauna del suelo lo que altera la dinámica de descomposición de materia orgánica y la disponibilidad de nutrientes.

Se establecieron microcosmos para estudiar el efecto de los MPs en un Andisol, evidenciando que MPs de poliamida (PA) y polietileno de baja densidad (LDPE) aumentaron el carbono orgánico total (TOC) y amonio (NH_4^+), acompañado de una disminución de fosfato (PO_4^{3-}), nitrato (NO_3^-) y disminución en la actividad β-glucosidasa. Además, se determinó que TOC y fosforo total (TP) pueden ser usados como parámetros químicos predictores de cambios microbiológicos en un Andisol contaminado con MPs.

Al estudiar los efectos en un sistema suelo-planta, se evidenció que plantas de *Raphanus sativus* crecidas en un Andisol con MPs incrementaron su biomasa y el contenido relativo de clorofila en los tratamientos con PA, LDPE y polipropileno (PP), sin embargo, hubo un aumento en el estrés oxidativo además de una alteración en los perfiles de compuestos bioactivos, como antocianinas y glucosinolatos en la raíz comestible, especialmente con PP. Además, se identificó al carbono de biomasa microbiana (MBC) y los polifenoles como parámetros de suelo predictores de cambio de *R. sativus* crecidos en suelo contaminado con MPs.

Estos resultados subrayan los riesgos a largo plazo de la contaminación por MPs, tanto para el suelo como para la calidad de los cultivos, lo que podría tener implicaciones para la seguridad alimentaria y la salud humana.

ABSTRACT

Microplastics (MPs) are plastics with a size between 0.1 μm and 5 mm, these can be intentionally manufactured to be small or result from the fragmentation of larger plastics. Their increasing presence in soils represents a significant environmental challenge, affecting the soil's physical, chemical, and biological properties. In conducting one of the earliest systematic reviews of the literature, it was found that MPs disrupt the nitrogen (N) cycle in multiple areas; serving as a substrate for microorganisms, altering microbial communities and enzymes, and affecting soil fauna, which in turn impacts organic matter decomposition and nutrient availability.

Microcosms were established to study the effect of MPs in an Andisol, showing that polyamide (PA) and low-density polyethylene (LDPE) MPs increased total organic carbon (TOC) and ammonium (NH_4^+), while reducing phosphate (PO_4^{3-}), nitrate (NO_3^-), and β-glucosidase activity. Additionally, TOC and total phosphorus (TP) were identified as chemical parameters that can serve as predictors of microbiological changes in Andisol contaminated with MPs.

When studying soil-plant system, it was found that *R. sativus* plants grown in an Andisol with MPs showed increased biomass and relative chlorophyll content in PA, LDPE, and polypropylene (PP) treatments. However, an increase in oxidative stress was also observed, along with changes in the profiles of bioactive compounds such as anthocyanins and glucosinolates in the edible root, particularly with PP. Microbial biomass carbon (MBC) and polyphenols were identified as key soil predictors of the responses of *R. sativus* grown in soil contaminated with MPs.

These findings highlight the long-term risks of MP contamination, both for soil health and crop quality, with potential implications for food security and human health.

I. INTRODUCCIÓN GENERAL

Desde el año 1600 a.C., los pueblos mesoamericanos utilizaban el látex del árbol *Castilla elastica* para obtener caucho, el cual tenía diversos usos por su plasticidad, como la elaboración de pelotas de goma y figuras humanas, entre otros (Hosler et al., 1999). Sin embargo, no fue hasta 1839 cuando Charles Goodyear desarrolló la vulcanización, un proceso que mejoró la dureza, elasticidad y durabilidad del caucho, demostrando la gran utilidad y versatilidad de los polímeros maleables (Andrady y Neal, 2009). A pesar de estos avances, el primer plástico sintético, la baquelita, se desarrolló recién en 1907. Esto trajo una expansión en el desarrollo de plásticos modernos durante la primera mitad del siglo XX, con la creación de al menos 15 nuevas clases de polímeros. Estos polímeros se obtienen mediante la polimerización de monómeros derivados de materias primas como combustibles fósiles: el carbón, el petróleo y el gas natural (Andrady y Neal, 2009; Frias y Nash, 2019). Los hitos más importantes en la evolución del uso del plástico se resumen en el gráfico de la figura 1.

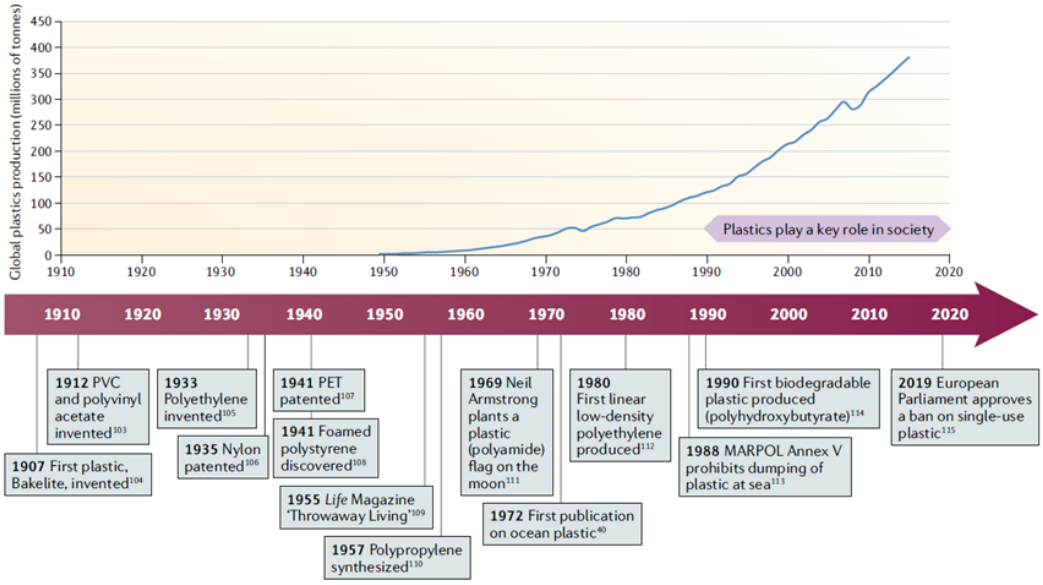

Figura 1. Línea de tiempo incluyendo la producción global de plásticos (Amaral-Zettler et al., 2020).

Los plásticos poseen una variedad de propiedades únicas que los hacen ampliamente versátiles y útiles en numerosas aplicaciones; pueden emplearse en un amplio rango de temperaturas, son resistentes a la corrosión, muy fuertes y duraderos. Además, su bajo costo de producción, diversidad y funcionalidad los convierten en materiales idóneos para diversos usos (Andrady y Neal, 2009; PlasticsEurope, 2022). Gracias a la diversidad de tipos y formas, los plásticos se han consolidado como uno de los materiales más empleados a nivel global, tanto en la industria, la agricultura y en la vida diaria en general. Su versatilidad abarca aplicaciones que van desde embalajes (como bolsas y botellas) hasta materiales de construcción, ropa y componentes electrónicos. Los plásticos más comunes, como el polietileno de baja y alta densidad (LDPE, HDPE), el polipropileno (PP), el cloruro de polivinilo (PVC), el poliestireno (PS) y el tereftalato de polietileno (PET), representan aproximadamente el 90% de la demanda mundial de plásticos (Andrady y Neal, 2009; PlasticsEurope, 2022).

Actualmente, vivimos en la era del plástico; el uso global actual de plástico es enorme y ha estado incrementando en los últimos años, alcanzando 368 millones de toneladas en 2019 (PlasticsEurope, 2022; Thompson et al., 2009). Por este mismo uso global es que los plásticos representan el 10% de los desechos generados en todo el mundo, y aunque algunos residuos plásticos son reciclados, la mayoría termina en el medio ambiente, como en vertederos y campos agrícolas (Barnes et al., 2009; Wang et al., 2019). Según un estudio realizado por Horton et al. (2017), se calcula que en ambientes continentales de la Unión Europea se liberan y retienen anualmente entre 473.000 y 910.000 toneladas métricas de desechos plásticos, estas cantidades corresponden entre 4 y 23 veces la cantidad estimada que se libera en los océanos. La contaminación plástica se considera un factor importante responsable del declive global de la biodiversidad amenazando ecosistemas de todo el mundo (Barnes et al., 2009; Qi et al., 2020). Sin embargo, no solo la abundancia y persistencia de los plásticos representan un grave riesgo ambiental, sino que en las últimas décadas también se ha puesto atención en los plásticos de tamaño

microscópico, conocidos como microplásticos (Scheurer y Bigalke, 2018; Steffen et al., 2015).

El término “microplásticos” (MPs) fue acuñado por Thompson et al. en 2004 para referirse a plásticos de tamaño microscópico, si bien el término hace alusión a tamaños menores a 1 mm, en el año 2008 se hizo una propuesta para ampliar el término de MPs a tamaños de hasta 5 mm (Arthur et al., 2009). En la actualidad la mayoría de los autores aceptan esta clasificación (De-la-Torre et al., 2020; Huang et al., 2020; Yu et al., 2020a), sin embargo, otros realizan una subdivisión más específica dentro de esta categoría de plásticos, dividiéndolos en MPs grandes (menores a 5 mm y mayores a 500 µm) y MPs pequeños (entre 500 µm y 100 nm) (Jiang et al., 2019; Möller et al., 2020; Vermeiren et al., 2020). Los MPs no solo son clasificados según su tamaño, sino que también según su origen, de esta forma podemos distinguir los MPs primarios como aquellos fabricados intencionalmente para ser de tamaño microscópico y pueden ser encontrados en productos de cuidado personal como pastas dentales, cosméticos, productos de limpieza, abrasivos industriales entre otros. Por otro lado, están los MPs secundarios que corresponden a fibras o fragmentos provenientes de desechos de plásticos, coberturas plásticas en la agricultura, fibras textiles, materiales plásticos domiciliarios, entre otros, los que fueron generados a partir de la fragmentación de plásticos más grandes, ya sea por procesos fotolíticos, mecánicos y/o degradación biológica (Cole et al., 2011; Duis y Coors, 2016; Rocha-Santos y Duarte, 2017).

Los MPs han generado una gran atención debido a su ubicuidad, disponibilidad y efectos negativos en diferentes ecosistemas (Rocha-Santos y Duarte, 2017; Scheurer y Bigalke, 2018). Estos pueden actuar como vectores de patógenos y absorber contaminantes como hidrocarburos aromáticos policíclicos (PAHs), bifenilos policlorados (PCBs), productos farmacéuticos, pesticidas, sustancias perfluoroalquiladas (PFAS) y metales pesados. Además, pueden acumularse en la cadena alimentaria mediante la absorción de las

plantas desde el suelo o por el consumo de biota del suelo contaminada (Riveros et al., 2022). La contaminación por MPs está catalogada como uno de los principales problemas ambientales por el Programa de las Naciones Unidas para el Medio Ambiente (UNEP) y ha ganado atención debido a sus efectos adversos sobre el suelo, la biota del suelo y los ecosistemas en general (Rocha-Santos y Duarte, 2017; Scheurer y Bigalke, 2018; UNEP, 2014).

Existen numerosas fuentes de entrada de MPs en los suelos y se han detectado en áreas industriales, suelos agrícolas, invernaderos, jardines domésticos, suelos costeros y llanuras aluviales con un amplio rango de concentraciones (Xu et al., 2020). Los suelos son componentes esenciales de los ecosistemas terrestres y están bajo fuerte presión debido a la contaminación por MPs. Rillig (2012) fue el primer investigador en abordar este problema en ecosistemas terrestres, documentando la presencia de cantidades significativas de MPs en los suelos debido principalmente a factores como su durabilidad y la limitación tecnológica existente para reciclar el plástico producido y desechado (Barnes et al., 2009). Si bien algunos desechos plásticos se reciclan, la mayoría termina en el medio ambiente en vertederos o en campos agrícolas (Wang et al., 2019).

Las principales fuentes de contaminación de MPs a los suelos agrícolas son los lodos activos provenientes de plantas de tratamiento de aguas residuales (utilizados como enmiendas orgánicas), y la fragmentación de plásticos utilizados en la agricultura moderna, mientras que otras formas de contaminación incluyen el uso de compost, riego, inundaciones, fragmentación de residuos plásticos y deposición atmosférica (Nizzetto et al., 2016; Riveros et al., 2022). El suelo agrícola es uno de los principales reservorios de MPs, A nivel mundial, la concentración en suelos agrícolas varía significativamente, con valores entre 0.34 y 1.27 × 10^7 partículas kg^{-1}, dependiendo del uso de plásticos en la agricultura, fertilizantes y gestión de residuos. China registra algunas de las concentraciones más altas, alcanzando hasta 111,010 partículas/kg,

especialmente en regiones con uso intensivo de coberturas plásticas. En Europa, Alemania y España reportan 3.7 y 3,607 partículas kg^{-1}, respectivamente, mientras que, en América del Norte, Canadá presenta 541 partículas kg^{-1}. Chile no está ajeno a esta situación, la contaminación en suelos agrícolas se ha estimado en 306 partículas kg^{-1}, ubicándose en un rango intermedio. Esta presencia de MPs se asocia principalmente al uso de lodos usados como enmiendas orgánicas, lo que resalta la necesidad de evaluar su impacto en los ecosistemas agrícolas y en la seguridad alimentaria (Shi et al., 2024). El suelo industrial no se queda atrás, En Australia, se han reportado niveles entre 0.03% a incluso 6.7% (p/p) de MPs en suelos altamente contaminados (Fuller y Gautam, 2016). Además, los MPs pueden persistir y acumularse durante largos períodos, afectando las propiedades fisicoquímicas y las funciones del suelo. Esta persistencia puede llevar a una contaminación compuesta con otros contaminantes del suelo, lo que tiene implicaciones significativas para la salud del suelo, la biodiversidad y la función del ecosistema (Nizzetto et al., 2016).

La presencia de MPs en el suelo desencadena cambios físicos (estabilidad de agregados, densidad aparente del suelo y dinámica del agua), químicos (disponibilidad de nutrientes, materia orgánica y pH), y microbiológicos (actividad microbiana y enzimática, y fauna del suelo) (Riveros et al., 2022). Además, los MPs ofrecen una nueva superficie para la colonización de microorganismos, dando lugar a la formación de un ecosistema conocido como "plastisfera" (De Souza Machado et al., 2019; Huang et al., 2019). Qi et al. (2020) incubaron un suelo arenoso con MPs de LDPE y observaron un aumento en la conductividad eléctrica. Además, Liu et al. (2017) realizaron un estudio de incubación en un Cambisol con la adición de MPs de PVC, y reportaron un aumento en los nutrientes orgánicos disueltos, como C, N, P, ácido fúlvico después de 30 días de incubación. En general, la presencia de MPs en el suelo se ha asociado con alteraciones en la disponibilidad, distribución y equilibrio estequiométrico de C, N y P, lo que influye en las

demandas relativas de los microorganismos a estos nutrientes (Fu et al., 2024; Li et al., 2024). Los MPs se descomponen muy lentamente, y ciertos microorganismos son capaces de aprovechar el C y/o N presente en ellos (Huang et al., 2019; Qi et al., 2020; Rillig et al., 2019, 2021). La incubación de suelos con MPs altera la estructura de las comunidades bacterianas del suelo, en este sentido, Wang et al. (2020) reportaron que 25 órdenes de bacterias se enriquecieron en suelos expuestos a MPs de LDPE, mientras que solo 18 órdenes se enriquecieron en el control sin la adición de MPs. Las comunidades bacterianas son los principales productores de enzimas en el suelo, por lo que los MPs al alterar la estructura bacteriana afectan la actividad enzimática del suelo. Por ejemplo, investigaciones han demostrado que los MPs pueden reducir la actividad de la ureasa, una enzima importante para el ciclo del nitrógeno, y la deshidrogenasa, una enzima involucrada en la actividad microbiana del suelo (Yu et al., 2020b).

Para los sistemas agrícolas la incorporación de nitrógeno es esencial para la fertilidad del suelo y por tanto para la productividad vegetal. La dinámica de este elemento en la biosfera comprende principalmente la fijación de nitrógeno, la mineralización, la amonificación (nitrificación), la desnitrificación y la oxidación anaeróbica del amonio (Annamox); en estos procesos las comunidades microbianas poseen un rol principal (Cerón y Aristizábal, 2012). Varios estudios han informado que la adición de MPs al suelo tiene un impacto en el ciclo del nitrógeno, principalmente por la alteración de la microbiota y con ella la abundancia de genes y por lo tanto las enzimas que se secretan al suelo (Riveros et al., 2022). Las alteraciones en las comunidades microbianas asociadas con el ciclo del N pueden llevar a una alteración del ciclo del N en el suelo (Wang et al., 2022, 2024a). Los estudios han demostrado que la adición de MPs puede provocar cambios en la abundancia de genes como lo es el *amoA*. Gao et al. (2021) encontraron que los MPs disminuyeron la abundancia de los genes *amoA* de bacterias oxidantes de amonio (AOB), pero no tuvieron efecto en los genes *amoA* de arqueas oxidantes de amonio (AOA), lo que

inhibió potencialmente las emisiones de N_2O al reducir las bacterias oxidantes de amonio durante la nitrificación del suelo. Además, los MPs en el suelo pueden influir en la abundancia del gen *nifH*, que es crucial para la fijación de N. Los estudios han demostrado que la adición de MPs puede aumentar la abundancia del gen *nifH* según el período de incubación, lo que potencialmente altera los microorganismos fijadores de N en el suelo (Rong et al., 2021). Esta disrupción puede tener consecuencias de gran alcance para la fertilidad del suelo y el crecimiento de las plantas, ya que el N es un nutriente crítico para el desarrollo de las plantas (Wang et al., 2024a).

Las investigaciones también han indicado que la contaminación por MPs puede tener efectos cascada sobre la absorción de agua, la disponibilidad de nutrientes, la absorción y el crecimiento general de las plantas, lo que finalmente conduce a una menor producción de cultivos (Cusworth et al., 2024; de Souza Machado et al., 2018; Wang et al., 2024a). Sin embargo, los estudios de los efectos en plantas siguen siendo limitados debido a las numerosas variables presentes en las investigaciones publicadas, lo que lleva a resultados controversiales. Los efectos en plantas dependen de numerosos factores, incluido el tipo de polímero, la forma, la concentración y la fotodegradación (De Souza Machado et al., 2019; Lozano et al., 2021, 2024; Shah et al., 2023). Con respecto a la biomasa y el desarrollo, algunos estudios han mostrado aumentos en plantas como *Daucus carota*, *Allium fistulosum* y *Glycine max* (De Souza Machado et al., 2019; Lozano et al., 2021; Shah et al., 2023). Sin embargo, este parámetro se redujo en estudios con *Lactuca sativa*, *Fragaria* × *ananassa* y *Solanum lycopersicum* (Nuamzanei et al., 2024; Pinto-Poblete et al., 2023; Wang et al., 2024b). Además, los efectos sobre la biomasa dependen de si se estudian las partes subterráneas o aéreas de la planta (De Souza Machado et al., 2019; Tong et al., 2023). Incluso al evaluar peso seco o fresco, los efectos pueden ser distintos. Por ejemplo, la biomasa seca de bulbos *A. fistulosum,* disminuyó en las plantas expuestas a PA, pero el contenido de agua aumento 2 veces. Lo contrario sucedió con la exposición a MPs de PES en donde el peso

seco de los bulbos casi se duplicó, sin embargo, el contenido de agua disminuyó con la exposición a PES, PET y PP (De Souza Machado et al., 2019). Además de alterar la morfología, se ha demostrado que las plantas cultivadas en suelos y sustratos contaminados con MPs exhiben una bioquímica alterada, especialmente en lo que respecta al estrés oxidativo. Los MPs inducen estrés celular al promover la acumulación de especies reactivas de oxígeno (ROS), lo que causa daño oxidativo, como lo demuestra el aumento del contenido de malondialdehído (MDA) en los tejidos de brásicas (López et al., 2022; Nuamzanei et al., 2024; Wang et al., 2024b,c). El estrés celular causado por la presencia de MPs desencadena una cascada de respuestas en las plantas, como alteraciones en las actividades de enzimas antioxidantes que contrarrestan las ROS, incluidas las peroxidasas (POD), ascorbato peroxidasa (APX) y superóxido dismutasa (SOD) (Ranauda et al., 2024; Wang et al., 2024b,c).

La importancia de estudiar los efectos en las plantas no solo es ecológica, sino también nutricional, ya que es crucial evaluar el impacto en las plantas que crecen en contacto con este contaminante generalizado, que luego será consumido por los humanos. Una planta ampliamente consumida, el rábano común (*Raphanus sativus L.*), es una especie con una parte comestible subterránea conocida por sus nutrientes y compuestos bioactivos, como los glucosinolatos y las antocianinas, que tienen efectos beneficiosos para la salud humana (López et al., 2022). Al día de hoy los estudios sobre esta especie cultivada en suelos contaminados con MPs son escasos. Botyanszká et al. (2022) estudiaron el crecimiento de *R. sativus* en suelos limosos contaminados con MPs (PS, PVC y HDPE) y encontraron un aumento en el peso fresco de las zonas aéreas y subterráneas, sin embargo, estos valores no fueron estadísticamente significativos. Por el contrario, Cui et al. (2022) observaron una reducción en el peso fresco de los brotes y las raíces de *R. sativus* cultivado en suelos contaminados con microplásticos (MPs) de PVC. Además, en las raíces, los MPs de PA y PVC incrementaron el contenido de MDA,

mientras que los MPs de PP redujeron la actividad de SOD y aumentaron la actividad de POD (Cui et al. 2022). Con respecto al efecto de los MPs en los compuestos bioactivos, López et al. (2022) estudiaron los brotes de *R. sativus* cultivados en fibra de coco con MPs de HDPE y observaron una disminución en el contenido total de glucosinolatos. Además, se evidenció que los MPs aumentaron el contenido total de antocianinas, la capacidad antioxidante (ORAC) y el contenido de MDA en los brotes.

La importancia de seguir investigando el efecto de los MPs en el suelo y las plantas radica en la falta de estudios concluyentes debido a la gran cantidad de variables que influyen en los sistemas biológicos, lo que ha generado resultados contradictorios. Este trabajo busca abordar una de las principales brechas en el conocimiento: el estudio de los efectos de los MPs en suelos del orden Andisol. A pesar de su relevancia en la agricultura chilena, donde representan aproximadamente el 50% de la superficie destinada a la producción de cereales, el conocimiento sobre efectos de los MPs en Andisoles sigue siendo escaso, tanto en lo que respecta a los tipos de MPs estudiados, las propiedades del suelo afectadas y las plantas que crecen en este tipo de suelos (Poblete-Grant et al., 2020; Staff, 2006).

Los Andisoles son suelos formados a partir de material volcánico y destacan por su extraordinaria capacidad de almacenamiento de carbono. Esta capacidad se debe a la presencia elevada de minerales de baja cristalinidad, como alofán y los materiales de tipo imogolita favoreciendo la inmovilización de fosfato mediante sorción y/o precipitación con cationes como Al y Fe. (Neculman et al., 2013). Estas propiedades de secuestro de C y la capacidad de inmovilizar fosfato hacen de los Andisoles un suelo único. Por ello, resulta crucial comprender de manera integral el impacto de los MPs en las propiedades de estos suelos y en las plantas que crecen en ellos, para así evaluar posibles efectos a largo plazo en la fertilidad del suelo y en la productividad agrícola.

HIPÓTESIS: La presencia de microplásticos en un Andisol disminuirá sus propiedades químicas (nutrientes) y biológicas (actividades enzimáticas), principalmente vinculadas al ciclo del nitrógeno. Esto tendrá un efecto negativo en el crecimiento de plantas de *R. sativus* (biomasa), un incremento del estrés oxidativo y una disminución de los compuestos bioactivos en esta planta.

OBJETIVO GENERAL

Evaluar el efecto los microplásticos en parámetros químicos (nutrientes) y propiedades microbiológicas (actividad enzimática) en un Andisol, así como en el crecimiento, estrés oxidativo y compuestos bioactivos de *R. sativus* cultivados en este suelo bajo condiciones controladas.

OBJETIVOS ESPECIFICOS

1. Evaluar el efecto de microplásticos de polietileno de baja densidad y poliamida en propiedades químicas y microbiológicas en un Andisol mediante una incubación de seis semanas, con énfasis en la disponibilidad de nutrientes y la actividad enzimática.

2. Identificar predictores químicos de suelo que permitan determinar cambios en el corto plazo en las propiedades microbiológicas de un Andisol contaminado con microplásticos.

3. Evaluar el efecto de microplásticos de poliamida, polietileno de baja densidad y polipropileno en las propiedades químicas (disponibilidad de nutrientes) y microbiológicas (actividad enzimática) en un Andisol, así como su influencia en el estrés oxidativo, la síntesis de compuestos bioactivos (glucosinolatos y antocianinas) y las alteraciones en el crecimiento de *R. sativus*.

4. Identificar predictores del suelo que permitan determinar cambios en las características de *R. sativus* crecidos en un Andisol contaminado con microplásticos.

REFERENCIAS

Amaral-Zettler, L. A., Zettler, E. R., y Mincer, T. J. (2020). Ecology of the plastisphere. Nature Reviews Microbiology, 18(3), 139-151. https://doi.org/10.1038/s41579-019-0308-0

Andrady, A. L., y Neal, M. A. (2009). Applications and societal benefits of plastics. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), 1977–1984. https://doi.org/10.1098/RSTB.2008.0304

Arthur, C., Baker, J. E, 1959-, y Bamford, H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA. https://repository.library.noaa.gov/view/noaa/2509

Barnes, D. K. A., Galgani, F., Thompson, R. C., y Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1526), 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Botyanszká, L., Šurda, P., Vitková, J., Lichner, y Igaz, D. (2022). Effect of microplastics on silty loam soil properties and radish growth. Journal of Hydrology and Hydromechanics, 70(3), 321–329. https://doi.org/10.2478/johh-2022-0018

Cole, M., Lindeque, P., Halsband, C., y Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: A review. In Marine Pollution Bulletin (Vol. 62, Issue 12, pp. 2588–2597). https://doi.org/10.1016/j.marpolbul.2011.09.025

Cui, M., Yu, S., Yu, Y., Chen, X., y Li, J. (2022). Responses of cherry radish to different types of microplastics in the presence of oxytetracycline. Plant Physiology and Biochemistry, 191, 1–9. https://doi.org/10.1016/j.plaphy.2022.09.012

Cusworth, S. J., Davies, W. J., McAinsh, M. R., y Stevens, C. J. (2024). A nationwide assessment of microplastic abundance in agricultural soils: The influence of plastic crop covers within the United Kingdom. Plants People Planet, 6(2), 304–314. https://doi.org/10.1002/ppp3.10430

de Souza Machado, A. A., Kloas, W., Zarfl, C., Hempel, S., y Rillig, M. C. (2018). Microplastics as an emerging threat to terrestrial ecosystems. Global Change Biology, 24(4), 1405–1416. https://doi.org/10.1111/gcb.14020

De Souza Machado, A. A., Lau, C. W., Kloas, W., Bergmann, J., Bachelier, J. B., Faltin, E., Becker, R., Görlich, A. S., y Rillig, M. C. (2019). Microplastics Can Change Soil Properties and Affect Plant Performance. Environmental Science and Technology, 53(10), 6044–6052. https://doi.org/10.1021/acs.est.9b01339

De-la-Torre, G. E., Dioses-Salinas, D. C., Castro, J. M., Antay, R., Fernández, N. Y., Espinoza-Morriberón, D., y Saldaña-Serrano, M. (2020). Abundance and distribution of microplastics on sandy beaches of Lima, Peru. Marine Pollution Bulletin, 151. https://doi.org/10.1016/j.marpolbul.2019.110877

Duis, K., y Coors, A. (2016). Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environmental Sciences Europe, 28(2), 1–25. https://doi.org/10.1186/s12302-015-0069-y

Cerón, E., y Aristizábal, F. (2012). Dinámica del ciclo del nitrógeno y fósforo en suelos. Revista Colombiana de Biotecnología, ISSN 0123-3475, ISSN-e 1909-8758, Vol. 14, Nº. 1, 2012, Págs. 285-295, 14(1), 285–295. https://dialnet.unirioja.es/servlet/articulo?codigo=4194085yinfo=resumenyidioma=SPA

Frias, J. P. G. L., y Nash, R. (2019). Microplastics: Finding a consensus on the definition. Marine Pollution Bulletin, 138, 145–147. https://doi.org/10.1016/J.MARPOLBUL.2018.11.022

Fu, B., Zhou, W., Chen, Y., Wu, Y., Gan, W., She, N., y Ma, Y. (2024). A bibliometric perspective on the occurrence and migration of microplastics in soils amended with sewage sludge. Water Environment Research, 96(6). https://doi.org/10.1002/wer.11054

Fuller, S., y Gautam, A. (2016). A Procedure for Measuring Microplastics using Pressurized Fluid Extraction. Environmental Science and Technology, 50(11), 5774–5780. https://doi.org/10.1021/acs.est.6b00816

Gao, B., Yao, H., Li, Y., y Zhu, Y. (2021). Microplastic Addition Alters the Microbial Community Structure and Stimulates Soil Carbon Dioxide Emissions in Vegetable-Growing Soil. Environmental Toxicology and Chemistry, 40(2), 352–365. https://doi.org/10.1002/etc.4916

Gao, H., Yan, C., Liu, Q., Ding, W., Chen, B., y Li, Z. (2019). Effects of plastic mulching and plastic residue on agricultural production: A meta-analysis. Science of The Total Environment, 651, 484–492. https://doi.org/10.1016/J.SCITOTENV.2018.09.105

Hosler, D., Burkett, S. L., y Tarkanian, M. J. (1999). Prehistoric polymers: Rubber processing in ancient Mesoamerica. Science, 284(5422), 1988–

1991. https://doi.org/10.1126/SCIENCE.284.5422.1988/ASSET/8CF8C196-AAAC-4B64-9ED4-826FC0499899/ASSETS/GRAPHIC/SE2297580001.JPEG

Huang, Y., Liu, Q., Jia, W., Yan, C., y Wang, J. (2020). Agricultural plastic mulching as a source of microplastics in the terrestrial environment. Environmental Pollution, 260. https://doi.org/10.1016/j.envpol.2020.114096

Huang, Y., Zhao, Y., Wang, J., Zhang, M., Jia, W., y Qin, X. (2019). LDPE microplastic films alter microbial community composition and enzymatic activities in soil. Environmental Pollution, 254, 1–10. https://doi.org/10.1016/j.envpol.2019.112983

Jiang, X., Chen, H., Liao, Y., Ye, Z., Li, M., y Klobučar, G. (2019). Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. Environmental Pollution, 250, 831–838. https://doi.org/10.1016/j.envpol.2019.04.055

Li, M., Ma, Q., Su, T., Wang, Z., y Tong, H. (2024). Effect of Polycaprolactone Microplastics on Soil Microbial Communities and Plant Growth. Journal of Polymers and the Environment, 32(3), 1039–1045. https://doi.org/10.1007/s10924-023-03028-0

Liu, H., Yang, X., Liu, G., Liang, C., Xue, S., Chen, H., Ritsema, C. J., y Geissen, V. (2017). Response of soil dissolved organic matter to microplastic addition in Chinese loess soil. Chemosphere, 185, 907–917. https://doi.org/10.1016/j.chemosphere.2017.07.064

López, M. D., Toro, M. T., Riveros, G., Illanes, M., Noriega, F., Schoebitz, M., García-Viguera, C., y Moreno, D. A. (2022). Brassica sprouts exposed to

microplastics: Effects on phytochemical constituents. Science of the Total Environment, 823. https://doi.org/10.1016/j.scitotenv.2022.153796

Lozano, Y. M., Gordillo-Rocha, H., Waldman, W. R., y Rillig, M. C. (2024). Photodegradation modifies microplastic effects on soil properties and plant performance. Journal of Applied Ecology, 61(1), 13–24. https://doi.org/10.1111/1365-2664.14514

Lozano, Y. M., Lehnert, T., Linck, L. T., Lehmann, A., y Rillig, M. C. (2021). Microplastic Shape, Polymer Type, and Concentration Affect Soil Properties and Plant Biomass. Frontiers in Plant Science, 12. https://doi.org/10.3389/fpls.2021.616645

Möller, J. N., Löder, M. G. J., y Laforsch, C. (2020). Finding Microplastics in Soils: A Review of Analytical Methods. Environmental Science and Technology, 54(4), 2078–2090. https://doi.org/10.1021/acs.est.9b04618

Neculman, R., Rumpel, C., Matus, F., Godoy, R., Steffens, M., y de la Luz Mora, M. (2013). Organic matter stabilization in two Andisols of contrasting age under temperate rain forest. Biology and Fertility of Soils, 49(6), 681–689. https://doi.org/10.1007/s00374-012-0758-2

Nizzetto, L., Futter, M., y Langaas, S. (2016). Are Agricultural Soils Dumps for Microplastics of Urban Origin? In Environmental Science and Technology (Vol. 50, Issue 20, pp. 10777–10779). American Chemical Society. https://doi.org/10.1021/acs.est.6b04140

Nuamzanei, Changmai, U., SK, S., Kumar, N., Borah, B., Chikkaputtaiah, C., Saikia, R., y Phukan, T. (2024). Impact of polyvinyl chloride (PVC) microplastic on growth, photosynthesis and nutrient uptake of Solanum

lycopersicum L. (Tomato). Environmental Pollution, 349, 123994. https://doi.org/10.1016/j.envpol.2024.123994

Pinto-Poblete, A., Retamal-Salgado, J., Zapata, N., Sierra-Almeida, A., y Schoebitz, M. (2023). Impact of polyethylene microplastics and copper nanoparticles: Responses of soil microbiological properties and strawberry growth. Applied Soil Ecology, 184. https://doi.org/10.1016/j.apsoil.2022.104773

PlasticsEurope. (2022). Plásticos – Situación en 2022. https://plasticseurope.org/knowledge-hub/plastics-the-facts-2022/

Poblete-Grant, P., Suazo-Hernández, J., Condron, L., Rumpel, C., Demanet, R., Malone, S. L., y Mora, M. de L. L. (2020). Soil available P, soil organic carbon and aggregation as affected by long-term poultry manure application to Andisols under pastures in Southern Chile. Geoderma Regional, 21, 1–10. https://doi.org/10.1016/j.geodrs.2020.e00271

Qi, R., Jones, D. L., Li, Z., Liu, Q., y Yan, C. (2020). Behavior of microplastics and plastic film residues in the soil environment: A critical review. In Science of the Total Environment (Vol. 703). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2019.134722

Qi, Y., Beriot, N., Gort, G., Huerta Lwanga, E., Gooren, H., Yang, X., y Geissen, V. (2020). Impact of plastic mulch film debris on soil physicochemical and hydrological properties. Environmental Pollution, 266, 115097. https://doi.org/10.1016/J.ENVPOL.2020.115097

Ranauda, M. A., Zuzolo, D., Maisto, M., Tartaglia, M., Scarano, P., Prigioniero, A., Sciarrillo, R., y Guarino, C. (2024). Microplastics affect soil-plant system: Implications for rhizosphere biology and fitness of sage (Salvia officinalis

L.). Environmental Pollution, 346. https://doi.org/10.1016/j.envpol.2024.123656

Redel, Y., Cartes, P., Demanet, R., Velásquez, G., Poblete-Grant, P., Bol, R., y Mora, M. L. (2016). Assessment of phosphorus status influenced by Al and Fe compounds in volcanic grassland soils. Journal of Soil Science and Plant Nutrition, 16(2), 490–506. https://doi.org/10.4067/S0718-95162016005000041

Rillig, M. C., De Souza Machado, A. A., Lehmann, A., y Klümper, U. (2019). Evolutionary implications of microplastics for soil biota. Environmental Chemistry, 16(1), 3–7. https://doi.org/10.1071/EN18118

Rillig, M. C., Leifheit, E., y Lehmann, J. (2021). Microplastic effects on carbon cycling processes in soils. PLoS Biology, 19(3). https://doi.org/10.1371/JOURNAL.PBIO.3001130

Riveros, G., Urrutia, H., Araya, J., Zagal, E., y Schoebitz, M. (2022). Microplastic pollution on the soil and its consequences on the nitrogen cycle: a review. Environmental Science and Pollution Research, 29(6), 7997–8011. https://doi.org/10.1007/s11356-021-17681-2

Rocha-Santos, T. A. P., y Duarte, A. C. (2017). Characterization and Analysis of Microplastics (Vol. 5). Elsevier.

Rong, L., Zhao, L., Zhao, L., Cheng, Z., Yao, Y., Yuan, C., Wang, L., y Sun, H. (2021). LDPE microplastics affect soil microbial communities and nitrogen cycling. Science of the Total Environment, 773. https://doi.org/10.1016/j.scitotenv.2021.145640

Scheurer, M., y Bigalke, M. (2018). Microplastics in Swiss Floodplain Soils. Environmental Science and Technology, 52(6), 3591–3598. https://doi.org/10.1021/acs.est.7b06003

Shah, T., Ali, A., Haider, G., Asad, M., y Munsif, F. (2023). Microplastics alter soil enzyme activities and microbial community structure without negatively affecting plant growth in an agroecosystem. Chemosphere, 322. https://doi.org/10.1016/j.chemosphere.2023.138188

Shi, W., Wu, N., Zhang, Z., Liu, Y., Chen, J., y Li, J. (2024). A global review on the abundance and threats of microplastics in soils to terrestrial ecosystem and human health. Science of The Total Environment, 912, 169469.

Staff, S.S. (2006). Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. US Government Printing Office.

Steffen, W., Richardson, K., Rockström, J., Cornell, S. E., Fetzer, I., Bennett, E. M., Biggs, R., Carpenter, S. R., De Vries, W., De Wit, C. A., Folke, C., Gerten, D., Heinke, J., Mace, G. M., Persson, L. M., Ramanathan, V., Reyers, B., y Sörlin, S. (2015). Planetary boundaries: Guiding human development on a changing planet. Science, 347(6223).

Thompson, R. C., Swan, S. H., Moore, C. J., y Vom Saal, F. S. (2009). Our plastic age. In Philosophical Transactions of the Royal Society B: Biological Sciences (Vol. 364, Issue 1526, pp. 1973–1976). Royal Society. https://doi.org/10.1098/rstb.2009.0054

Tong, Y., Ding, J., Xiao, M., Shahbaz, M., Zhu, Z., Chen, M., Kuzyakov, Y., Deng, Y., Chen, J., y Ge, T. (2023). Microplastics affect activity and spatial distribution of C, N, and P hydrolases in rice rhizosphere. Soil Ecology Letters, 5(3). https://doi.org/10.1007/s42832-022-0138-2

UNEP. (2014). United Nations Environment Programme - Annual Report 2014. https://www.unep.org/resources/annual-report/unep-2014-annual-report

Vermeiren, P., Muñoz, C., y Ikejima, K. (2020). Microplastic identification and quantification from organic rich sediments: A validated laboratory protocol. Environmental Pollution, 262. https://doi.org/10.1016/j.envpol.2020.114298

Wang, J., Huang, M., Wang, Q., Sun, Y., Zhao, Y., y Huang, Y. (2020). LDPE microplastics significantly alter the temporal turnover of soil microbial communities. Science of the Total Environment, 726, 1–8. https://doi.org/10.1016/j.scitotenv.2020.138682

Wang, J., Liu, X., Li, Y., Powell, T., Wang, X., Wang, G., y Zhang, P. (2019). Microplastics as contaminants in the soil environment: A mini-review. In Science of the Total Environment (Vol. 691, pp. 848–857). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2019.07.209

Wang, Q., Adams, C. A., Wang, F., Sun, Y., y Zhang, S. (2022). Interactions between microplastics and soil fauna: A critical review. In Critical Reviews in Environmental Science and Technology (Vol. 52, Issue 18, pp. 3211–3243). https://doi.org/10.1080/10643389.2021.1915035

Wang, W., Xie, Y., Li, H., Dong, H., Li, B., Guo, Y., Wang, Y., Guo, X., Yin, T., Liu, X., y Zhou, W. (2024b). Responses of lettuce (Lactuca sativa L.) growth and soil properties to conventional non-biodegradable and new biodegradable microplastics. Environmental Pollution, 341. https://doi.org/10.1016/j.envpol.2023.122897

Wang, W., Zhang, Z., Gao, J., y Wu, H. (2024a). The impacts of microplastics on the cycling of carbon and nitrogen in terrestrial soil ecosystems:

Progress and prospects. In Science of the Total Environment (Vol. 915). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2024.169977

Wang, X., Gao, W., Cai, K., Pan, W., y Tu, Z. (2024c). Response of Tobacco (*Nicotiana tabacum L.)* Growth to Soil Microplastic Pollution. Polish Journal of Environmental Studies, 33(5), 5357–5365. https://doi.org/10.15244/pjoes/182894

Xu, B., Liu, F., Cryder, Z., Huang, D., Lu, Z., He, Y., Wang, H., Lu, Z., Brookes, P. C., Tang, C., Gan, J., y Xu, J. (2020). Microplastics in the soil environment: Occurrence, risks, interactions and fate–A review. Critical Reviews in Environmental Science and Technology, 50(21), 2175–2222. https://doi.org/10.1080/10643389.2019.1694822

Yu, H., Fan, P., Hou, J., Dang, Q., Cui, D., Xi, B., y Tan, W. (2020b). Inhibitory effect of microplastics on soil extracellular enzymatic activities by changing soil properties and direct adsorption: An investigation at the aggregate-fraction level. Environmental Pollution, 267, 1–11. https://doi.org/10.1016/j.envpol.2020.115544

Yu, Q., Hu, X., Yang, B., Zhang, G., Wang, J., y Ling, W. (2020a). Distribution, abundance and risks of microplastics in the environment. Chemosphere, 249. https://doi.org/10.1016/j.chemosphere.2020.126059

Zhang, G. S., y Liu, Y. F. (2018). The distribution of microplastics in soil aggregate fractions in southwestern China. The Science of the Total Environment, 642, 12–20. https://doi.org/10.1016/J.SCITOTENV.2018.06.004

II. ARTICULOS CIENTÍFICOS

CAPITULO 1. Microplastic pollution on the soil and its consequences on the nitrogen cycle: a review

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Abstract

Microplastics (MPs) correspond to plastics between 0.1 μm and 5 mm in diameter, and these can be intentionally manufactured to be microscopic or generated from the fragmentation of larger plastics. Currently, MP contamination is a complicated subject due to its accumulation in the environment. They are a novel surface and a source of nutrients in soils because MPs can serve as a substrate for the colonization of microorganisms. Its presence in soil triggers

physical (stability of aggregates, soil bulk density, and water dynamics), chemical (nutrients availability, organic matter, and pH), and biological changes (microbial activity and soil fauna). All these changes alter organic matter degradation and biogeochemical cycles such as the nitrogen (N) cycle, which is a key predictor of ecological stability and management in the terrestrial ecosystem. This review aims to explore how MPs affect the N cycle in the soil, the techniques to detect it in soil, and their effects on the physicochemical and biological parameters, emphasizing the impact on the main bacterial groups, genes, and enzymes associated with the different stages of the N cycle.

Keywords Plastisphere; Soil microbiome; Enzyme activities; Biogeochemical cycles; Microplastic identification; Soil fauna; Microbial nitrogen genes

1. INTRODUCTION

Plastics have a range of unique properties and have numerous applications; they can be used at an extensive range of temperatures, they are corrosion resistant, very strong, and though. Furthermore, their low cost, diversity, and utility make them suitable for various applications (Table 1) (Andrady and Neal 2009; PlasticsEurope 2021). Nowadays, we are in the plastic age; the current global usage of plastic is enormous and has been increasing in recent years, reaching 368 million tons in 2019 (Thompson et al. 2009; PlasticsEurope 2021). Plastics represent 10% of waste generated around the world, while some plastic wastes are recycled, and the majority end up in the environment like landfills and agriculture fields (Barnes et al. 2009; Wang et al. 2019). According to Horton et al. (2017), between 473,000 and 910,000 tons of plastic waste are released and retained annually in continental environments of the European Union. These quantities correspond to between 4 and 23 times the estimated amount that is released in the oceans. Plastic pollution is considered to be a major factor responsible for the global decline in biodiversity. This is a threat to the soil system's functioning and has been documented in

ecosystems worldwide (Barnes et al. 2009; Qi et al. 2020). Therefore, the abundance and persistence of plastics and microplastics (MPs) is a severe environmental risk (Scheurer and Bigalke 2018; Steffen et al. 2015).

Table 1. Types of plastics commonly used worldwide (Rocha-Santos and Duarte, 2017; PlasticsEurope, 2021). *: No information

PLASTIC TYPE	APPLICATION	RELATIVE DENSITY	DEMAND (%)
Polypropylene (PP)	Packaging, bottle caps, ropes, carpets, laboratory equipment, drinking straws	0.83 – 0.85	19.4
Low-density polyethylene (LDPE)	Packaging, general-purpose containers, shower curtains, floor tiles.	0.91 – 0.93	17.4
High-density polyethylene (HDPE)	Milk containers, detergent bottles, tubing	0.94	12.4
Polyvinyl chloride (PVC)	Pipes, window frames, flooring, shower curtains	1.38	10
Polyurethane (PUR)	Building insulation, pillows, and mattresses, insulating foams for fridges	0.871 – 0.42	7.9
Polyethylene terephthalate (PET)	Soft drink bottles, food packaging, thermal insulation, blister packs	1.38	7.9
Polystyrene (PS)	Packaging foam, disposable cups, food containers, CDs, building materials	1.05	6.2
High impact polystyrene (HIPS)	Electronics, cups in vending machines, refrigerator liners	1.08	*
Polyamides (PA - nylon)	Textiles, toothbrush bristles,	1.13 – 1.35	*

	fishing lines, automotive		
Acrylonitrile butadiene styrene (ABS)	Musical instruments, printers, drainage pipes, protective equipment	1.06 – 1.08	*
Polycarbonate (PC)	CDs, DVDs, construction materials, electronics, lenses	1.20 – 1.22	*
Polyester (PES)	Textiles	1.4	*

The term “MPs” was coined by Thomson et al. in 2004, to refer to microscopic-sized plastics and have often been defined as particles between 5 mm and 100 nm in diameter. MPs are classified according to their origin; in this way, we can distinguish primary and secondary MPs. Primary MPs are those intentionally manufactured microscopic and can be found in personal care products like toothpaste, cosmetics, and cleaning products. On the other hand, secondary MPs are originated from the fragmentation of larger plastic products, such as plastic mulch films and household garbage (Duis and Coors 2016; Qi et al. 2020; Rocha-Santos and Duarte 2017; Wang et al. 2019). MPs contain mixtures of chemical additives, fillers, residual monomers, catalysts, and non-intentionally added substances (NIAS). Also, they act as a vector for pathogens and absorb contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexane (HCH), pharmaceuticals, pesticides, perfluoroalkyl substances (PFAS), and heavy metals. Furthermore, they accumulate in the food web by direct uptake from the soil or by consumption of contaminated soil biota (Besseling et al. 2017; Fendall and Sewell 2009; Hodson et al. 2017; Huerta Lwanga et al. 2017; Rochman et al. 2013; Wang et al. 2019).

MP pollution is listed as one of the top environmental problems by the United Nations Environment Programme (UNEP) and have gained attention due to their adverse effects on the soil, soil biota, and ecosystems in general. These effects are produced due to their small size and ubiquity (Rocha-Santos and Duarte 2017; Scheurer and Bigalke 2018; UNEP 2014). There are numerous sources of MP entry to soils (Fig. 1) and have been detected in industrial areas, agricultural soils, greenhouses, home gardens, coastal soils, and alluvial plains with a wide range of concentrations, which are well summarized in the study of Xu et al. (2020).

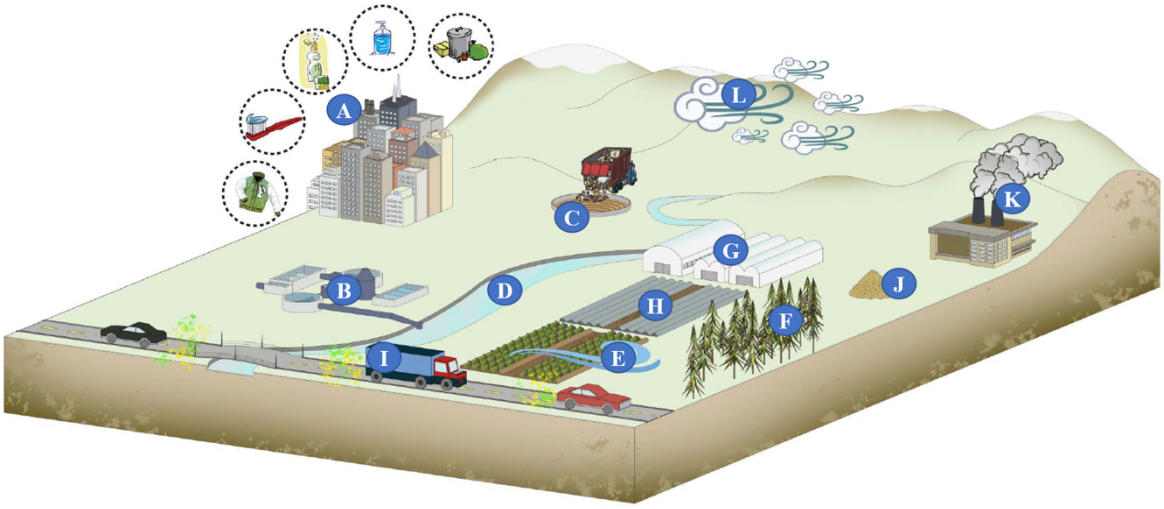

Fig. 1. Main sources of dispersion and entry of MPs to soils. A: Use and disposal of household products that have primary and secondary MPs. B: Wastewater treatment plants produce sewage sludge which contains MPs, due to their high nutrient load they are used to fertilize agricultural and forestry fields. C: Household garbage is disposed of in landfills, where plastics are fragmented forming MPs. D: MPs in watercourses reach the adjacent soil by irrigation. E: Soil erosion by wind disperses the MPs in the environment. F: Forest's foliage traps MPs present in the air, with the fall of leaves or the rain they are deposited on soils. G: Plastics fragmentation of greenhouses generates MPs. H: Plastic fragmentation of mulch-film generates MPs. I: Car tires release MPs due to physical wear of the tires, as well as the wear of the brakes. J: Organic

amendments such as compost, made with household or municipal waste will contribute MPs to the soils. K: Industrial zones are highly contaminated with MPs. L: Air masses transport MPs, which pollute soils by atmospheric deposition.

It has been estimated that up to 430,000 and 300,000 tons of MPs enter each year to agricultural land in Europe and North America, respectively (Nizzetto et al. 2016). Moreover, China has reported between 50 and 260 kg ha^{-1} of plastic in farmland soils after 30 years of extensive use of agricultural plastic films (Liu et al. 2014). In Australia, concentrations as high as 7% of MPs have been reported in highly contaminated topsoils (Fuller and Gautam 2016).

2. DETERMINATION OF MPS IN SOILS

MP pollution has been documented in various environments, and their determination is highly challenging. Therefore, is essential to choose correct methodologies in the stages of sampling, processing, detection, and quantification of MPs (Fig. 2) (Moller et al. 2020; Zhang 2007). The soil is a heterogeneous matrix comprised of minerals with a range of particle sizes, distributions, and organic matter at varying stages of decomposition. Also, the distribution and quantity of MPs can vary considerably. Therefore, the first stage in determining MPs in soils is the sampling, which must be representative and always avoid adding MPs from the sampling or transport materials (IAEA 2004; Moller et al. 2020; Yang et al. 2021).

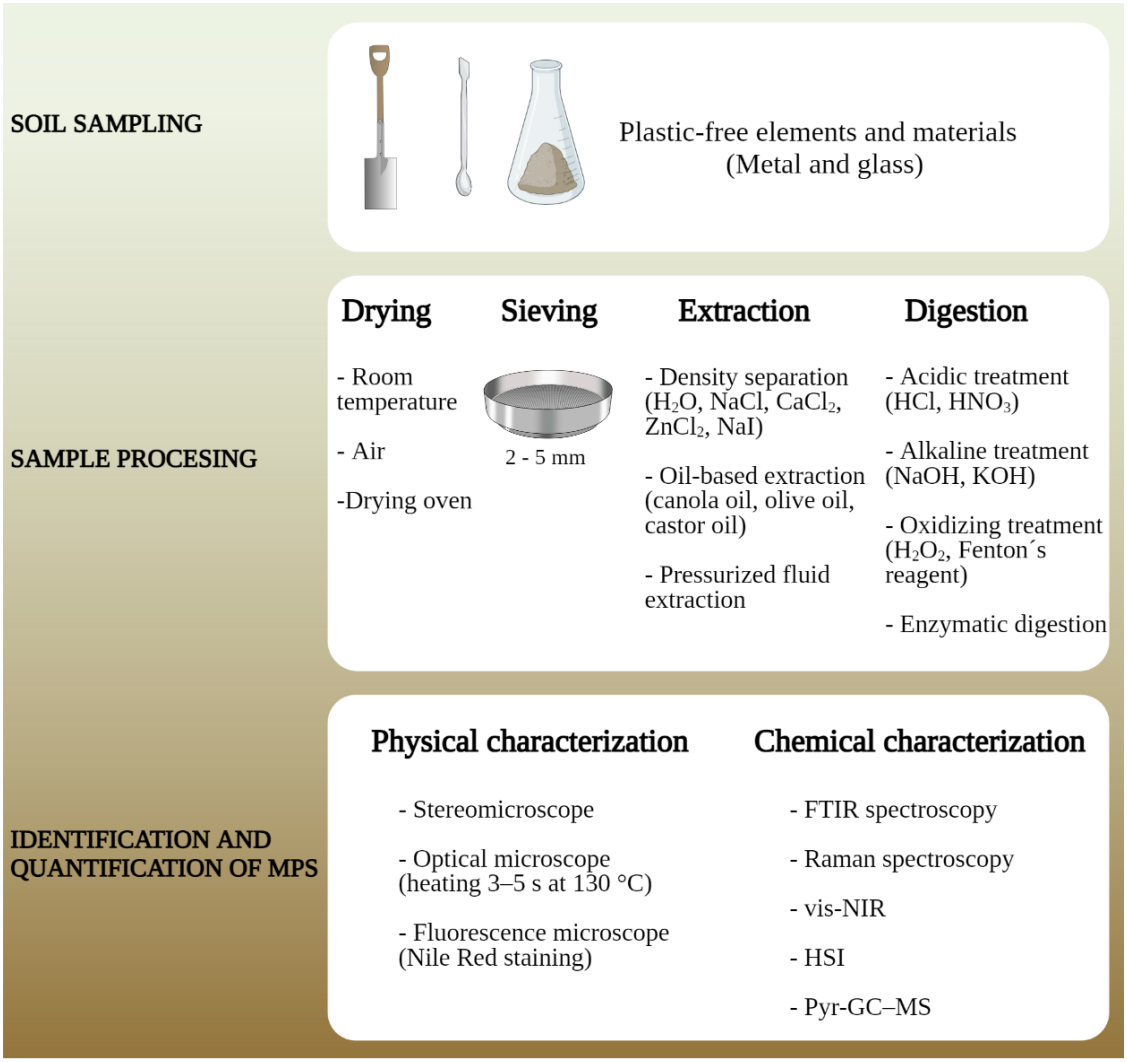

Fig. 2. Methodologies used in the main stages of MPs determination in soils.

2.1. Sample processing

To date, there is no consensus methodology for soil processing; the analytical methods for MPs research vary among research groups. First, the sample must be dry, and the purpose is to analyze a known quantity of mass to normalize by MP abundance (g, mg, or particles) per kilogram of dry soil (Moller et al. 2020; Yang et al. 2021). Then, the soil must be sieved (5 mm), in order to separate stones, roots, or other more prominent elements. Is recommended to disrupt the soil aggregates and pass them through the sieve to recover MPs from the soil aggregate fractions (Moller et al. 2020; Yang et al. 2021; Zhang

and Liu 2018). After sieving, the MPs must be isolated from the soil matrix, and several methods exist (Fig. 2). Density fractionation methods are widely used to extract MPs from complex matrices such as soil and compost. This technique uses solutions with a similar density to plastics, and plastic particles have a lower density than sediments and soil (typically 2.65–2.7 g cm^{-3}) (Li et al. 2020a; Rocha-Santos and Duarte 2017). The best results have been using saturated NaI solution (density 1.8 g cm^{-3}), an expensive reagent. However, it can reach a density that allows the vast majority of MPs to be separated without damaging them (Scheurer and Bigalke 2018; Yang et al. 2021; Zhang and Liu 2018). Oil-based extraction techniques take advantage of most plastics' lipophilic properties. This methodology consists of mixing the sample with water and some oil, then MPs are separated from the matrix by shaking. Due to its oleophilic surface, MPs remain in the oil, from where they can be filtered and extracted (Crichton et al. 2017; Mani et al. 2019; Moller et al. 2020; Scopetani et al. 2020). The oilbased extracting technique has several advantages; spiked polymers are not chemically altered during treatment, and it requires minimal reagents and essential laboratory equipment. However, filters and MPs need to be carefully rinsed with hexane to remove oil traces and the interaction is not strong enough to extract fluorinated plastics like polytetrafluoroethene (PTFE) from solid samples (Crichton et al. 2017; Mani et al. 2019; Moller et al. 2020; Scopetani et al. 2020). Fuller and Gautam (2016) developed an extraction method based on pressurized fluid extraction (PFE); this technique uses solvents at subcritical temperature and pressure conditions as an alternative. This method is fully automatized and fast, as it does not require sample purification. However, it is a destructive method, it does not allow to extract MPs larger than 30 μm, and only enable massquantitative analysis, not providing information on number, size, and shape of the polymer particles (Blasing and Amelung 2018; Fuller and Gautam 2016; Moller et al. 2020). To guarantee a reliable identification and quantification of MPs in soil, they must be purified from any biogenic material present (alive and non-living). Soil organic matter (SOM) should be removed because it interferes with some MP identification techniques

such as Fourier transform infrared (FTIR) and Raman spectroscopy (Blasing and Amelung, 2018). Various methodologies have been developed (Hurley et al. 2018; Scheurer and Bigalke 2018; Yang et al. 2021) (Fig. 2). Hurley et al. (2018) obtained the best results with Fenton's reagent, which is an oxidation reagent that uses H_2O_2 in the presence of a catalyst (Fe^{2+}). This method is performed at room temperature, it is low cost, fast, and effectively destroys highly chlorinated aromatic or inorganic compounds, typically recalcitrant in H_2O_2. Recently, Mbachu et al. (2021) developed a simple protocol for soil samples based on the application of cellulase, hemicellulase, lipase, and protease enzymes that digest the natural components of lignocellulosic biomass. This method proved to be effective reducing approximately 90% of organic matter. However, the authors used plant materials to simulate organic matter; nevertheless, it will be relevant to study this method in different soil samples.

2.2. Identification and quantification of MPs

There are several methods to determine MPs in soil samples. From simple visual sorting (MPs are identified by color, shape, or surface texture), to more complex techniques where MPs are determined by their chemical composition (Li et al. 2020a; Zhang et al. 2018). All these techniques are summarized with their advantages and limitations in Table 2. Nowadays, FTIR spectroscopy techniques are the most popular to identify and quantify MPs and are a promising tool for automated MP analysis. This technique provides information regarding MP abundance, shape, size, and precise identification of polymer types by recording the spectral chemical fingerprint of samples and comparing them with spectral databases. It has been used to detect MPs down to 5–10 μm (Chen et al. 2020a; Li et al. 2020a; Moller et al. 2020; Yang et al. 2021). Another common technique is Raman spectroscopy; this technique identifies substances with aromatic bonds, where FTIR has weaker intensity. Therefore, the combination of FTIR and Raman spectroscopy would be optimal for complete and reliable chemical characterization of MPs (Chen et al. 2020a; Kappler et al. 2016). Another promising tool for MP determination in soil

samples is visible-near-infrared spectroscopy (vis-NIR). This technique, which allows to be implemented in portable devices, measures the reflectance spectrum of a sample, that can be used to the identify its chemical composition (Corradini et al. 2019b). Hyperspectral imaging (HSI) has also been used in soils by Shan et al. in 2018. In this technique, the spectrum, which can be obtained in the vis-NIR or middle infrared (MIR) region, is recorded in each pixel of an image, giving spatial context to chemical information (Moller et al. 2020). On the other hand, Watteau et al. (2018) used pyrolysisgas chromatography/mass spectrometry (Pyr-GC–MS); to determine MPs in soil amended with municipal solid waste composts. This technique decomposes the sample in an inert gas at high temperature, then separates using gas chromatography, and finally analyzes by mass spectrometry the composition of the MPs (Junhao et al. 2021).

Table 2. Most common techniques used to MPs determination in soils.

METHOD	ADVANTAGES	LIMITATIONS	REFERENCES
Visual sorting	It is fast and inexpensive to implement for soil samples. Can identify, record the physical characteristics and abundance of MPs. Can be optimized using the heating method (130° C for 3-5 s) to differentiate MPs from other particles. It can be optimized using Nile Red to stain the MPs and visualize them with fluorescence microscopy.	Can´t determine the composition of the MPs and is less suitable for particles with a diameter smaller than 50 µm. Visual identification under a light microscope is extremely prone to bias, with error rates ranging from 20% to 70%, because particles of natural origin could look like synthetic fragments. Not all plastic particles surpass the bright threshold.	(Erni-Cassola et al., 2017; Piehl et al., 2018; Zang et al., 2018; Crew et al., 2020; Möller et al., 2020; Vermeiren et al., 2020; Yang et al 2021)
Fourier transform infrared spectroscopy (FTIR)	It allows identification of polymer types, their abundance, shape, and size. Non-destructive technique. micro-FTIR spectroscopy allows the identification of particles ranges from 10 to 500 µm.	Can't analyze samples wet samples. Irregularly shaped MPs will generate light scattering which cause non linearities in the spectrum and differences in the effective path length (can be avoided using ATR).	(Bläsing and Amelung, 2018; Chen et al., 2020a; Möller et al., 2020; Yang et al., 2021)
Near infrared spectroscopy (vis-NIR)	No pre-treatment required; fast and cheap sample analysis.	It is mostly used for pollution hotspots due to its low accuracy (10 g kg^{-1}) and detection limit of around 15 g kg^{-1}. This can be avoided using chemometric analysis, (it requires prior calibration). Water interference Black particles often result in unidentifiable spectra, due to the high absorption of infrared radiation.	(Becker et al., 2017; Corradini et al., 2019b; Li et al., 2020a)

Raman spectroscopy	It allows identification of polymer types, their abundance, shape, and size. It allows MPs identification down to a pixel resolution of 500 nm and could be improved up to 100 nm with silver colloid for surface-enhanced Raman spectroscopy (SERS). It can analyze wet samples and simultaneously identify fillers or pigments.	Background fluorescence of biological, organic, and inorganic (e.g., clay minerals) contaminations in the polymers may strongly interfere with the real spectra, making them unidentifiable. Poor signal to noise ratio (can be avoided using SERS)	(Käppler et al., 2016; Lv et al., 2020; Möller et al., 2020; Yang et al., 2021)
Hyper spectral imaging (HSI.)	No pre-treatment required, have the potential for automated identification analysis in soil samples. Nondestructive identification method, giving information on the spatial position, size, and chemical composition.	Method limited by the particle size. Is only capable of scanning MPs (0.5–5 mm) and can only be applied to the soil surface, whereas polymer particles situated in deeper soil strata are neglected. Highly expensive	(Shan et al., 2018; Möller et al., 2020; Yang et al., 2021)
Pyrolysis coupled with gas chromatography–mass spectrometry (Pyr-GC–MS.)	Easy to analyze samples with organic plastics additives in one run without the use of solvents and hence background contamination can be avoided.	Per run only one particle with a certain weight can be assessed and its database is available only for selected polymers. The method requires sample preparation and choice of pyrolysis type, so it is difficult for researchers to obtain similar results. No amount or shape of MPs can be identified	(Watteau et al., 2018; Baruah et al., 2021; Junhao et al., 2021)
Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)	It can provide information on particle sizes and their distribution.	MP analysis is susceptible to interference from natural organic matter present in the soil.	(Du et al., 2020)

3. ACCUMULATION OF MPS IN AGRICULTURAL ENVIRONMENT

Soils are essential components of terrestrial ecosystems and have intense pressure due to MP contamination. Rillig in 2012 was the first to expose this problem; he documented that there is a large accumulation of MPs in the environment mainly due to factors such as its durability and the existing technological limitation to discard or recycle the plastic produced (Barnes et al. 2009). MP's presence in the soil trigger changes in physical and chemical parameters, which can alter the degradation of organic matter (Liu et al. 2017). Qi et al. (2020) incubated soil with low-density polyethylene (LDPE) MPs for 4 months. They observed an electroconductivity increase, which is relevant because along with pH, and it affects the mobility of nutrients and heavy metal absorption by plants (Marschner and Rengel 2012; Zeng et al. 2011). It has also been shown that MPs can adsorb heavy metals on their surface. Soil incubation experiments with high-density polyethylene (HDPE) MPs demonstrated adsorption of zinc and an increased soil desorption capacity of cadmium (Cd). This suggests that MPs can increase the percentage of exchangeable Cd (Hodson et al. 2017; Wang et al. 2020a).

MP's presence also produces alterations of soil physical parameters; in agricultural soils, it has been shown that 72% of the MPs were associated with soil aggregates (Zhang and Liu 2018). de Souza-Machado et al. (2018) and de Souza-Machado et al. (2019) demonstrated that polyamide (PA), polyester (PES) fibers, and PA microspheres decrease the water-stable aggregates, unlike the HDPE, polyethylene terephthalate (PET), polypropylene (PP), and polystyrene (PS) fragments that did not show statistically significant results. This indicates that the shape of the microplastic, especially the microfibers, is an essential factor influencing the soil aggregates and would decrease the soil's structural stability (Zhang and Liu 2018). In addition, PA, PS, and HDPE increased the evapotranspiration in the soil. Evapotranspiration is relevant for numerous processes like microbial activity, precipitation, and the associated latent heat flux that helps to control surface temperatures (de Souza Machado et

al. 2019; Jung et al. 2010). Plastics are often less dense than many minerals present in soil; therefore, there is an bulk density parameter decreased by the addition of HDPE, PET, PP, and PS MPs at a concentration of 2% w/w (de Souza-Machado et al. 2018, 2019). However, experiments with lower concentrations of PS microfibers (0.3%) did not alter soil bulk density significantly (Zhang et al. 2019c). Due to MP pollution, a decrease in bulk density alters the soil pore structure, which may reduce penetration resistance for plant roots, enhance soil aeration, and influence water transport, increasing evaporation rate. In addition, physical and chemical parameters affect soil water dynamics, decomposition of organic matter, and biogeochemical cycles (de Souza Machado et al. 2019). Regarding agricultural soil's contamination, the most important MP entry-ways are sludge from sewage treatment and the use of plastic covers. There are also other ways of contamination, such as the use of organic amendments, compost, irrigation, flooding, fragmentation of plastic waste, and atmospheric deposition (Blasing and Amelung 2018; Ng et al. 2018; Nizzetto et al. 2016; Xu et al. 2020).

3.1. Sewage sludge

Agricultural soils are one of the main reservoirs of MPs, and the application of sludge from water treatment plants corresponds to the highest entry of MPs (Nizzetto et al. 2016). Sewage sludge is widely used as fertilizer because its richness in organic and inorganic plant nutrients is economically advantageous to increase yields in agricultural applications (Blasing and Amelung 2018; Nizzetto et al. 2016). Nizzetto et al. (2016) estimated that through direct application of sewage sludge, between 125 and 850 tons MPs per million inhabitants are added annually to European agricultural soils. In addition, MPs accumulate in soils with successive sludge applications over time, thus increasing their concentration. Moreover, fibers have been found in agricultural soils where sewage sludge was applied 15 years ago, and these fibers were still maintaining their original properties (Corradini et al. 2019a; Zhang and Liu 2018; Zubris and Richards 2005). Comparing MP concentrations of sewage sludge

from different countries, Chile has an average of 34,000 MP particles kg^{-1} (Corradini et al. 2019a), Spain has an average of 50,000 MP particles kg^{-1} (Van den Berg et al. 2020), and Canada has up to 11,469 MPs kg^{-1} (Crossman et al. 2020). MP polymer differs too. For example, Ren et al. (2020) found that 41% of MPs particles in sewage sludge from Yangling in China were PVC. In contrast, Crossman et al. (2020) reported mainly PS (44%) in sewage sludge from Ontario, Canada. The differences in concentration and resin are because the regions have different dietary habits, human activities, industrial manufactures, and different wastewater treatment processes (Zhou et al. 2020b).

3.2. Fragmentation plastic covers or mulch film

Plastic films are covering around 128,652 km^2 of agricultural land worldwide. The 80% of the mulched surface is found in China with estimated applications of around 700,000 t $year^{-1}$, where the growth rate is approximately 25% per year (Espi et al. 2006; Zhang et al. 2019a). Plastic mulch films are widely used in intensive production systems because they contribute to modify soil temperatures, improve the water content reducing evapotranspiration, increase rooting, control weeds, and significantly increase the productivity of crops. However, plastic polymers efficiently accumulate other harmful pollutants from the surrounding environment during its use, including several persistent, bioaccumulative, and toxic substances like PCBs, dioxins, DDTs, and PAHs (Nizzetto et al. 2016). For example, Ramos et al. (2015) evidenced a concentration of deltamethrin in mulch film (584–2284 μg pesticide g^{-1} plastic) higher than the concentration in soil (13–32 μg pesticide g^{-1} soil). Furthermore, there was a recalcintrant effect on the degradation of deltamethrin adsorbed in PE film. This aspect could be very concerning because PE and PP MPs have been found in agricultural soil where plastic mulch was applied for at least 20 years and with unknow consequences for soil biota and/or biodiversity (Piehl et al. 2018). Recent studies in soils with plastic covers and mulch films have shown up to 18,760 MPs per kilogram of soil. Moreover, soils with mulch film have more than twice MPs compared to non-mulch since the remaining plastic

decomposes into smaller pieces under the action of various physical, chemical, and biological factors (Zhang and Liu 2018; Huang et al. 2020; Zhou et al. 2020a).

3.3. Atmospheric deposition

The third most important entryway of MPs to soils is through atmospheric deposition. Atmospheric deposition is understood as the flux of substances from the atmosphere onto the earth's surface. Due to their small size and relatively low density (compared to other natural sediments), MPs are easily transported by air masses. Moreover, MPs can be transported to remote locations as has happened to MPs found in the Alps, the Pyrenees, and even the Arctic (Allen et al. 2019; Bergmann et al. 2019; Dris et al. 2016; Evangeliou et al. 2020; Klein and Fisher 2019). Currently, studies of the presence of MPs in atmospheric deposition have focused mainly on urban centers due to the possible impact on human health (Liu et al. 2019). In monitoring carried out throughout the year in Creteil (France), the atmospheric deposition of MPs ranged from 2 to 355 particles m-2 per day, indicating a high annual variability (Dris et al. 2016). In the case of the Hamburg metropolitan area (Germany), an average abundance of 275 particles m-2 per day has been reported, similar to the Chinese city of Dongguan, where up to 313 MPs particles m-2 per day were found (Cai et al. 2017; Klein and Fisher 2019). An essential source of MPs into the atmosphere is road traffic. Cars release MPs due to physical wear of tires, as well as the wear of brakes (Kole et al. 2017). Dowarah et al. in 2020 studied the abundance of MPs in road dust in 16 sites in India, finding 227 particles per 100 g of dust, where most were fibers (92%). An alarming fact about this situation is there is a correlation between changes in the dominant wind direction and the number of MPs measured during the same period (Klein and Fisher 2019). It has also been shown that the wind can erode the soil, such as uncovered agricultural soil, and drag MPs that can be re-suspended to the atmospheric load and be transported to remote sites (Rezaei et al. 2019).

4. IMPACT OF MPS ON SOIL FAUNA

Soil fauna is the total population of endopedonic (living inside the soil) and amphihabitant animals (living for a time in the soil and then outside) (Bunnenberg and Taeschner 2000). Studies show that MPs and soil contaminated with MPs negatively affect soil fauna, and the magnitude of its impact depends on several factors such as the species, concentration, size, and polymers present (Huerta Lwanga et al. 2016; Pflugmacher et al. 2020). Additionally, MPs can indirectly affect soil fauna by changing the soil's physicochemical parameters (Kim et al. 2020). Decomposition of SOM is performed in 90% by microorganisms such as bacteria and fungi. Also, decomposition is facilitated by ants, termites, earthworms, and others, which create channels, pores, aggregates, and mounds that influence the gases and water transport (Brussaard 1997; Garcia-Palacios et al. 2013). According to the body width, soil fauna is classified into three categories. The macrofauna (fauna of size >2 mm in diameter) are recognized as litter transformers by converting organic matter into organic structures (fecal pellets) (Xu et al. 2020). Selonen et al. (2020) studied the effect of soil contaminated with 0.02 to 1.5% w/w of PS microfibers on *Porcellio scaber* and observed that contaminated soil decreases feeding activity and allocates energy resources from proteins and lipids to carbohydrates, suggesting a potential depletion in energy reserves. Prendergast-Miller et al. (2019) also evidenced the effects of PS fibers in *Lombricus terrestris*. Treatments of 1% w/w showed a 1.5-fold lower cast production and a change in stress biomarker genes responses (24.3-fold increase metallothionein expression and a 9.9-fold decline in heat shock protein-70 expression). On the other hand, the significantly higher concentration of LDPE MPs (<150 um, 28% w/w) increased mortality and decreased the growth rate of *L. terrestris* (Huerta Lwanga et al. 2016). Otherwise, Song et al. (2019) demonstrated that PET microfibers can be ingested and depurated throughout the digestive system of terrestrial snails *Achatina fulica*. This behavior caused effects like villi damage, decreased food intake, excretion rate, glutathione peroxidase content, and total antioxidant capacity (T-AOC). In the case of

mesofauna (fauna with a size between 100 μm and 2 mm in diameter), studies have focused on the species *Enchytraeus crypticus* and *Folsomia candida*. Pflugmacher et al. (2020) showed that an increase in the concentration of HDPE MPs of 0 to 8% w/w in soil resulted in an increased *E. crypticus* mortality from 2 to 14%, respectively. Furthermore, when enchytraeids are exposed to soils with different concentrations of MPs, they preferred an environment with lower MP dose or an MP-free environment. MP particles used in this study (4 mm) were too large to be consumed by the oligochaete. It probably changed the soil structure, which resulted in unfavorable conditions for the Enchytraeids (Pflugmacher et al. 2020). Similar behaviors were evidenced in *F. candida*; springtails exhibited avoidance behaviors at 0.5 and 1% of PE MPs (w/w), and the avoidance rate was 59 and 69%, respectively. Other effects in springtails (1% MPs in soil w/w) were a decrease in the reproduction rate (70.2%) and an increase in mortality (26%) compared to the control group (Ju et al. 2019). Lin et al. (2020) studied a high-dose of MP addition (15 g m−2), finding a decrease of abundance of oribatid mites, dipteran larvae, lepidopteran larvae, and hymenoptera ants. However, Barreto et al. (2020) found no effects on the abundance and species richness of the groups Oribatida, Prostigmata, Astigmata, Mesostigmata, and Collembola in a loamy sand soil with addition of PE and PP MPs (0.4% w/w). These different results can be explained because the use of different MPs and concentrations in both studies. Regarding microfauna (fauna of size <200 μm in diameter), *in vitro* experiments using *Caenorhabditis elegans* nematode, show that effects depend on MPs' concentration, size, polymer content, and additives. MPs triggered a decrease in offspring and survival rates and produce more oxidative stress, intestinal damage, and shorter defecation intervals than the control group (Lei et al. 2018; Schopfer et al. 2020). Recently Kim et al. (2020) studied the effect of soils contaminated with PS nanospheres or microspheres on *C. elegans*, finding that offspring number significantly decreased at concentrations of 10 mg kg^{-1} of soil, and nematodes were more sensitive to MPs (530 nm) than nanoplastics (42 nm). Moreover, a principal component analysis showed that soil composition

and properties like bulk density, cation exchange capacity, clay, and sand content significantly affect the toxicity induced by these 530-nm-sized PS particles (Kim et al. 2020). Gut microbiota present in soil fauna (springtail *F. candida* and oligochaete *E. crypticus)* has also been studied. Insects exposed to soils with MPs had a different structure of gut microbial community than insects in soils without MPs; gut microbes play a vital role in host reproduction, nutrient supply, and immunity (Ju et al. 2019; Zhu et al. 2018). Exposure to HDPE MPs increased the relative abundance of *Bradyrhizobiaceae*, *Ensifer*, and S*tenotrophomonas*, all associated with N fixation (Ju et al. 2019). It is estimated that biological fixation contributes globally with 180 million metric tons of ammonia per year, and these fixation processes are performed by a great variety of bacteria that have nitrogenases (Tilak et al. 2005).

5. EFFECT OF MPS ON THE SOIL MICROBIOTA AND NITROGEN CYCLE

MPs are a novel surface and serve as a substrate for microorganism colonization; this ecosystem which in marine environments was called “Plastisphere” (Zettler et al. 2013) is also present in soils. Recently, next-generation sequencing (NGS) analysis of MP surface from soils evidenced different microbial communities, with lower richness and evenness in MPs compared to microbial community of soil (Huang et al. 2019; Yi et al. 2020). Also, there was differences in the microbiome on PET and LDPE, suggesting that chemical properties of MPs play an important role directing the evolution of the soil microbiome (Huang et al. 2019; Ng et al. 2021; Wang et al. 2021). MPs in soil serve as a “special microbial accumulator” as well, enriching the bacterial groups involved in their own biodegradation (Zhang et al. 2019b). An example of this colonization is the phylum Actinobacteria, which is the most sensitive to MP addition, because it decreases in the soil, but is enriched on the surfaces of PE (Huang et al. 2019; Yi et al. 2020; Wang et al. 2020b). *Actinomycetes* produce extracellular polymers such as dextran, glycogen, levan, and *N*-acetylglucosamine-rich slime polysaccharides, facilitating their attachment to

plastic surfaces for subsequent microbial action (Amobonye et al. 2020). N dynamics in the biosphere include biological processes such as, N fixation, mineralization, nitrification, denitrification, and anaerobic oxidation of ammonium. Its incorporation is essential for soil fertility and, therefore, for plant productivity. Microbial communities play a significant role in these processes, and when soil microbial ecology is disturbed, biological processes such as nutrient cycling will be affected (Ceron and Aristizabal 2012; Rong et al. 2021). Several studies have reported that MP addition to the soil could have an impact on the N cycle at different levels; altering the microbiota and the abundance of genes, and therefore, the enzymes that catalyze the different stages of the N cycle (Fei et al. 2020; Huang et al. 2019; Qi et al. 2020; Rong et al. 2021; Wang et al. 2020b). There is consensus that phyla *Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, and *Proteobacteria* are significantly more abundant in soils with the addition of PE and PP, and the composition of microbial communities plays a fundamental role in SOM decomposition (Fei et al. 2020; Huang et al. 2019; Rong et al. 2021; Yi et al. 2020; Wang et al. 2020b). In *Proteobacteria*, there are the families *Burkholderiaceae* (which is documented as a N-fixing bacteria), *Pseudomonaceae* (with the ability to promote both nitrification and denitrification), and *Xanthobacteraceae* (with fixing-N capacity). These three families increased their abundance in loamy and sandy soils with the addition of LDPE (1 and 5% w/w), PVC (1 and 5% w/w), and PP (2% w/w) (Fei et al. 2020; Yi et al. 2020; Wiegel 2006). Furthermore, in the study of Qian et al. (2018), the MP addition produced an increase in nitrite-oxidizing bacteria belonging to the Phylum *Nitrospirae*, which also participate in soil nitrification in agricultural ecosystems. On the other hand, the phylum *Acidobacteria* decreased with the addition of LDPE MPs at both 1% and 5%. Some members of this group have been reported as nitrate reducers (Kielak et al. 2016; Qian et al. 2018). Regarding silty loam soil, exposure of LDPE MPs of 150–200 μm (2 and 7% w/w) affected the soil bacterial diversity and structure. It triggered a shift in the abundance of some bacterial genera involved in soil N-cycling processing. Bacterial diversity significantly increased with 2% of LDPE MPs amendment at

day 7 and significantly decreased in soils with 7% w/w of LDPE MP amendment at day 60 (Rong et al. 2021). Besides, a high concentration of LDPE MPs (7% w/w) altered the structure of nitrogen-cycling bacterial community. This increased the proportions of *Mycobacterium*, *Gordonia*, and *Rhodococcus*, but decreased the proportion of *Azoarcus* compared to control (Rong et al. 2021). In general, MPs alter microbial communities of the soil and these changes mainly depend on the polymer's shape, quantity, and composition (de Souza Machado et al. 2018; Xu et al. 2020).

Other ubiquitous microorganisms in which the influence of MPs has also been studied are arbuscular mycorrhizal fungi (AMF). Studies showed that MPs alter symbiosis with roots. MPs of PES and PP increased the root colonization ~8 and ~1.4 times, respectively, but PET reduced root colonization ~50% (De Souza Machado et al. 2019). In addition, next-generation sequencing (NGS) analysis evidenced that depending on the type and dose, MPs also alter the structure and diversity of the AMF community (Wang et al. 2020a). MPs in soil have the potential to alter the role of AMF in the nitrogen cycle, like improving soil structure and nitrogen retention (the global AM fungal N pool may be at least 70% of that in the root pool) (Hodge and Fitter 2010). This role is connected to key ecosystem services important for soil and, eventually, human health (Leifheit et al. 2021). In conclusion, the addition of MPs affects different stages of the nitrogen cycle, as seen in Fig. 3.

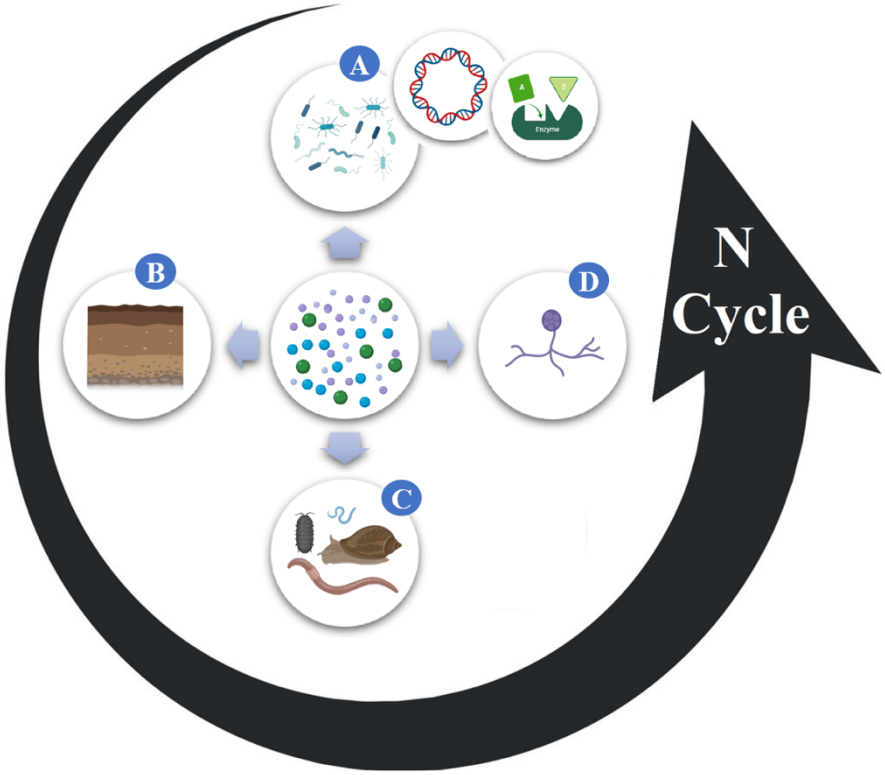

Fig. 3. Factors affected by MPs that alter the soil nitrogen cycle. A: Bacterial communities, genes and enzymes. B: Soil physiochemical parameters (soil aggregate fractions, evapotranspiration, soil bulk density, electroconductivity). C: Soil Fauna (macrofauna, mesofauna and microfauna). D: Arbuscular mycorrhizal fungi.

5.1. Effect of MPs on genes related to the nitrogen cycle

The alteration of bacterial communities due to MP addition changes the abundance of bacterial genes related to the nitrogen cycle. To date, there are few studies on the effect on these genes. Qian et al. in 2018 studied the use of plastic film and its effect on soil communities involved in the nitrogen cycle. They found that the abundance of the *nifH* gene increased by approximately 48%; this gene is used as a marker in the nitrogen fixation stage. On the other hand, the abundance of *amoA* (marker gene related to the nitrification stage) decreased by 9.8%. Regarding the *nosZ* and *nirS* genes, these genes increased 80 and

83%, respectively, but the abundance of *nirK* decreased 37% (Qian et al. 2018). The abundance of the *nirS* and *nosZ* genes was positively correlated with the activity of nitrate reductase. However, it showed no correlation with *nirK* gene abundances, indicating that the marker genes of the denitrification stage *nirS*type and *nosZ*-type contribute more to nitrate reduction and are more active. This suggests that functional communities involved in denitrification respond differently to soils covered with plastic (Iqbal et al. 2020; Qian et al. 2018).

5.2. Nitrogen fixation

The study of the MP effects on the abundance of nitrogen cycle marker genes is a recent topic in soils as in other ecosystems as sediments and freshwater systems. However, to date, it has been shown that soils with LDPE MPs at 0.5% (w/w) did not produce significant effects on the abundance of marker genes of the nitrogen fixation stage, such as the *nifD*, *nifH*, and *nifK* genes (Feng et al. 2022). But high doses of LDPE (7% w/w) promoted the abundance of *nifH* gene (Rong et al. 2021). These results can be associated with the increase of certain genera related to nitrogen fixation, such as the genus *Burkholderiaceae* that significantly increased after MP addition (LDPE 1% and 5% w/w and PVC 5% w/w) (Fei et al. 2020). Furthermore, mass balance calculation of total nitrogen at the beginning and at the end of a microcosm experient with freshwater suggested a possible N input caused by biological nitrogen fixation produced by biofilms on PP MPs (Chen et al. 2020b).

5.3. Nitrification

Regarding the nitrification stage, studies with soil are based on the abundance of *amoA* gene, which codes for the ammonia monooxygenase enzyme that oxidizes ammonia (NH_3^+) to hydroxylamine (NH_2OH) (Seeley et al. 2020). Rong et al. (2021) showed that addition of LDPE MPs (2% w/w) promoted the bacterial *amoA* gene abundance on day 15, but not the following days. These results showed a positive correlation with nitrifying bacteria *Nitrosopira* (r = 0.662, p = 0.007). Moreover, the addition of high-dosage LDPE

MPs (7% w/w) promoted the bacterial and archaeal *amoA* genes abundance on day 60. However, the addition of LDPE MPs (2% and 7% w/w) also produced a decrease in the *amoA* gene abundance of archaeas on day 15. This suggests that LDPE MPs can occasionally inhibit the abundance of ammoniaoxidizing archaea (AOA)-*amoA* gene (Rong et al. 2021). The *amoA* gene abundance has also been studied in other environments such as sediments and freshwater. In sediment studies with MPs, it has been shown that abundance of ammonia-oxidizing bacteria (AOB)-*amoA* gene increased from day 7 to day 16, suggesting enhanced nitrification potential with time (Seeley et al. 2020). Furthermore, in freshwater systems, the addition of MPs with biofilms further increased nitrification ability in the system (Chen et al. 2020b).

5.4. Denitrification

Regarding the denitrification stage, the addition of high doses of LDPE MPs to the soil (7% w/w) promoted the abundance of *nirK* gene on day 90 and *nirS* genes on days 7 and 15. However, the stimulative effects on *nirS* gene were temporary and decreases by day 90. The results about *nirK* gene abundance are positively correlated with the abundances of denitrifying bacterias *Pseudomonas*, *Stenotrophomonas*, *Brachybacterium*, and *Achromobacter* ($r > 0.5$, $p = 0.5$) (Rong et al. 2021). On the other hand, Ren et al. (2020) studied the effect of the MP addition to a fertilized soil on the emission of greenhouse gases, concluding that LDPE MPs (5% w/w) decreased the emission of N_2O by changing the abundances of microbes related to N_2O emissions. The impact of MPs on the nitrogen cycle has also been studied in sediments, where microcosm experiment with sediment and PVC MPs exhibited a decrease of relative abundance of *nirS* gene and a low potential rate of denitrification too (Seeley et al. 2020). However, in experiments adding MPs to activate sludge and MPs with biofilms at a freshwater system, the denitrification has been promoted (Chen et al. 2020b; Li et al. 2020b). Studies conclude that addition of MPs to the soil produces effects on the nitrogen cycle and additional

studies are required to measure the real impact on the different stages of the nitrogen cycle.

5.5. Effect of MPs on soil enzymatic activity

Bacterial communities are the main enzyme producers in soils, and MPs alter the bacterial structure and affect the soil enzymatic activity (de Souza Machado et al. 2018; Xu et al. 2020; Zhang and Liu 2018). Urease catalyzes the conversion of urea to ammonium that will be oxidize in the nitrification process. The effects on this enzyme depend mainly on the MPs used, concentration, and experiment extension time. In the study of Yi et al. (2020), a higher urease activity was observed in soils treated with MPs of LDPE and PP at 2% (w/w) on day 14, but this activity decreased by 31% on day 29 compared to the control. However, in a different study, urease activity was stimulated in soil with LDPE MPs during the 90 days of the experiment, although this effect was probably due to the lower concentration of MPs that was used (0.0076% w/w) (Huang et al. 2019). Alterations in the community structure, gene expression, and synthesized enzymes result in variations in the nitrogen content in soil. For example, in the study of Liu et al. (2017), when MPs of PVC at 7 and 28% (w/w) were added, the total dissolved nitrogen (TDN) and dissolved organic nitrogen (DON) content increased significantly after days 7 and 14, respectively. However, between days 7 and 30, the addition of MPs did not produce significant changes in NO_3^- and NH_4^+ compared to control. On the other hand, Yan et al. (2020) showed that paddy soil with concentrations of 1% of PVC had a 13% lower NO_3^- content than soil without MPs. These contradictory results are likely because different concentrations of PVC or soils were used, and therefore, there were different physical, chemical, and biological characteristics. It has also been shown that MPs alter the nitrogen cycle directly too, by enriching the soil with nitrogen, particularly when PA MPs are added because their composition is rich in nitrogen (de Souza Machado et al. 2019).

6. CONCLUSIÓN, CHALLENGES, AND FUTURE DIRECTIONS

It is important to know the MP load that the soils contain; for this purpose, a representative sampling and suitable processing of soils must be performed. Then, a combination of FTIR and Raman spectroscopy would be optimal for complete and reliable chemical characterization of MPs. MPs are classified as emerging pollutants and like any pollutant, and it alters the ecosystem it enters. It has been shown that the addition of MPs to soils alters biogeochemical cycles, such as the nitrogen cycle, and does it directly by adding MPs that have nitrogen in their chemical structure. However, these alterations can be indirectly too, i.e., by modifying the microbiota/enzymes that catalyze reactions in the different stages of the nitrogen cycle in the soil, by changing the soil fauna that is responsible for facilitating the decomposition of organic matter, and/or by altering the physicochemical parameters of the soil such as evapotranspiration, electrical conductivity, and/or the proportion of microaggregates. The global effects of MPs on the nitrogen cycle are still unknown, since the studies to date have been performed under soil plant (leguminous) in laboratory conditions. Also, field experiments to study the changes in the nitrogenous species should be performed for better understanding of the N-biological stages and processes afected. This is necessary since the consumption of plastic is increasing, and with it, the accumulation of MPs, as their natural degradation, is limited. These analyses would show strong evidence that could be used to conduct appropriate agronomic practices and public policies to reduce the consumption and disposal of plastics to mitigate their effects. Additionally, understanding the impact of MPs on the nitrogen cycle is important because this cycle is a key predictor of ecological stability and management in the terrestrial ecosystem.

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Authors' contributions

GR compiled information and wrote the manuscript, which was reviewed and edited by MS, HU, JA and EZ. The final manuscript was read and approved by all authors.

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REFERENCES

Allen S, Allen D, Phoenix VR, Le Roux G et al (2019) Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nat Geosci 12:339-344. https://doi.org/10.1038/s41561-019-0335-5

Amobonye A, Bhagwat P, Singh S, Pillai S (2020) Plastic biodegradation: frontline microbes and their enzymes. Sci Total Environ 143536. https://doi.org/10.1016/j.scitotenv.2020.143536

Andrady AL, Neal MA (2009) Applications and societal benefits of plastics. Philos Trans R Soc B Biol Sci 364:1977-1984. https://doi.org/10.1098/rstb.2008.0304

Barnes DK, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc B Biol Sci 364:1985-1998. https://doi.org/10.1098/rstb.2008.0205

Barreto C, Rillig M, Lindo Z (2020) Addition of polyester in soil affects litter decomposition rates but not microarthropod communities. Soil Org 92:109-119. https://doi.org/10.25674/so92iss2pp109

Baruah A, Sharma A, Sharma S, Nagraik R (2021) An insight into different microplastic detection methods. Int J Environ Sci Technol 1-10. https://doi.org/10.1007/s13762-021-03384-1

Becker W, Sachsenheimer K, Klemenz M (2017) Detection of black plastics in the middle infrared spectrum (MIR) using photon up-conversion technique for polymer recycling purposes. Polymers 9:435. https://doi.org/10.3390/polym9090435

Bergmann M, Mützel S, Primpke S, Tekman MB et al (2019) White and wonderful? Microplastics prevail in snow from the Alps to the Arctic Sci Adv. 5:eaax1157. https://doi.org/10.1126/sciadv.aax1157

Bläsing M, Amelung W (2018) Plastics in soil: Analytical methods and possible sources. Sci Total Environ 612:422-435. https://doi.org/10.1016/j.scitotenv.2017.08.086

Besseling E, Quik JT, Sun M, Koelmans AA (2017) Fate of nano-and microplastic in freshwater systems: A modeling study. Environ Pollut 220;540-548. https://doi.org/10.1016/j.envpol.2016.10.001

Brandon J, Goldstein M, Ohman MD (2016) Long-term aging and degradation of microplastic particles: comparing in situ oceanic and experimental weathering patterns. Mar Pollut Bull 110:299– 308. https://doi.org/10.1016/j.marpolbul.2016.06.048

Brussaard L (1997) Biodiversity and ecosystem functioning in soil. Ambio 563-570.

Bunnenberg C, Taeschner M (2000) Soil fauna transport versus radionuclide migration. Radiat Prot Dosim 92;35-38. https://doi.org/10.1093/oxfordjournals.rpd.a033280

Cai L, Wang J, Peng J, Tan Z, Zhan Z, Tan X, Chen Q (2017) Characteristic of microplastics in the atmospheric fallout from Dongguan city, China: preliminary research and first evidence. Environ Sci Pollut Res 24;24928-24935. https://doi.org/10.1007/s11356-017-0116-x

Cerón LE, Aristizábal FA (2012) Nitrogen and phosphorus cycles dynamics in Soils. Rev Colomb Biotecnol 14:285-295.

Chen Y, Wen D, Pei J, Fei Y, Ouyang D, Zhang H, Luo Y (2020a) Identification and quantification of microplastics using Fourier Transform Infrared Spectroscopy: Current status and future prospects. Curr Opin Environ Sci Health 18:14-19. https://doi.org/10.1016/j.coesh.2020.05.004

Chen X, Chen X, Zhao Y, Zhou H et al (2020b). Effects of microplastic biofilms on nutrient cycling in simulated freshwater systems. Sci Total Environ 719: 137276. https://doi.org/10.1016/j.scitotenv.2020.137276

Corradini F, Meza P, Eguiluz R, Casado F, Huerta-Lwanga E, Geissen V (2019a) Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. Sci Total Environ 671:411-420. https://doi.org/10.1016/j.scitotenv.2019.03.368

Corradini F, Bartholomeus H, Lwanga EH, Gertsen H, Geissen V (2019b) Predicting soil microplastic concentration using vis-NIR spectroscopy. Sci Total Environ 650:922-932. https://doi.org/10.1016/j.scitotenv.2018.09.101

Crew A, Gregory-Eaves I, Ricciardi A (2020) Distribution, abundance, and diversity of microplastics in the upper St. Lawrence River. Environ Pollut 260:113994 https://doi.org/10.1016/j.envpol.2020.113994

Crichton EM, Noël M, Gies EA, Ross PS (2017) A novel, density-independent and FTIR-compatible approach for the rapid extraction of microplastics from aquatic sediments. Anal. Methods 9, 1419-1428. https://doi.org/10.1039/C6AY02733D

Crossman J, Hurley RR, Futter M, Nizzetto L (2020) Transfer and transport of microplastics from biosolids to agricultural soils and the wider environment. Sci Total Environ. 724:138334. https://doi.org/10.1016/j.scitotenv.2020.138334

de Souza Machado AA, Lau CW, Till J, Kloas W et al (2018) Impacts of microplastics on the soil biophysical environment. Environ Sci Technol 52:9656-9665. https://doi.org/10.1021/acs.est.8b02212

de Souza Machado AA, Lau CW, Kloas W, Bergmann J, Bachelier JB, Faltin E, Becker R, Gorlich AS, Rillig MC (2019) Microplastics can change soil properties and affect plant performance. Environ. Sci Technol 53:6044-6052. https://doi.org/10.1021/acs.est.9b01339

Dris R, Gasperi J, Saad M, Mirande C, Tassin B (2016) Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? Mar Pollut Bull Mar Pollut Bull 104:290-293. https://doi.org/10.1016/j.marpolbul.2016.01.006

Du C, Wu J, Gong J, Liang H, Li Z (2020) ToF-SIMS characterization of microplastics in soils. Surf Interface Anal 52:293-300. https://doi.org/10.1002/sia.6742

Duis K, Coors A (2016) Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ Sci Eur 28:2. https://doi.org/10.1186/s12302-015-0069-y

Erni-Cassola G, Gibson MI, Thompson RC, Christie-Oleza JA (2017) Lost, but found with Nile red: a novel method for detecting and quantifying small microplastics (1 mm to 20 μm) in environmental samples. Environ. Sci. Technol. 51:13641-13648. https://doi.org/10.1021/acs.est.7b04512

Espi E, Salmeron A, Fontecha A, García Y, Real AI (2006) Plastic films for agricultural applications. J Plast Film Sheeting. 22:85-102. https://doi.org/10.1177/8756087906064220

Evangeliou N, Grythe H, Klimont Z, Heyes C et al (2020) Atmospheric transport is a major pathway of microplastics to remote regions. Nat Commun 11:1-11. https://doi.org/10.1038/s41467-020-17201-9

Feng Y, Han L, Li D, Sun M et al (2022) Presence of microplastics alone and co-existence with hydrochar unexpectedly mitigate ammonia volatilization from rice paddy soil and affect structure of soil microbiome. J Hazard Mater. 422: 126831. https://doi.org/10.1016/j.jhazmat.2021.126831

Fei Y, Huang S, Zhang H, Tong Y et al (2020) Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil. Sci Total Environ 707:135634. https://doi.org/10.1016/j.scitotenv.2019.135634

Fendall LS, Sewell MA (2009) Contributing to marine pollution by washing your face: microplastics in facial cleansers. Mar Pollut Bull 58:1225-1228. https://doi.org/10.1016/j.marpolbul.2009.04.025

Fuller S, Gautam A (2016) A procedure for measuring microplastics using pressurized fluid extraction. Environ Sci Technol 50:5774-5780. https://doi.org/10.1021/acs.est.6b00816

Gao Y, Xie Y, Jiang H, Wu B, Niu J (2014) Soil water status and root distribution across the rooting zone in maize with plastic film mulching. Field Crops Res 156:40–47. https://doi.org/10.1016/j.fcr.2013.10.016.

García-Palacios P, Maestre FT, Kattge J, Wall DH (2013) Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. Ecol Lett 16:1045-1053. https://doi.org/10.1111/ele.12137

Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Natl Acad Sci 107:13754-13759. https://doi.org/10.1073/pnas.1005874107

Hodson ME, Duffus-Hodson CA, Clark A, Prendergast-Miller MT, Thorpe KL (2017) Plastic bag derived-microplastics as a vector for metal exposure in terrestrial invertebrates. Environ Sci Technol 51:4714-4721. https://doi.org/10.1021/acs.est.7b00635

Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C (2017) Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci Total Environ 586:127-141. https://doi.org/10.1016/j.scitotenv.2017.01.190

Huang Y, Zhao Y, Wang J, Zhang M, Jia W, Qin X (2019) LDPE microplastic films alter microbial community composition and enzymatic activities in soil. Environ Pollut 254:112983. https://doi.org/10.1016/j.envpol.2019.112983

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

Huerta Lwanga E, Gertsen H, Gooren H, Peters P, Salánki T, Van Der Ploeg M, Besseling E, Koelmans AA, Geissen V (2016) Microplastics in the terrestrial ecosystem: implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). Environ Sci Technol 50:2685-2691. https://doi.org/10.1021/acs.est.5b05478

Huerta Lwanga E, Mendoza Vega J, Ku Quej V, de los Angeles Chi J et al (2017) Field evidence for transfer of plastic debris along a terrestrial food chain. Sci Rep 7:1-7. https://doi.org/10.1038/s41598-017-14588-2

Hurley RR, Lusher AL, Olsen M, Nizzetto L (2018) Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. Environ Sci Technol 52:7409-7417. https://doi.org/10.1021/acs.est.8b01517

IAEA (International Atomic Energy Agency) (2004) Soil sampling for environmental contaminants. IAEA-TECDOC-1415, IAEA, Vienna

Iqbal S, Xu J, Allen SD, Khan S, Nadir S, Arif MS, Yasmeen T (2020) Unraveling consequences of soil micro-and nano-plastic pollution for soil-plant system with implications for nitrogen (N) cycling and soil microbial activity. Chemosphere 127578. https://doi.org/10.1016/j.chemosphere.2020.127578

Ju H, Zhu D, Qiao M (2019) Effects of polyethylene microplastics on the gut microbial community, reproduction and avoidance behaviors of the soil springtail, Folsomia candida. Environ. Pollut. 247:890-897. https://doi.org/10.1016/j.envpol.2019.01.097

Jung M, Reichstein M, Ciais P, Seneviratne SI et al (2010) Recent decline in the global land evapotranspiration trend due to limited moisture supply. Nature 467:951-954. https://doi.org/10.1038/nature09396

Junhao C, Xining Z, Xiaodong G, Li Z, Qi H, Siddique KH (2021) Extraction and identification methods of microplastics and nanoplastics in agricultural soil: A review. J Environ Manage 294:112997. https://doi.org/10.1016/j.jenvman.2021.112997

Käppler A, Fischer D, Oberbeckmann S, Schernewski et al (2016) Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both?. Anal Bioanal Chem 408:8377-8391. https://doi.org/10.1007/s00216-016-9956-3

Kielak AM, Barreto CC, Kowalchuk GA, van Veen JA, Kuramae EE (2016) The ecology of Acidobacteria: moving beyond genes and genomes. Front Microbiol 7:744. https://doi.org/10.3389/fmicb.2016.00744

Kim SW, Kim D, Jeong SW, An YJ (2020) Size-dependent effects of polystyrene plastic particles on the nematode Caenorhabditis elegans as related to soil physicochemical properties. Environ Pollut 258:113740. https://doi.org/10.1016/j.envpol.2019.113740

Klein M, Fischer EK (2019) Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. Sci Total Environ 685:96-103. https://doi.org/10.1016/j.scitotenv.2019.05.405

Kole P, Löhr AJ, Van Belleghem F, Ragas A (2017) Wear and tear of tyres: a stealthy source of microplastics in the environment. Int J Environ Res Public Health 14:1–4. https://doi.org/10.3390/ijerph14101265

Lei L, Wu S, Lu S, Liu M et al (2018) Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode *Caenorhabditis elegans*. Sci Total Environ. 619:1-8. https://doi.org/10.1016/j.scitotenv.2017.11.103

Leifheit EF, Lehmann A, Rillig MC (2021) Potential effects of microplastic on arbuscular mycorrhizal fungi. Front Plant Sci 12. https://doi.org/10.3389/fpls.2021.626709

Li J, Song Y, Cai Y (2020a) Focus topics on microplastics in soil: analytical methods, occurrence, transport, and ecological risks. Environ Pollut 257:113570. https://doi.org/10.1016/j.envpol.2019.113570

Li L, Song K, Yeerken S, Geng S et al (2020b). Effect evaluation of microplastics on activated sludge nitrification and denitrification. Sci Total Environ 707: 135953. https://doi.org/10.1016/j.scitotenv.2019.135953

Lin D, Yang G, Dou P, Qian et al (2020) Microplastics negatively affect soil fauna but stimulate microbial activity: insights from a field-based microplastic addition experiment. Proc Royal Soc B 287:20201268. https://doi.org/10.1098/rspb.2020.1268

Liu EK, He WQ, Yan CR (2014) 'White revolution' to 'white pollution'— agricultural plastic film mulch in China. Environ Res Lett 9:091001 2014. https://doi.org/10.1088/1748-9326/9/9/091001.

Liu H, Yang X, Liu G et al (2017) Response of soil dissolved organic matter to microplastic addition in Chinese loess soil. Chemosphere 185:907-917. https://doi.org/10.1016/j.chemosphere.2017.07.064.

Liu K, Wang X, Wei N, Song Z, Li D (2019) Accurate quantification and transport estimation of suspended atmospheric microplastics in megacities: Implications for human health. Environ Int 132:105127. https://doi.org/10.1016/j.envint.2019.105127

Lv L, He L, Jiang S, Chen J et al (2020) In situ surface-enhanced Raman spectroscopy for detecting microplastics and nanoplastics in aquatic environments. Sci Total Environ 728:138449. https://doi.org/10.1016/j.scitotenv.2020.138449

Marschner P, Rengel Z (2012) Nutrient availability in soils. In Marschner's mineral nutrition of higher plants (pp. 315-330). Academic Press https://doi.org/10.1016/B978-0-12-384905-2.00012-1.

Mani T, Frehland S, Kalberer A, Burkhardt-Holm P (2019) Using castor oil to separate microplastics from four different environmental matrices. Anal Methods 11:1788-1794. https://doi.org/10.1039/C8AY02559B

Mbachu O, Jenkins G, Pratt C, Kaparaju P (2021) Enzymatic purification of microplastics in soil. MethodsX 8:101254. https://doi.org/10.1016/j.mex.2021.101254

Möller JN, Löder MG, Laforsch C (2020) Finding microplastics in soils: a review of analytical methods. Environ Sci Technol 54:2078-2090. https://doi.org/10.1021/acs.est.9b04618

Ng EL, Lwanga EH, Eldridge SM, Johnston P et al (2018) An overview of microplastic and nanoplastic pollution in agroecosystems. Sci. Total Environ. 627:1377-1388. https://doi.org/10.1016/j.scitotenv.2018.01.341

Ng EL, Lin SY, Dungan AM, Colwell JM, Ede S et al (2021) Microplastic pollution alters forest soil microbiome. J Hazard Mater 409:124606. https://doi.org/10.1016/j.jhazmat.2020.124606

Nizzetto L, Futter M, Langaas S (2016) Are agricultural soils dumps for microplastics of urban origin? Environ Sci Technol 50:10777–10779. https://doi.org/10.1021/acs.est.6b04140

Prendergast-Miller MT, Katsiamides A, Abbass M, Sturzenbaum SR, Thorpe KL, Hodson ME (2019) Polyester-derived microfibre impacts on the soil-dwelling earthworm Lumbricus terrestris. Environ Pollut 251:453-459. https://doi.org/10.1016/j.envpol.2019.05.037

Pflugmacher S, Huttunen JH, Wolff MAV, Penttinen OP et al (2020) Enchytraeus crypticus avoid soil spiked with microplastic. Toxics 8:10. https://doi.org/10.3390/toxics8010010

Piehl S, Leibner A, Löder MG, Dris R, Bogner C, Laforsch C (2018) Identification and quantification of macro-and microplastics on an agricultural farmland. Sci Rep 8:1-9. https://doi.org/10.1038/s41598-018-36172-y

PlasticsEurope (2021) Plásticos – Situación en 2020 Un análisis de los datos sobre producción, demanda y residuos de plásticos en Europa. https://www.plasticseurope.org/es/resources/publications/4803-plasticos-situacion-en-2020 (accessed 06 july 2021)

Qi Y, Ossowicki A, Yang X, Lwanga EH et al (2020) Effects of plastic mulch film residues on wheat rhizosphere and soil properties. J Hazard Mater 387:121711. https://doi.org/10.1016/j.jhazmat.2019.121711

Qian H, Zhang M, Liu G, Lu T et al (2018) Effects of soil residual plastic film on soil microbial community structure and fertility. Water Air Soil Pollut 229:261. https://doi.org/10.1007/s11270-018-3916-9

Ramos L, Berenstein G, Hughes EA, Zalts A, Montserrat JM (2015) Polyethylene film incorporation into the horticultural soil of small periurban production units in Argentina. Sci Total Environ 523:74-81. https://doi.org/10.1016/j.scitotenv.2015.03.142

Ren X, Sun Y, Wang Z, Barceló D, Wang Q, Zhang Z, Zhang Y (2020) Abundance and characteristics of microplastic in sewage sludge: A case study of Yangling, Shaanxi province, China. Case Stud Chem Environ Eng 2:100050. https://doi.org/10.1016/j.cscee.2020.100050

Rezaei M, Riksen MJ, Sirjani E, Sameni A, Geissen V (2019) Wind erosion as a driver for transport of light density microplastics. Sci Total Environ 669:273-281. https://doi.org/10.1016/j.scitotenv.2019.02.382

Rillig MC (2012) Microplastic in terrestrial ecosystems and the soil?. Environ Sci Technol 46:6453–6454. https://doi.org/10.1021/es302011r

Rillig MC, Ziersch L, Hempel S (2017) Microplastic transport in soil by earthworms. Sci Rep 7:1-6. https://doi.org/10.1038/s41598-017-01594-7

Rillig MC, de Souza Machado AA, Lehmann A, Klümper U (2019) Evolutionary implications of microplastics for soil biota. Environ Chem 16:3-7. https://doi.org/10.1071/EN18118

Rocha-Santos TAP, Duarte AC (2017) Comprehensive Analytical Chemistry. Characterization and analysis of Microplastics. Vol 75 1st Oxford: Elsevier.

Rochman CM, Hoh E, Kurobe T, Teh SJ (2013) Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci Rep 3:1-7. https://doi.org/10.1038/srep03263

Rong L, Zhao L, Zhao L, Cheng Z, Yao Y, Yuan C, Wang L, Sun H (2021) LDPE microplastics affect soil microbial communities and nitrogen cycling. Sci Total Environ 773:145640. https://doi.org/10.1016/j.scitotenv.2021.145640

Scheurer M, Bigalke M (2018) Microplastics in Swiss floodplain soils. Environ Sci Technol 52:3591-3598. https://doi.org/10.1021/acs.est.7b06003

Schöpfer L, Menzel R, Schnepf U, Ruess L et al (2020) Microplastics effects on reproduction and body length of the soil-dwelling nematode Caenorhabditis elegans. Front Environ Sci 8:41. https://doi.org/10.3389/fenvs.2020.00041

Scopetani C, Chelazzi D, Mikola J, Leiniö V et al (2020) Olive oil-based method for the extraction, quantification and identification of microplastics in soil and compost samples. Sci Total Environ 733:139338. https://doi.org/10.1016/j.scitotenv.2020.139338

Seeley ME, Song B, Passie R, Hale R C (2020) Microplastics affect sedimentary microbial communities and nitrogen cycling. Nat Commun 11: 1-10. https://doi.org/10.1038/s41467-020-16235-3

Selonen S, Dolar A, Kokalj AJ, Skalar T et al (2020) Exploring the impacts of plastics in soil–The effects of polyester textile fibers on soil invertebrates. Sci Total Environ 700:134451. https://doi.org/10.1016/j.scitotenv.2019.134451

Shan J, Zhao J, Liu L, Zhang Y, Wang, X, Wu F (2018) A novel way to rapidly monitor microplastics in soil by hyperspectral imaging technology and chemometrics. Environ Pollut 238:121-129. https://doi.org/10.1016/j.envpol.2018.03.026

Song, Y., Cao, C., Qiu, R., Hu, J et al (2019) Uptake and adverse effects of polyethylene terephthalate microplastics fibers on terrestrial snails (Achatina fulica) after soil exposure. Environ Pollut 250:447-455. https://doi.org/10.1016/j.envpol.2019.04.066

Steffen W, Richardson K, Rockström J. Cornell SE et al (2015) Planetary boundaries: Guiding human development on a changing planet. Science 347:6223. https://doi.org/10.1126/science.1259855

Steinmetz Z, Wollmann C, Schaefer M, Buchmann C et al (2016) Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation?. Sci Total Environ 550:690-705. https://doi.org/10.1016/j.scitotenv.2016.01.153

Thompson RC, Swan SH, Moore CJ, Vom Saal FS (2009) Our plastic age. Philos Trans R Soc Lond B Biol Sci 364:1526. https://doi.org/10.1098/rstb.2009.0054

Tilak KVBR, Ranganayaki N, Pal KK, De R et al (2005) Diversity of plant growth and soil health supporting bacteria. Curr Sci 136-150. http://www.jstor.org/stable/24110439

UNEP (2014) ANNUAL REPORT 2014 https://www.unep.org/resources/annual-report/unep-2014-annual-report (accessed 06 july 2021).

van den Berg P, Huerta-Lwanga E, Corradini F, Geissen V (2020) Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils. Environ Pollut 261:114198. https://doi.org/10.1016/j.envpol.2020.114198

Vermeiren P, Muñoz C, Ikejima K (2020) Microplastic identification and quantification from organic rich sediments: A validated laboratory protocol. Environ Pollut 262:114298. https://doi.org/10.1016/j.envpol.2020.114298

Wang J, Liu X, Li Y, Powell T et al (2019) Microplastics as contaminants in the soil environment: a mini-review. Sci Total Environ 691:848-857. https://doi.org/10.1016/j.scitotenv.2019.07.209

Wang F, Zhang X, Zhang S, Zhang S, Sun Y (2020a) Interactions of microplastics and cadmium on plant growth and arbuscular mycorrhizal fungal communities in an agricultural soil. Chemosphere 126791. https://doi.org/10.1016/j.chemosphere.2020.126791

Wang J, Huang M, Wang Q, Sun Y, Zhao Y, Huang Y (2020b) LDPE microplastics significantly alter the temporal turnover of soil microbial communities. Sci Total Environ 726:138682. https://doi.org/10.1016/j.scitotenv.2020.138682

Wang J, Peng C, Li H, Zhang P, Liu X (2021) The impact of microplastic-microbe interactions on animal health and biogeochemical cycles: A mini-review. Sci Total Environ 145697. https://doi.org/10.1016/j.scitotenv.2021.145697

Watteau F, Dignac MF, Bouchard A, Revallier A, Houot S (2018) Microplastic detection in soil amended with municipal solid waste composts as revealed

by transmission electronic microscopy and pyrolysis/GC/MS. Front Sustain Food Syst 2:81. https://doi.org/10.3389/fsufs.2018.00081

Wiegel J (2006) The genus Xanthobacter. The prokaryotes 5:290-314. Third Edition. Springer, New York. https://doi.org/10.1007/0-387-30745-1

Xu B, Liu F, Cryder Z, Huang D et al (2020) Microplastics in the soil environment: occurrence, risks, interactions and fate–a review. Crit Rev Environ Sci Technol 50:2175-2222. https://doi.org/10.1080/10643389.2019.1694822

Yan Y, Chen Z, Zhu F, Zhu C et al (2020) Effect of polyvinyl chloride microplastics on bacterial community and nutrient status in two agricultural soils. Bull Environ Contam. Toxicol 1-8. https://doi.org/10.1007/s00128-020-02963-1

Yang L, Zhang Y, Kang S, Wang Z, Wu C (2021) Microplastics in soil: A review on methods, occurrence, sources, and potential risk. Sci Total Environ 146546. https://doi.org/10.1016/j.scitotenv.2021.146546

Yi M, Zhou S, Zhang L, Ding S (2020) The effects of three different microplastics on enzyme activities and microbial communities in soil. Water Environ Res 93:24-32. https://doi.org/10.1002/wer.1327

Zeng F, Ali S, Zhang H, Ouyang Y, Qiu B, Wu F Zhang G (2011) The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environ Pollut 159:84-91. https://doi.org/10.1016/j.envpol.2010.09.019

Zettler ER, Mincer TJ, Amaral-Zettler LA (2013) Life in the “plastisphere”: microbial communities on plastic marine debris. Environ Sci Technol 47:7137-7146. https://doi.org/10.1021/es401288x

Zhang C (2007) Fundamentals of environmental sampling and analysis. John Wiley & Sons. Hoboken, New Jersey, U.S.A.

Zhang GS, Liu YF (2018) The distribution of microplastics in soil aggregate fractions in southwestern China. Sci Total Environ 642:12-20. https://doi.org/10.1016/j.scitotenv.2018.06.004

Zhang S, Yang X, Gertsen H, Peters P, Salánki T, Geissen V (2018) A simple method for the extraction and identification of light density microplastics from soil. Sci Total Environ 616:1056-1065. https://doi.org/10.1016/j.scitotenv.2017.10.213

Zhang XY, Yao SY, Tian YQ, Li JS (2019a) Comparison of plastic film, biodegradable paper and bio-based film mulching for summer tomato production: soil properties, plant growth, fruit yield and fruit quality. Sci Hortic 249:38e48. https://doi.org/10.1016/j.scienta.2019.01.037

Zhang M, Zhao Y, Qin X, Jia W, Chai L, Huang M, Huang Y (2019b) Microplastics from mulching film is a distinct habitat for bacteria in farmland soil. Sci Total Environ 688:470-478. https://doi.org/10.1016/j.scitotenv.2019.06.108

Zhang GS, Zhang FX, Li XT (2019c) Effects of polyester microfibers on soil physical properties: Perception from a field and a pot experiment. Sci Total Environ 670:1-7. https://doi.org/10.1016/j.scitotenv.2019.03.149

Zhou B, Wang J, Zhang H, Shi H et al (2020a) Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, east China: Multiple sources other than plastic mulching film. J Hazard Mater 388:121814. https://doi.org/10.1016/j.jhazmat.2019.121814

Zhou G, Wang Q, Zhang J, Li Q et al (2020b) Distribution and characteristics of microplastics in urban waters of seven cities in the Tuojiang River basin, China. Environ Pollut 189:109893. https://doi.org/10.1016/j.envres.2020.109893

Zhu D, Chen QL, An XL, Yang XR et al (2018) Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition. Soil Biol Biochem 116:302-310. https://doi.org/10.1016/j.soilbio.2017.10.027

Zubris KAV, Richards BK (2005) Synthetic fibers as an indicator of land application of sludge. Environ Pollut 138:201-211. https://doi.org/10.1016/j.envpol.2005.04.013

CAPITULO 2. Effects of low-density polyethylene and polyamide microplastics on the microbiological and chemical characteristics of an Andisol

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Abstract

Microplastics (MPs) are a significant threat to soils. However, there is scarce information on the impact of MPs on soil properties, particularly in volcanic ash derived soils. The objective of this study was to evaluate the impact of polyamide (PA) and low-density polyethylene (LDPE) MPs on the biological and chemical characteristics of an Andisol from central Chile. Twenty-one parameters were evaluated, including pH, electrical conductivity (EC), total

organic carbon (TOC), dissolved organic carbon (DOC), total phosphorus (TP), available phosphorous (AP), available nitrogen (AN), inorganic nitrogen forms (NH_4^+, NO_2^- and NO_3^-), carbohydrates (CHO), polyphenols (POL), humic substances, soil basal respiration(SBR) and activities of soil enzymes such as dehydrogenase, β-glucosidase, phosphatase and urease. For this, a microcosm was set up in clay pots in an incubation chamber at 21°C and 60% soil moisture, with the addition of PA and LDPE at doses of 1% and 3% w/w; a control treatment consisting of microcosm without MPs was also included. After 6 weeks of incubation, PA addition resulted in an increase in TOC and NH_4^+ by up to 32% and 26%, respectively, and a decrease in NO_3^- by 22%. AP decreased by 15%–19% with the addition of PA and LDPE. Similarly, acid phosphatase and β-glucosidase activities decreased by 15% and 26% with PA and LDPE, respectively. The distance-based linear model (DistLM) was used to analyse relationships in chemical and biological datasets. The analysis revealed that TOC and TP were primary components in the best model for predicting microbiological changes ($R2$ = 0.469, AICs = 16.026), indicating that MPs accumulation affects soil carbon cycling and P content. Overall, the results show that MPs addition alters soil chemical and microbiological properties in Andisols, with varying effects depending on the type and dose of MPs, with the highest dose (3%) producing the most marked negative effects.

KEYWORDS

Biochemical soil properties, microcosms, plastisphere, soil pollution, volcanic soils

1. INTRODUCTION

The term “microplastics” (MPs) was first used by Thompson et al. (2004) to refer to microscopic-sized plastics, being commonly defined as particles between 100 nm and 5 mm in size (de Souza Machado, Kloas, et al., 2018; Duis & Coors, 2016; Xu et al., 2020). MPs have gained great attention due to their small particle size, ubiquity, availability, and negative effects on different

ecosystems (Rocha-Santos & Duarte, 2017; Scheurer & Bigalke, 2018). In fact, they can act as a vector for pathogens and absorb contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pharmaceuticals, pesticides, perfluoroalkyl substances (PFAS) and heavy metals. Furthermore, they can accumulate in the food chain by plant uptake from the soil or by consumption of contaminated soil biota (Riveros et al., 2022). Soils are essential components of terrestrial ecosystems and have strong pressure due to MPs contamination. Rillig (2012) was the first researcher who addressed this problem, documenting the presence of significant amounts of MPs in terrestrial ecosystems and the soil. MPs accumulation is primarily due to factors such as their durability and the current limitations in technology for discarding or recycling plastic (Barnes et al., 2009; Rillig, 2012). Annually, up to 300,000 and 430,000 tons of MPs enter agricultural lands in North America and Europe, respectively (Nizzetto et al., 2016). The most important MPs inputs are active sludge from sewage treatment plants used as organic amendments, and the fragmentation of plastics used in modern agriculture, while other ways of contamination include the use of compost, irrigation, flooding, fragmentation of plastic waste and atmospheric deposition (Nizzetto et al., 2016; Riveros et al., 2022).

The presence of MPs in the soil triggers physical (aggregate stability, soil bulk density and water dynamics), chemical (nutrient availability, organic matter and pH), and microbiological changes (microbial and enzymatic activity and soil fauna) (Riveros et al., 2022). Qi et al. (2020) incubated a sandy soil with LDPE and observed an increase in electroconductivity. Furthermore, Liu et al. (2017) conducted an incubation study on a Cambisol (FAO soil classification) from China, with the addition of PVC-type MPs and reported an increase in dissolved organic nutrients, such as C, N and P, and fulvic acid content after 30 days of incubation. In soils, MPs are a novel surface for the colonization of microorganisms and can act as a source of their nutrients (de Souza Machado et al., 2019; Huang et al., 2019). MPs decompose very slowly, but there are

certain microbial genotypes that can utilize the C and/or nitrogen contained within them (Huang et al., 2019; Qi et al., 2020; Rillig et al., 2019, 2021).

Soil incubation with MPs has been shown to alter the structure of soil bacterial communities. In this sense, Wang et al. (2020) reported that 25 orders of bacteria were enriched in soils exposed to LDPE, while only 18 orders were enriched in the control without MPs addition. Bacterial communities are the main producers of enzymes in the soil and thus MPs alter the bacterial structure and therefore, affect soil enzymatic activity. Several studies have reported on the impact of MPs on soil enzymatic activities, describing effects that vary depending on the soil, incubation time and/or MPs. Urease activity increased in loamy soils amended with LDPE (Huang et al., 2019). A similar effect was observed in a study conducted in loamy and sandy soils incubated with 2% (w/w) membranous polyethylene (PE) or fibrous polypropylene (PP) on day 14 but then decreased on day 29 (Yi et al., 2021). In the same study, dehydrogenase activity decreased in soil incubated with membranous PE or microsphere PP on day 14, but an increase was recorded on day 29. The addition of fibrous PP resulted in a decrease in soil phosphatase activity on day 14 and a subsequent increase on day 29; conversely, this enzymatic activity decreased with the addition of microsphere PP. Recent studies in Andisols show that urease and dehydrogenase activities were not altered with high-density polyethylene (HDPE) incubations, probably because the doses of MPs were low (0.2 g MPs kg−1 soil) or the parameters were not sensitive to MPs (Pinto-Poblete et al., 2022, 2023).

Andisols account for <1% of the Earth's land surface but play an important role in Chilean agriculture, occupying approximately 50% of the country's land area under cereal production (Poblete-Grant et al., 2020; Staff, 2006). Andisols have a high C storage capacity due to their elevated content of short-range-order minerals like allophane and imogolite-type materials (Neculman et al., 2013). They have specific andic properties, such as high

content of short-range order minerals, favouring phosphate immobilization through sorption and/or precipitation with cations such as Al and Fe (Redel et al., 2016).

Polyethylene and PA are commonly used in agriculture. PA has nitrogen in its structure since it is partly synthesized with reagents derived from ammonia. Pinto-Poblete et al. (2022, 2023) studied the effects of the addition of HDPE–MPs and trace metals (Cd and Cu) on properties of an Andisol mixed with sand on a soil–plant system and evidenced a synergistic effect of both pollutants, reporting an increase in acid phosphatase and dehydrogenase activity with respect to the control and the soil with only MPs. Knowledge of the effects of MPs on Andisols is still limited in terms of types of MPs and soil properties analysed, and thus, experimental studies are required to gain a comprehensive understanding of the impact of MPs. Therefore, the aim of this study was to elucidate the short-term effect of LDPE (a plastic intensively used in agriculture) and PA (a plastic that has nitrogen in its structure since it is synthesized in part with reagents derived from ammonia) on chemical and microbiological properties of an Andisol. Chemical predictors were also determined to identify changes at the microbiological level in soils polluted with MPs. To the best of the authors' knowledge, this is the first report to consider 21 soil properties, including POL content, for determining the effects of MPs on the soil.

2. MATERIALS AND METHODS

2.1. Study site and experimental design

Soil was collected from a location with no recent history of anthropogenic activity, located near Chillan (Lat. 36°31′59.4″ S; Long 71°55′35.7″ W). The soil corresponds to an Andisol (USDA, 2010), Diguillin series (Typic Melanoxerand) (Zagal et al., 2002). A total of 15 kg of soil was collected with a metal shovel (excluding the O horizon) and transported in metal containers to the laboratory

to be air-dried and sieved through a 2 mm mesh for the analysis of initial chemical soil properties and MPs.

For quantification, MPs particles were isolated from soils with a saturated solution of sodium chloride (density approx. 1.23 g cm−3) according to the methodology of Jorquera et al. (2022) with modifications. For this purpose, 50 g of air-dried soil was mixed with 200 mL of NaCl solution (33% w/v). Subsequently, they were shaken for 60 min and allowed to settle overnight. For particle collection, 100 mL of supernatant was filtered through a Whatmann No. 40 filter, and then the filters were dried at 60°C and kept in a glass Petri dish until optical inspection. Visual sorting was performed under a stereomicroscope (Euromex StereoBlue, Holland) with a magnification range between 0.7X, and 4.5X. Particles were isolated using fine metal tweezers, following the criteria of Jorquera et al. (2022). Fourier-transform infrared spectroscopy (FTIR) was performed on 20 MPs-like particles, using an FTIR spectrometer and a microscope system (Spotlight 400, Perkin Elmer). The MPs-like particles were placed on KBr discs to obtain spectra by transmission and compared with reference spectra from the library of the equipment. A baseline correction, subtracting CO_2 and humidity (H_2O) signals, was performed automatically on the equipment. Each spectrum was an average of 18–80 scans, acquired with an area of 100 × 100 μm^2, spectral range of 4000–550 cm^{-1}, spectral resolution of 4 cm^{-1}, and spectral interval of 2 cm^{-1}.

2.2. MPs materials

LDPE films and PA ropes were purchased from agricultural supply stores (LDPE: Polimaq Ltda, Concepcion – Chile; PA: Sodimac S.A., Concepcion – Chile). LDPE films were crushed using an Ultra-turraxR homogenizer (Ultra-TurraxT25, IKA, USA) to obtain particles of the size range of MPs, while particle size was determined by Mastersizer 3000R equipment; 0.25–3.5 mm). PA particles were obtained by manually cutting PA ropes using scissors to obtain particles of 2–5 mm; particle size was determined using a vernier calliper. To

confirm the polymer type, MPs were analysed using an FTIR spectrometer and microscope system (Spotlight 400, Perkin Elmer) (Figure S1). MPs samples were slightly pressed and analysed by a micro-ATR Germanium crystal to collect FTIR images of 400 × 400 μm2. Each pixel of the FTIR image corresponded to 6.25 × 6.25 μm2 of the sample and contained a spectrum in the range of 4000–750 cm−1, with a resolution of 6 cm−1 (3 cm−1 data interval). Each spectrum had an average of 16 scans.

2.3. Incubation experiment

The soil used for the microcosm experiment had the following initial chemical properties (mg kg−1 soil): 37.3 mg of available N, 8.5 mg of available Olsen P, 195.7 mg of available K, 3.84 cmol of exchangeable Ca, 0.7 cmol of exchangeable Mg, 8.7% organic matter, and pH 5.6. Moreover, the FTIR analysis revealed that there were no plastic particles present in the soil, the 20 MPs-like particles analysed had a lignocellulosic composition (Figure S2). MPs particles (LDPE and PA) were UV sterilized (254 nm) for an hour to minimize microbial contamination and mixed with soil using a metal trowel at two different doses, 1% and 3% w/w (dry weight, which can be regarded as an environmentally relevant concentration (Fuller & Gautam, 2016). Clean soil without MPs was used as control. Each microcosm was performed in a 500 mL sterilized clay pot with 350 g of air-dried soil for each pot (14% humidity). A completely randomized design was used, with four replicates per treatment. The experiment was maintained in darkness at 21°C in a plant growth chamber (JSPC-420C, JSR Corporation, Gonju, Korea) at the University of Concepcion, Concepcion, Chile. During the experiment, pots were watered every 2 days to maintain soil moisture at 60% by adjusting weight loss due to evapotranspiration. After 6 weeks of incubation, the soil was taken from the centre of the pot to perform soil analysis.

2.4. Soil chemical parameters

Once soil–MPs incubation was finished, soil samples were air-dried to determine chemical characteristics. Soil pH was determined on a soil: water suspension of 1:2.5 using a digital pH metre (Model edgeR HI2020, Hanna Instruments, USA), EC was determined on a soil: water suspension of 1:5 using a digital conductivity metre (Model edgeR HI2030, Hanna Instruments, USA). TOC and DOC (water-soluble fraction) were determined by wet oxidation using the method modified by Mingorance et al. (2007). AP was analysed using the Olsen–Watanabe method (Olsen, 1954). CHO was quantified from a cold extraction on a soil: water suspension of 1:10 for 1 h under agitation using the anthrone–sulphuric acid method (Brink et al., 1960). POL content was measured in the same extract, using the Folin-Ciocalteau reagent by the Folin–Denis method (Pascal, 1968). CHO (625 nm) and POL (750 nm) absorbances were measured with a spectrophotometer, Spectronic Helios Gamma UV– Vis (Thermo Fisher Scientific, Waltham, Massachusetts, USA). To determine total phosphorus (TP), dried soils were individually ground with a mortar and pestle, and then subjected to microwave acid digestion. Samples were analysed by inductively coupled plasma-mass spectrometry ICP-MS (iCAP™ TQ Thermo Fisher Scientific, Bremen, Germany). NO_3^-), NO_2^-, and NH_4^+ concentrations were analysed in a filtered extract (soil: water, 1:20), which was acid digested with concentrated acids (9 mL of HNO_3^- (65%), 3 mL of HCl (30%), 1 mL of HF (40%) and completed with 25 mL with ultrapure water) in a microwave oven (Milestone UltraWave, 230°C). Subsequently, the digested sample was analysed by an ion chromatography system (CH-930 COMPACT IC FLEX, Metrohm, Switzerland) equipped with an anion column (Metrosep A Supp 7, 250 × 4 mm, Eluent: Na_2CO_3 (3.6 mM), Flow: 0.8 mL min−1, 45°C) and a cation column (Metrosep C6–150/4.0 mm, Eluent: HNO_3 (3 mM) + oxalic acid (1 mM), Flow: 1.1 mL min−1, 45°C). AN was calculated as the sum of NH_4^+ and NO_3^-.

2.5. Isolation and quantification of soil humic fractions

The isolation and quantitative analysis of soil humic fractions were based on the methods of Duchafour and Jacquin (1975) and Dorado et al. (2003).

Subsequently, the organic carbon content of the different fractions obtained was determined using the wet oxidation method described by Walkley and Black (1934). For the analysis, 5 g of soil sample was suspended in H_3PO_4 (2 M). The floating organic particles were separated by centrifugation, filtration, and washing with distilled water. The pellet was successively extracted with $Na_4P_2O_7$ (0.1 M), shaking for 3 h, centrifuged at 4500 rpm for 30 min (Digicen 21, Ortoalresa, Spain), and then extracted with NaOH (0.1 M). The process was repeated three times. The total humic extract (THE) was obtained by aggregating the successive alkaline supernatants after centrifugation. Two aliquots of this extract were taken and precipitated with H_2SO_4 (1:1 by volume) and centrifuged at 3000 rpm. The quantitative estimation of humic acids (HAs) was made from the insoluble fraction, while acid-soluble fulvic acids (FAs) were estimated by difference. The results obtained from the isolation and quantification of HAs and FAs were then used to compare the humification degree between the different treatments (HAs/FAs).

2.6. Enzymatic and microbial activity assays

Soil basal respiration was measured in 100-mL hermetically sealed glass vials by placing 5 g of soil sample at 60% water holding capacity. The vials were incubated in darkness at 28°C for 38 days. The CO2 produced by microbial respiration was measured every day for the first 4 days and then every 4 or 5 days, using an infrared gas analyser (IRGA S151; Qubit Systems Inc., Canada). The results were expressed as mg C-CO_2 kg−1 dry soil day−1. Dehydrogenase activity was analysed using 0.5 g of sample, as the reduction of 0.2 mL of p-iodonitrotetrazolium chloride to iodonitrotetrazolium formazan (INTF) at room temperature (Garcia et al., 1997). The INTF produced was extracted with a 1:1 (v:v) mixture of ethanol and dimethylformamide and measured spectrophotometrically at 490 nm. The activity was quantified by reference to a calibration curve constructed with data obtained by incubating INTF standards under the same conditions described above and expressed as μmol INTF g^{-1} h^{-1}. Urease activity was determined as described by Nannipieri et al. (1980) with

slight modifications. Briefly, urea was used as substrate and borate was used as a buffer (Borate buffer pH 10.0; di-Sodium tetraborate 15%). After 2 h at 37°C, a volume of 2.5 mL of 2 M KCl was added to the mixtures. In the control, urea solution was added to the mixtures after the addition of KCl. NH_4^+ was determined by the colorimetric indophenol method at 490 nm, as described by Nannipieri et al. (1979). The enzymatic activity was expressed as μmol N- NH_4^+ g^{-1} h^{-1}. β-glucosidase activity was analysed following the method described by Eivazi' and Tabatabai (1988). The method involves extraction and calorimetric determination of the p-nitrophenol released when 0.5 g of soil is incubated with 2.5 mL of buffered p-Nitrophenyl-β-D-glucopyranoside solution at 37°C for 1 h. Alkaline phosphatase (MUB buffer pH 11) and acid phosphatase (MUB buffer pH 6.5) activities were determined according to Tabatabai and Bremner (1969). This method involves colorimetric estimation of the 4-nitrophenol released by phosphatase activity when soil is incubated with buffered sodium p-nitrophenyl phosphate solution at 3°C for 1 h. The activity was quantified by reference to a calibration curve constructed with p-nitrophenol determined at 400 nm and expressed as μmol PNF g^{-1} soil h^{-1}.

2.7. Statistical analysis

Significant differences in soil chemical and microbiological properties between the different MPs treatments, doses applied, and natural control soils were analysed by permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) ($p > 0.05$), using permutation tests to obtain the p values. A sample similarity matrix was performed using Euclidean Distance to test the effects of the MPs treatments and doses for each individual variable. Subsequently, pairwise test comparisons were performed using a multivariate analogue of the t-test and probability levels were estimated by permutation (Eldridge et al., 2016), using a total of 999 permutations. Differences were considered significant at 95% probability.

A principal component analysis (PCA) and Pearson correlation test (*r*) were performed to explore the relationships between the different variables studied (chemical, microbiological indicators and humus fractions) for each MPs treatment and dose, and the control was unmodified with MPs. Additionally, the distance-based linear model (DistLM) was used to determine the relative importance of each physical, chemical, and humus fraction on microbiological properties (enzymatic activity and SBR). For the DistLM routine, "marginal" tests of the relationship between the response variable (enzyme activity and basal soil respiration) and an independent variable were performed to identify the independent variables that explain the variability in the different treatments. Subsequent "sequential" tests of the individual variables were then performed to assess the significant contribution to the explained variance of the response variable. The AICc (Akaike Information Criterion) (Akaike, 1974) was adopted to select the best model, and a step-by-step procedure was followed to build the model. The statistical package PRIMER + PERMANOVA (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) for Windows was used for PERMANOVA, PCA, and DistLM analysis. Pearson correlations were performed using Statgraphic Centurion XVIII-X64 software (StatPoint Technologies, Inc., Warrenton, VA, USA).

3. RESULTS

3.1. Effects of MPs on soil chemical properties

The results of the PERMANOVA analysis revealed significant differences ($p < 0.05$) in the Andisol samples incubated with MPs for 6 weeks (Table 1; Table S1). The presence of MPs caused significant changes in the chemical properties of the soil, including alterations in pH, EC, and levels of soil nutrients, such as C, N and P. These changes were particularly evident in the LDPE 3% and PA 3% treatments. Moreover, LDPE 1% and 3% (w/w) increased the recorded pH values by 1.1% and 0.8%, respectively. Overall, the treatments with

MPs 3% (w/w) (LDPE and PA) decreased electroconductivity by 22% and 20%, respectively, compared to the control treatment. Soils with PA showed increases of 7% and 32% in TOC concentrations with PA of 1% and 3%, respectively. However, LDPE 1% decreased TOC content by 7% compared to control soil. Additionally, a 9% decrease in DOC content was observed in the treatments with LDPE 1% and PA 3%. In addition, POL content decreased between 39% and 52% in all the MPs treatments. In the case of nitrogenous species, MPs 3% (w/w) showed increases of 29% and 26% in NH_4^+ for LDPE and PA, respectively. However, PA 3% decreased contents of NO_2^- (31%) and NO_3^- (22%) compared to the control treatment. In relation to AN content, a 25% increase was observed in LDPE 1%. When evaluating P content, TP decreased 9% in the soil with LDPE 3% (w/w), while AP decreased between 15% and 19% in PA (1% and 3%) and LDPE (3% w/w) compared to control.

Table 1. Chemical properties in control soil and soils with MPs addition.

Soil properties	Treatments				
	CONTROL	LDPE 1%	PA 1%	LDPE 3%	PA 3%
pH	6.39 ± 0.03 a	6.46 ± 0.03 b	6.41 ± 0.03 ac	6.44 ± 0.02 bc	6.42 ± 0.04 abc
EC (mS cm^{-1})	42.9 ± 2.98 a	42.2 ± 5.66 a	39.6 ± 1.55 a	33.6 ± 3.23 b	34.6 ± 2.81 b
TOC (%)	4.83 ± 0.07 a	4.50 ± 0.12 b	5.16 ± 0.21 c	4.73 ± 0.14 ab	6.37 ± 0.38 d
DOC (g-C kg^{-1})	1.66 ± 0.08 a	1.51 ± 0.06 b	1.57 ± 0.13 ab	1.63 ± 0.05 a	1.51 ± 0.04 b
CHO (mg-C kg^{-1})	17.3 ± 2.71 ab	18.3 ± 0.62 a	16.7 ± 0.64 b	16.5 ± 1.78 ab	19.1 ± 1.76 a
POL (mg kg^{-1})	15.7 ± 2.26 a	7.57 ± 1.62 b	9.17 ± 0.91 b	9.65 ± 2.68 b	8.82 ± 0.91 b
AN (mg kg^{-1})	51.5 ± 3.02 a	64.9 ± 1.11 b	52.3 ± 3.07 a	56.4 ± 7.49 ab	54.9 ± 3.61 a
NH_4^+ (mg kg^{-1})	30.4 ± 4.24 ab	38.1 ± 4.83 ac	29.3 ± 0.64 b	39.3 ± 3.85 c	38.5 ± 1.74 c
NO_2^- (mg kg^{-1})	0.71 ± 0.14 a	0.53 ± 0.06 ab	0.47 ± 0.10 b	0.55 ± 0.07 ab	0.49 ± 0.03 b
NO_3^- (mg kg^{-1})	21.1 ± 1.43 ab	26.9 ± 4.64 a	23.0 ± 3.29 ab	17.0 ± 4.77 bc	16.5 ± 2.00 c
TP (mg kg^{-1})	1055 ± 34.3 a	989 ± 41.4 ab	1012 ± 19.8 a	959 ± 34.1 b	1005 ± 29.9 ab
AP (mg kg^{-1})	15.6 ± 0.35 a	16.2 ± 1.82 ab	13.3 ± 1.17 c	13.3 ± 1.54 bc	12.6 ± 0.67 c

Average of four replicates (average ± standard deviation). EC: electrical conductivity; TOC: total organic carbon; DOC: dissolved organic carbon; CHO:

carbohydrates; POL: polyphenols; AN: available nitrogen; NH_4^+: Ammonium; NO_2^-: Nitrite; NO_3^-: Nitrate; TP: total phosphorus; AP: available phosphorus.

3.2. Effects of MPs on the abundance of soil humic fractions

The addition of MPs 1% did not produce statistically significant changes in the content of humic substances in the soils, but differences were found in HAs and FAs with MPs 3% (Figure 1a) ($p < 0.05$). With respect to the control treatment, LDPE 3% (w/w) decreased THE, HAs and FAs substances by 38%, 28%, and 64%, respectively. Furthermore, HAs content decreased by 24% soil with PA 3% (w/w), while FAs content recorded a 108% increase compared to control ($p < 0.05$). A lower humification degree was observed in the soils with MPs 3%, with reductions of 39% (LDPE) and 23% (PA) compared to the control ($p < 0.05$).

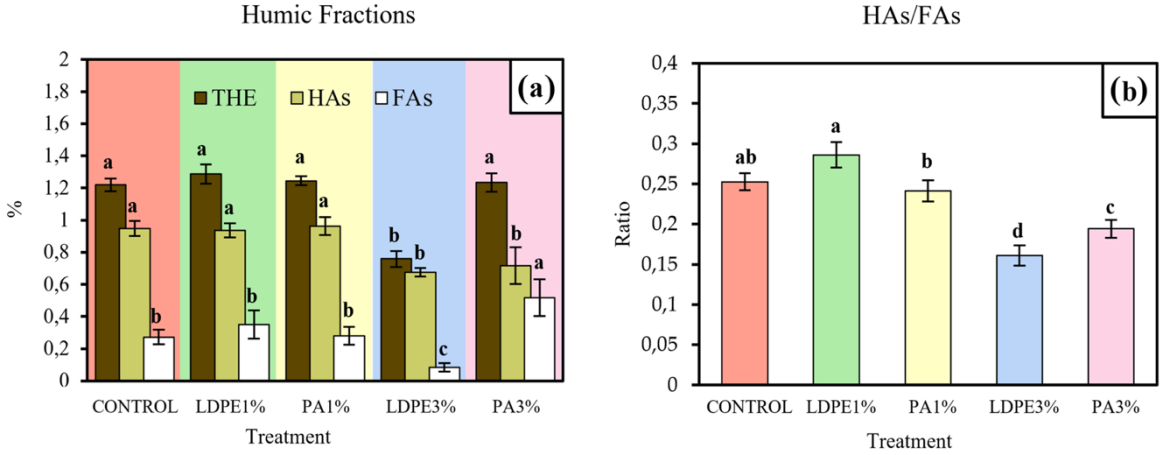
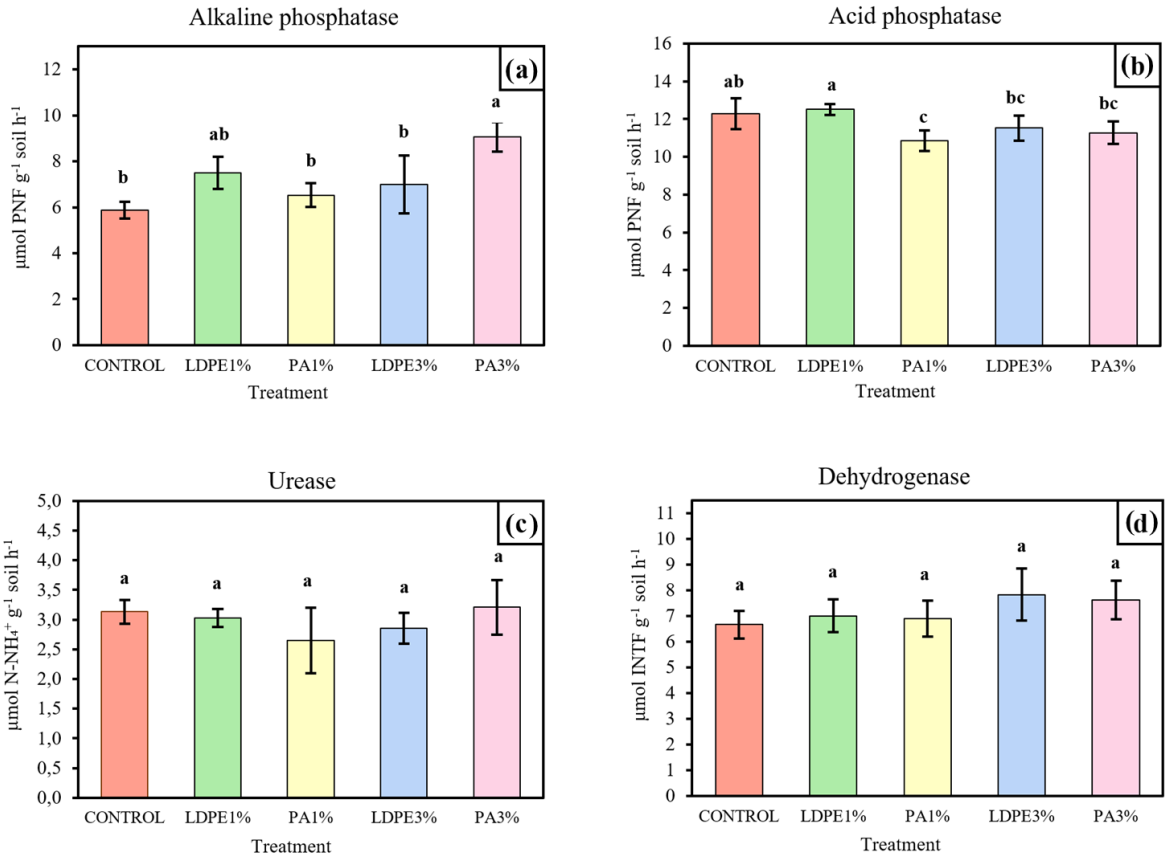


Figure 1 Humic substances in control soils and soils with MPs addition. (a) Humic fractions; (b) HAs/FAs. CONTROL: Soil without MPs addition; LDPE1%: Soil with 1% of LDPE-MPs added (w/w); PA1%: Soil with 1% of PA-MPs added (w/w); LDPE3%: Soil with 3% of LDPE-MPs added (w/w); PA3%: Soil with 3% of PA-MPs added (w/w). HAs: Humic acid fraction; FAs: Fulvic acid fraction; HAs/FAs: Humification ratio. Lowercase letters indicate significant differences ($p < 0.05$) between the different treatments.

3.3. Effects of MPs on soil microbiological properties

After 6 weeks of incubation, some microbiological properties in the soil microcosms with MPs were altered compared to the control soil (Figure 2) (PERMANOVA; $p < 0.05$). The addition of PA at 3% (w/w) drastically increased alkaline phosphatase activity by 54% compared to the control (Figure 2a). Furthermore, acid phosphatase activity decreased by 12% in PA 1% ($p < 0.05$), and with a tendency to decrease in the treatments with MPs 3%, with no statistically significant differences (Figure 2b). In terms of β-glucosidase activity, PA resulted in decreases of 21% and 15% with doses of 1% and 3%, respectively, whereas LDPE 3% resulted in a 26% decrease with respect to the control ($p < 0.05$) (Figure 2e). The urease and dehydrogenase activities did not show statistically significant changes after 6 weeks of MPs incubation (Figure 2c,d). With respect to the control, SBR values were 3.7% higher in soil with PA 3% ($p < 0.05$), while values were 4.7% and 4.9% lower with LDPE 1% and 3%, respectively (Figure 2f). When comparing treatments with 3% MPs addition, PA produced 9% more CO_2 than LDPE ($p < 0.05$).



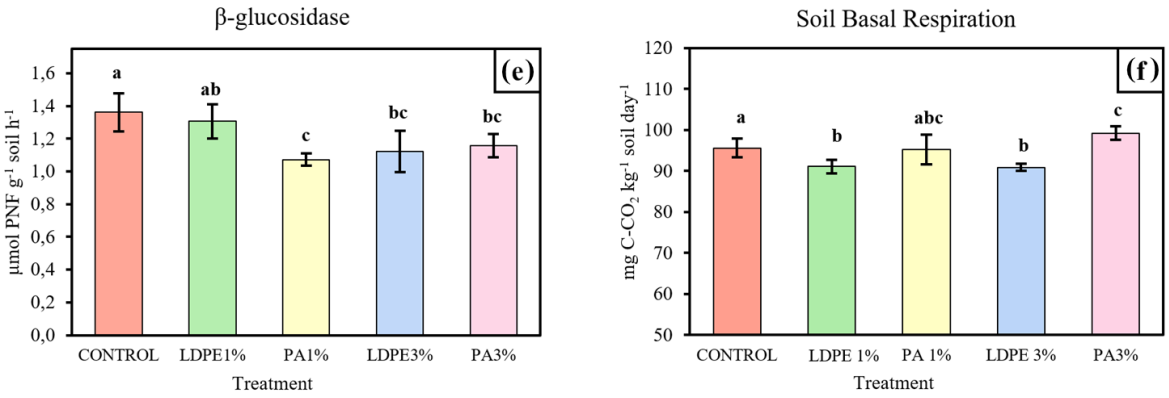


Figure 2 Microbiological properties of control soil and soils with MPs addition. (a) Alkaline phosphatase; (b) Acid phosphatase; (c) Urease; (d) Dehydrogenase; (e) β-glucosidase; (f) Soil basal respiration. CONTROL: Soil without MPs addition; LDPE1%: Soil with 1% of LDPE-MPs added (w/w); PA1%: Soil with 1% of PA- MPs added (w/w); LDPE3%: Soil with 3% of LDPE-MPs added (w/w); PA3%: Soil with 3% of PA-MPs added (w/w); PA: Polyamide. Lowercase letters indicate significant differences ($p < 0.05$) between the different treatments.

3.4. Relationships between chemical and microbial soil properties

A PCA biplot was performed by integrating all the variables evaluated on the microcosm experiments, the overall effects of the MPs under study (LDPE and PA) and their different doses (1% and 3%). The PCA biplot of the first two principal components calculated from the chemical and microbiological properties explained 45.6% of the total variability, with PC1 and PC2 explaining 28.1% and 17.5% of the variance, respectively. Interestingly, chemical and microbiological eigenvectors clearly differentiated soil samples according to the different treatments (Figure 3). The LDPE 1%, PA 1%, LDPE 3%, PA 3% treatments and the control soil formed independent clusters in different regions of the factor space. The horizontal axis (PC1) differentiated soil without MPs (control; lower left cluster) from soils with the addition of PA 1% (centre), followed by MPs 3% (LDPE and PA; clusters to the right). The vertical axis differentiates the control soil (lower left cluster) from soils with LDPE 1% and 3%

(upper right and left clusters) and PA 3% (lower right cluster) (Figure 3). The chemical and microbiological properties with the largest positive loadings on PC1 (NH_4^+, dehydrogenase, alkaline phosphatase and TOC) mostly influenced the scores of soils with LDPE and PA added at 3%. In contrast, variables with more negative loadings (AP, NO_3^-, Acid phosphatase, NO_2^-, HAs/FAs, EC, HAs, β-glucosidase, POL, THE and TP) mainly influenced the scores of the control and LDPE 1%. Likewise, chemical and microbiological properties with loadings with opposite signs (inverse correlation) in PC2 showed clear differences between soils with the addition of LDPE (3%) and PA (3%) (Figure 3).

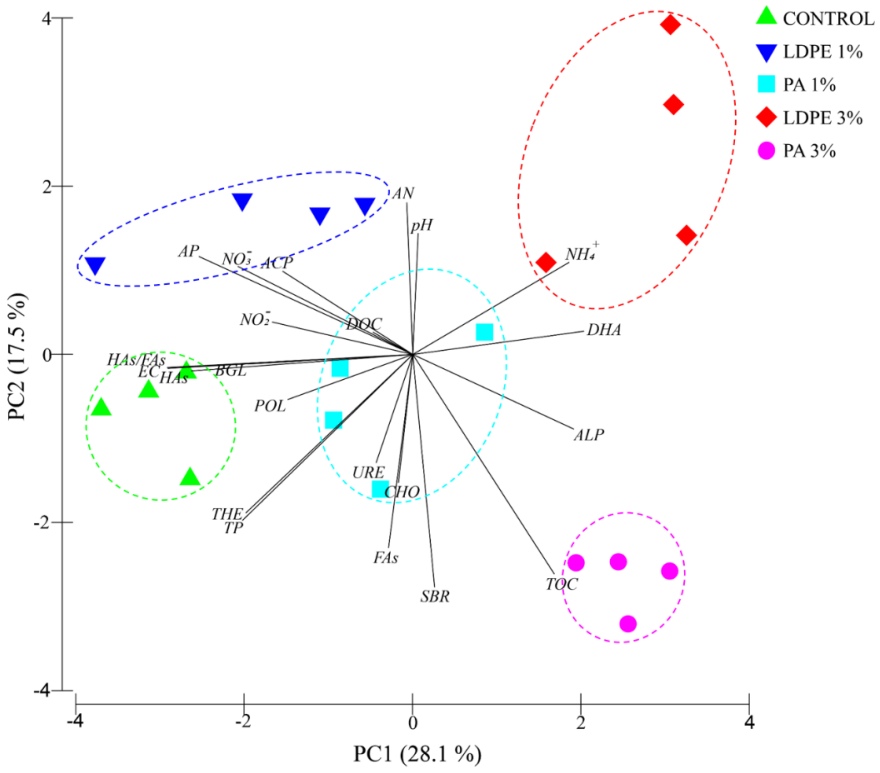


Figure 3 Ordination of principal component analysis of the different soil treatments and their chemical and microbiological properties. CONTROL: Soil without MPs addition; LDPE1%: Soil with 1% of LDPE-MPs added (w/w); PA1%: Soil with 1% of PA-MPs added (w/w); LDPE3%: Soil with 3% of LDPE-MPs added (w/w); PA3%: Soil with 3% of PA-MPs added (w/w). TOC: Total organic

carbon; AP: available phosphorus; TP: total phosphorus; THE: total humic extract; HAs: humic acids; FAs: fulvic acids; HAs/FAs: humification degree; CHO: carbohydrates; POL: polyphenols; BGL: β-glucosidase; ALP: Alkaline phosphatase; ACP: Acid phosphatase; URE: Urease; DHA: Dehydrogenase; DOC: Dissolved organic carbon; SBR: Soil basal respiration; NH_4^+: Ammonium; NO_2^-: Nitrite; NO_3^-: Nitrate; pH: pH; EC: electrical conductivity; AN: Available nitrogen.

Pearson correlations (*r*) analysis (Figure S3) showed different patterns between microbiological and chemical properties for the control soil, and soil modified with different types and doses of MPs. Soils with MPs presented a higher number of significant ($p < 0.05$) positive correlations than the control soils. However, these correlations were less strong in soils with MPs (Figure S3a,b). For example, both presented significant positive correlations ($p < 0.05$) of β-glucosidase with acid phosphatase activity ($r = 0.99$ in control soil, and $r = .57$ in soils with MPs), while other enzymatic activities showed different correlations; urease activity presented significant positive correlations with DOC in the control soil ($r = 0.98$) and with FAs ($r = 0.55$) in soil with MPs. TOC also showed significant positive correlations with SBR and alkaline phosphatase activity in soils amended with MPs as opposed to control soils where no significant correlations were found (Figure S3a,b). For soils amended with MPs, LPDE 1% showed significant positive correlations between alkaline phosphatase activity and HAs, and between that parameter and AN ($r = 0.98$ and 0.97, respectively). In addition, dehydrogenase activity had a strong correlation with SBR ($r = 0.95$) and acid phosphatase activity ($r = 0.96$) (Figure S3c). However, PA 1% did not show any significant correlation between microbiological and chemical properties (Figure S3d). The highest MPs dose (3%) favoured some of the correlations between microbiological and chemical properties. In particular, SBR increased with LPDE 3%, showing highly significant positive relationships with DOC, HAs, CHO and TP as opposed to control soils, where no significant correlations were found with SBR (Figure S3a,e). PA 3% showed highly significant positive correlations in HAs and FAs with alkaline ($r = 0.98$) and acid

phosphatase (r = 0.99), respectively. In addition, urease activity also showed significant positive correlations with FAs for both LPDE 3% and PA 3% (Figure S3e,f).

The DistLM analysis indicated that TOC, AP, TP, FAs and POL significantly influenced the microbiological properties of soil amended with MPs when chemical variables were analysed independently from microbiological properties (Table 2). On the contrary, THE, HAs, humification degree (HAs/FAs), CHO, DOC, NH_4^+, NO_2^-, NO_3^-, pH, EC and AN did not have a statistically significant (p < 0.05) effect on microbiological properties (Table 2). The best model for predicting changes in the microbiological activity of soil with MPs (R2 = 0.467, AICs = 16.026; Table 2) was composed of TOC and TP. Distance-based redundancy analysis (dbRDA) for soil microbiological properties showed that the percentage variation explained by dbRDA1 was 69.3% out of the fitted model and 14.4% out of the total variation, while the percentage of total variation explained by dbRDA2 was 30.7% out of the fitted model and 16.1% out of total variation (Figure 4). The dbRDA1 axis clearly discriminated the biological activity of each of the treatments and their different doses. The obtained results revealed that TOC and TP have a strong influence on the soil biological activity, especially in PA-amended soils. The model clearly separated the PA 3% groups from the cluster formed by the LDPE 1% and 3% treatments, which were shown to be in a position close to the control samples, while PA 1% was in an intermediate position between those treatments (Figure 4).

Table 2. DistLM analysis showing chemical independent variables that significantly influence soil microbiological properties with MPs incubation.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	*P*	Prop.
TOC	16.451	7.181	0.001	0.28518
AP	7.558	2.7138	0.027	0.13101
TP	9.609	3.5976	0.006	0.16657
THE	4.311	1.4536	0.234	7.47E-02
HAs	5.898	2.0497	0.111	0.10223
FAs	7.983	2.8909	0.031	0.13838
HAs/FAs	5.737	1.9878	0.107	9.95E-02
CHO	2.946	0.96884	0.457	5.11E-02
POL	8.584	3.1465	0.021	0.1488
DOC	3.385	1.122	0.369	5.87E-02
NH_4^+	6.109	2.1319	0.079	0.1059
NO_2^-	6.163	2.153	0.096	0.10683
NO_3^-	5.955	2.0718	0.096	0.10322
pH	1.558	0.49952	0.754	2.70E-02
EC	7.793	2.8114	0.036	0.13509
AN	4.472	1.5126	0.208	7.75E-02

SEQUENTIAL TESTS

Variable	AICc	SS (trace)	Pseudo-F	*P*	Prop.	Cumul.
TOC	19.178	16.451	7.181	0.001	0.28518	0.28518
TP	16.026	10.606	5.8862	0.001	0.18385	0.46902

Best solution

AICc	R^2	RSS	No.Vars	Selections
16.026	0.46902	30.631	2	1;3

Percentage of variation explained by individual axes

Axis	% explained variation out of fitted model		% explained variation out of total variation	
	Individual	Cumulative	Individual	Cumulative
1	69.31	69.31	32.51	32.51
2	30.69	100	14.39	46.9

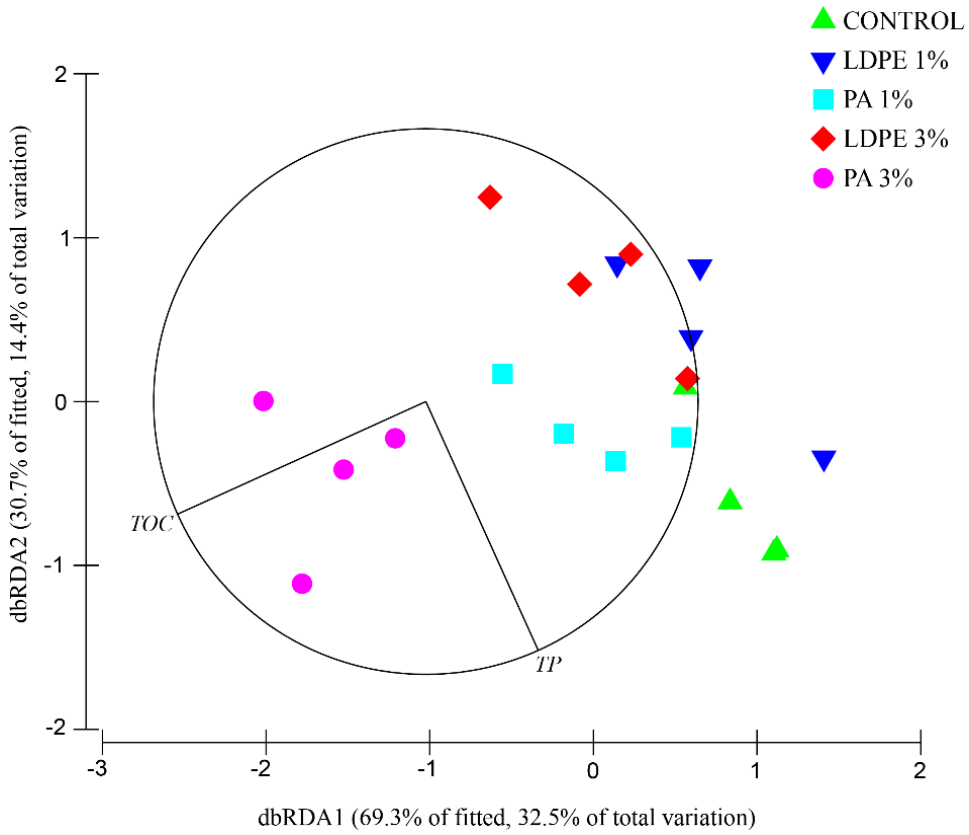


Figure 4 Distance-based Redundancy analysis (dbRDA) bi-plot of different soil treated with MPs and control soil. Footnotes: TOC: total organic carbon; TP: Total phosphorus.

4. DISCUSSION

4.1. Changes in soil chemical parameters driven by MPs

The results of this study showed that MPs can alter the chemical properties of an Andisol in a short period of exposure (6 weeks). LDPE addition increased soil pH by 1.1% compared to the control treatment, leading to a 15% increase in ion number (OH^-) (Fassbender et al., 2021). This agrees with Zhao et al. (2021), who showed that soil pH increased with PE foams on day 31. This phenomenon is attributed to the leaching of chemical compounds from MPs, which may alter the soil biota and affect pH levels (Zhao et al., 2021). Soil pH is considered to be the “master variable” of soil chemistry due to its impact on

numerous chemical reactions. Moreover, it has a strong influence on the selection of soil microbial communities because pH affects nutrient availability and may also impose physiological restrictions on the growth of soil bacterial communities (Miralles et al., 2021; Penn & Camberato, 2019). Regarding EC, levels decreased in MPs by 3% (LDPE and PA), which differs from the results of Qi et al. (2020), who incubated a sandy soil with LDPE of 1% for 4 months and reported an increase in EC values compared to the control (control = 103 μS cm^{-1}; 1%-LDPE = 179 μS cm^{-1}). It has been described that soil EC is proportional to the number of ions in the soil solution. Hence, it is important to identify which ions varied in the soil since ions, such as Na^{+}, and Cl^{-}, may also cause specific toxicity effects on plants, impair their nutritional balance, and/or decrease the permeability of soils, with further indirect deleterious effects on crops (Hossain et al., 2020; Visconti & de Paz, 2016). TOC content in the PA 1% and 3% treatments increased, but it decreased in LDPE 1%. This result agrees with Yu et al. (2020), who conducted a study on agricultural soil with PE addition. As PE contains about 90% C, MPs may influence soil carbon storage due to their degradation (Huang et al., 2021; Shi, Wang, et al., 2022). Huang et al. (2021) reported a 1.6% weight loss in PE films after 50 days of soil incubation, while degradation was confirmed by FTIR spectroscopy (Huang et al., 2021). Furthermore, PA has nitrogen in its composition and thus changes in soil N induce a variation of the carbon/nitrogen (C/N) ratio, which in turn affects soil microbial respiration (de Souza Machado et al., 2019; Li & Liu, 2022). Regarding DOC, levels decreased in the LDPE 1% and PA 3% treatments. This agrees with Yu et al. (2020), who found that DOC decreased in soil incubated with PE (28% w/w). However, Meng et al. (2022) and Ren et al. (2020) reported no changes in DOC in soils with LDPE after 30 and 105 days of incubation, respectively. Our findings differ from most other studies because we used a different incubation period, tested different doses of MPs, and used soils with different pH, and textures. Different types of soils have different responses to MPs pollution (Shi, Sun, et al., 2022; Shi, Wang, et al., 2022; Yi et al., 2021). MPs may alter microbial community structure and function, change the

biodegradation of litter and soil organic matter, trigger rapid depletion of soil nutrients and affect carbon pools such as DOC content, which microorganisms use as an immediate source of C (Liu et al., 2016; Zhang et al., 2022). Moreover, when MPs enter the soil, soil structure and the physical protection of soil aggregates on organic C are destroyed, resulting in a decrease in soil organic carbon (Yu et al., 2020). DOC is a key variable since it is the most active fraction of SOM and plays an important role in various biogeochemical processes (biodegradation and sorption) (Gao et al., 2021; Ren et al., 2020; Shi, Wang, et al., 2022).

POL content decreased in all the treatments with MPs. To the best of our knowledge, this is the first study evaluating polyphenols in the soil after MPs addition. Polyphenols play an important role in the soil by serving as substrates for microbial respiration or by selecting certain microbes (Schmidt et al., 2013). Phenolic compounds can directly affect the composition and activity of decomposer communities, thus influencing decomposition rates and nutrient cycling, especially nitrogen, due to binding with proteins, (Fan et al., 2017; Hattenschwiler & Vitousek, 2000). In the present study, MPs incubation did not statistically alter the carbohydrate content of the soil. This key finding agrees with molecular studies that have demonstrated that PE–MPs incubation (50 days) decreases carbohydrate metabolism compared to soil without MPs (Fei et al., 2020; Huang et al., 2019). Same as for polyphenol content determination, to our knowledge, this work is the first report on the effects of MPs on soil carbohydrate content. It should be noted, however, that the extraction method using cold water allowed quantifying monosaccharides, oligosaccharides and non-structural polysaccharides, which represent only a subset of total soil carbohydrates. Further research on the effect of MPs on soil carbohydrates is needed as they represent an important C and energy source for soil microorganisms (Gunina & Kuzyakov, 2015).

The effect of MPs on nitrogenous species showed that the ammonium concentration increased only with the addition of PA and LDPE at 3%. These results differ from other studies available in the literature. For instance, Shi, Sun, et al. (2022) reported that the addition of PA, PE or polyethylene terephthalate (PET) at a rate of 0.5% w/w did not produce significant effects on ammonium content. On the contrary, Liu et al. (2017) indicated that an increase in ammonium content was evidenced with PP addition only at 7% w/w, but not at higher doses of 24.1% w/w. Meng et al. (2022) reported that a 46-day incubation period for LDPE–MPs added at doses of 0.5%, 1.5% and 2.5% did not result in any statistically significant changes compared to the control. However, ammonium content increased with and addition of 1%, but it decreased at a 2% dose, suggesting that the response of soil ammonium content to LDPE addition is ambiguous. In turn, these results indicate that further studies are required to fully understand the effect of LDPE–MPs on this parameter. Regarding PA addition, there is evidence that low doses (0.5% or 1%) do not seem to produce any effects on ammonium content, but it is affected by a higher dose of 3%. PA contains nitrogen in its chemical composition, and this could potentially lead to an increase in ammonia levels (de Souza Machado et al., 2019; Li & Liu, 2022).

In the present study, LDPE showed no effects on the nitrate or nitrite content of the soil. However, PA 3% resulted in a decrease in both parameters. Likewise, Yan et al. (2021) reported lower nitrate contents in paddy soil amended with 1% PVC–MPs incubated for 35 days, with decreases ranging from 10% to 13% with respect to the control. However, another study showed that LDPE and PA-MPs additions did not significantly alter soil nitrate between 7 and 105 days of incubation (Liu et al., 2017; Meng et al., 2022; Shi, Sun, et al., 2022). A decrease in nitrate and nitrite contents could be attributed to the effect of PA addition, which increased microbial activity (SBR) and favoured organic C accumulation. A possible alternative explanation is that an increase in C-substrates allows heterotrophic microbial growth (NH_4^+ immobilization) to dominate over autotrophic growth (NH_4^+ oxidation), thus inhibiting NO_3^-

accumulation (Liu et al., 2017). Moreover, different incubation temperatures may have different responses to MPs addition. In addition, Shi, Wang, et al. (2022) reported a significant impact on soil nitrogen transformation and availability at 25°C, while there were no significant difference in NO_3^-, NH_4^+, mineral nitrogen, net cumulative N ammonification, net cumulative N nitrification or net cumulative N mineralization at 15°C.

The decrease in total P in the soils with LDPE 3%, has also been evidenced in recent studies with PVC–, PS– and LDPE–MPs (Yu et al., 2020, 2021). Yu et al. (2020) reported a decrease of 30% with LDPE–MPs at 28% w/w. However, this behaviour was observed only in non-aggregated silt and clay fractions (particles <53 μm) and did not change in the larger micro-aggregate fractions (>53 μm). This indicates that aggregate-size fractions respond differently to MPs exposure. MPs can reduce soil bulk density and soil micropores, which can hinder soil ventilation and thus prevent phosphorus release (Chia et al., 2022; de Souza Machado et al., 2019). With respect to available P, levels decreased with PA 1% and 3% (w/w), as well as with LDPE 3%. This agrees with Yu et al. (2020), who reported that LDPE significantly decreased Olsen P by 43%, 38% and 29% in three tested soil aggregates. However, this outcome may be influenced by the type of soil evaluated. In this sense, Yan et al. (2021) demonstrated that the addition of PVC 1% reduced the available P content in a paddy soil, but it did not have any significant impact on red soil. In our study, the reduction in the amount of available P may be explained by lower effective solubilization or mineralization. The behaviour of inorganic P is influenced by the equilibrium between the mineralization of organic material and its immobilization by chemoautotrophic bacteria (Liu et al., 2017; Yan et al., 2021). Moreover, as microbes consume more nutrients such as carbon and phosphorus from the surrounding environment, the availability of these nutrients decreases. Additionally, when there is a rise in soil C limitation, it typically leads to an increase in P limitation (Zhang et al., 2022). The various

responses in AP content indicate that soil type is a critical factor when studying the effects of MPs.

During incubation with PA and LDPE 3%, the humic fractions were reduced, except for FAs, which increased in the PA 3% treatment. Furthermore, the analysis of humification degree (HAs/FAs) revealed a decrease in treatments containing MPs by 3%. This suggests that a higher proportion of FAs was present in comparison to HAs. Our results provide additional information to the limited information published on the effects of MPs on soil humic fractions. The decrease observed in LDPE 3% could be negative for the soil since soil humic substances contribute to improving the structure and composition of OM (Soria et al., 2022). An increase in FAs would be given by the ability of PA to absorb it (Tang et al., 2021). A previous study in PVC by Liu et al. (2017) demonstrated that high levels of PVC–MPs (28% w/w) facilitated the accumulation of fulvic acids, indicating a positive correlation between them.

4.2. Changes in soil microbiological activity driven by MPs

It has been proven that MPs can affect the microbiological activity of soils (Liu et al., 2017; Yan et al., 2021; Yu et al., 2020). The present study revealed that MPs addition altered some enzymatic activities and SBR in the short term. First, PA 3% promoted alkaline phosphatase activity, being partially consistent with Yi et al. (2021), who reported an increase in alkaline phosphatase activity in soil with fibrous PP and a decrease with microsphere PP on day 29 compared to the control soil. In the case of acid phosphatase activity, lower values were observed in the PA 1% treatment compared to the control. This finding agrees with Zhao et al. (2021), who also reported a decrease in acid phosphatase activity with PET–MPs at a concentration of 0.4% w/w from day 3 of incubation. Conversely, Pinto-Poblete et al. (2022, 2023) and Fei et al. (2020) evidenced an increase in acid phosphatase activity with HDPE–, LDPE– and PVC–MPs incubations. It is important to consider that phosphatase activity can also vary depending on the incubation period. In a study conducted by Ya et al. (2022), an

increase in phosphatase activity was observed on day 7 of soil incubation with LDPE 1%. However, when analysing its activity on day 35, a decrease in phosphatase activity was observed compared to the control treatment. These differences highlight the need for further research to determine the impact of MPs on phosphatase activities. Phosphatase enzymes catalyse the hydrolysis of phosphate mono and diesters in the soil, converting organic P into inorganic P. This inorganic form is a fundamental element for plants, microorganisms and ecosystem functions (Wang et al., 2022).

Urease and dehydrogenase enzymatic activities were not affected by MPs addition. This agrees with Pinto-Poblete et al. (2022, 2023), who also studied MPs incubations in an Andisol and reported that these parameters were not sensitive to MPs contamination. However, other studies have described that changes in the activities of both enzymes were observed after a short period of incubation with additions of PVC, LDPE and PP in loamy and sandy soils (Fei et al., 2020; Yi et al., 2021). Allophane, which is a predominant clay in Andisols, and its ability to immobilize enzymes (Calabi Floody et al., 2009) probably inhibit urease and dehydrogenase after a short period of soil incubation in the presence of MPs. However, more studies are needed to confirm this.

MPs addition decreased β-glucosidase enzyme activity, which agrees with the results reported by Yu et al. (2020). β-glucosidase is involved in the degradation of cellulose in soils and regulates the supply of an important energy source for microorganisms (Turner et al., 2002). This impact could directly influence soil microbial communities and their activity. The effects of MPs on soil enzyme activities show great variability, probably due to the heterogeneous chemical, physical and biological nature of soils. In fact, even different aggregate fractions have different responses to some enzymatic activities in the presence of MPs (Yu et al., 2020). Microbes produce extracellular enzymes that degrade soil organic matter through hydrolytic or oxidative processes. These enzymes have high-catalytic capacities and play a critical role in controlling the

cycling of soil nutrients (C, N and P). Therefore, they have the potential to serve as indicators of soil quality (Huang et al., 2019; Yan et al., 2021; Yu et al., 2020). Yu et al. (2020) concluded that the effects of MPs on soil enzymatic activities include a combination of direct (i.e., through changes in enzyme substrates and physicochemical niches) and indirect (i.e., through changes in physical and chemical soil properties). Furthermore, Huang et al. (2019) concluded that these indirect effects may alter soil microbial communities, which are the main producers of enzymes in the soil (de Souza Machado, Lau, et al., 2018; Xu et al., 2020; Zhang & Liu, 2018).

LDPE decreased SBR, probably because MPs compete with soil microorganisms for physicochemical niches to reduce microbial activity (Yu et al., 2020), or by the reduction of labile substrates (Zhao et al., 2021). However, PA 3% resulted in an increased SBR. This can be explained by the fact that the N and C in PA serve as nutrients that increase microbial activity (de Souza Machado et al., 2019; Shi, Wang, et al., 2022). Pinto-Poblete et al. (2022, 2023) found no changes in SBR when they amended an Andisol with a lower dose of HDPE–MPs (0.2 g MPs kg^{-1} soil). Similarly, Shi, Sun, et al. (2022) reported no differences using PA and LDPE at a dose of 0.5%, probably due to the low dose of MPs. Ren et al. (2020) showed an increase in CO2 from the respiration of soil microorganisms with LDPE added at 5%, but this soil also had N fertilization (150 kg N ha^{-1}). Based on these findings, we suggest that the presence of MPs in environmentally significant amounts, along with an external source of nitrogen, may elevate SBR, thus increasing the production of CO_2. However, further studies are required to validate this hypothesis.

4.3. Effect of MPs on the interrelationships of soil chemical and microbiological properties

The clear separation of the eigenvectors in the treatments (Figure 3) shows that MPs have varying effects on an Andisol depending on the dose and polymer used as described in previous studies (Shi, Sun, et al., 2022; Shi,

Wang, et al., 2022; Yan et al., 2021; Yi et al., 2021; Yu et al., 2020). Soil properties such as NH_4^+, dehydrogenase, alkaline phosphatase and TOC mostly influenced soil scores with both LPDE and PA 3% (Figure 3). The influence of MPs on TOC is also evidenced in Figure S3b, which shows a positive correlation between SBR (CO2 emissions) and TOC. Other authors have also found a correlation between TOC, CO2 emissions and SBR, indicating that TOC plays a crucial role in the dynamics of CO2 fluxes and SBR (Soria, Ortega, et al., 2021; Soria, Rodriguez-Berbel, et al., 2021). Similarly, a positive correlation between TOC and alkaline phosphatase activity was observed by Soria, Ortega, et al. (2021). Alkaline phosphatase activity may promote soil fertility and has a critical role in releasing soil inorganic P (Li et al., 2021). In our study, LDPE 1% and 3% showed a positive correlation between SBR and dehydrogenase activity, DOC, HAs and CHO (Figure S3c,e). As previously indicated, PE has approximately 90% of C in its composition, and thus it can change carbon input, carbon dioxide release and carbon conversion processes (Rillig et al., 2021; Wang et al., 2022).

The results of the DistLM analysis, which modelled the behaviour of microbiological properties in the soils incubated with MPs, suggest that the most influential parameters on enzymatic activities and SBR were TOC followed by TP (Table 2; Figure 4). Even though MPs are a rich-carbon source, in most cases, they correspond to a tiny proportion of the total soil organic matter carbon, while C degradation by certain microbial groups, such as actinobacteria, directly and/or indirectly influence microbiological properties (Guo et al., 2021; Rillig et al., 2021; Shi, Wang, et al., 2022). MPs may increase soil C storage when they accumulate in soils, which is the most accessible substrate for microbes, thus influencing soil microbial processes (Guo et al., 2021; Rillig et al., 2021; Shi, Wang, et al., 2022). MPs can alter microbiota and in turn the structure of microbial communities, triggering rapid changes in soil nutrients and carbon pools. These changes alter biogeochemical cycles, such as the soil carbon cycle (Rillig et al., 2021; Zhang et al., 2022). In addition, CHO and POL are C organic sources that soil microbial communities use as a source of energy, producing

more enzymes to mineralize organic matter and affecting SBR (CO2) (Abaecherli & Popa, 2005; Miralles et al., 2021). According to the DistLM analysis, TP influenced microbiological properties, such as enzyme activities, and SBR. This is consistent with a previous study with PS and PVC–MPs, where redundancy analysis (RDA) showed that total P content is an important factor that models bacterial communities and could influence microbiological properties because it is an element used for the synthesis of many biomolecules like nucleic acids, phospholipids and ATP (Li et al., 2021; Tapia-Torres et al., 2016; Yu et al., 2021).

5. CONCLUSIONS

The impact of MPs pollution in terrestrial ecosystems is of great concern, leading to increased research in this field. In this study, we evaluated the effects of different MPs (LPDE and PA) at 1% and 3% doses on the chemical and microbiological properties of an Andisol based on a 6-week incubation of microcosms. Our findings suggest that the effects of LPDE and PA addition (doses of 1% and 3%) on the soil chemical properties of an Andisol vary depending on the type and dose of MPs. Overall, MPs modified soil chemical parameters (pH and EC), nutrient availability (TOC, DOC, NH_4^+, NO3−, NO_2^-, TP, AP), humic substances (HAs and FAs), and significantly altered soil enzymatic activities, decreasing β-glucosidase and acid phosphatase, but increasing alkaline phosphatase activity. Changes in soil microbiological properties were observed due to MPs pollution as evidenced by the variation in TP and TOC contents. Knowledge of the impact of MPs on the nutrient cycling, quality and functionality of soils can help improve management for preventing MPs contamination. However, more research is needed to evaluate the short- and longterm impact of MPs contamination and determine if the methodology used herein can be applied to different types of plastic polymers on other orders of soils from different environments.

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Author Contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by Gustavo Riveros with the help of Ana Villafuerte and Paloma Gomez. Data analyses were performed by Gustavo Riveros and Rocio Soria. The first draft of the manuscript was written by Gustavo Riveros. Review and Editing were performed by Rocio Soria, Isabel Miralles, Mauricio Schoebitz and Raul Ortega. All authors read and approved the final manuscript.

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REFERENCES

Abaecherli, A., Popa, V.I., 2005. Lignin in crop cultivations and bioremediation. Environ Eng Manag J 4, 273–292. https://doi.org/http://dx.doi.org/10.30638/eemj.2005.030

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans Automat Contr 19, 716–723. https://doi.org/10.1109/TAC.1974.1100705

Anderson, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences 58, 626–639. https://doi.org/10.1139/cjfas-58-3-626

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Brink, R.H., Dubach, P., Lynch, D.L., 1960. Measurement of carbohydrates in soil hydrolyzates with anthrone. Soil Sci 89, 157–166. https://doi.org/10.1097/00010694-196003000-00006

Calabi Floody, M., Theng, B.K.G., Reyes, P., Mora, M.L., 2009. Natural nanoclays: applications and future trends – a Chilean perspective. Clay Miner 44, 161–176. https://doi.org/10.1180/claymin.2009.044.2.161

Chia, R.W., Lee, J.Y., Jang, J., Kim, H., Kwon, K.D., 2022. Soil health and microplastics: a review of the impacts of microplastic contamination on soil properties. J Soils Sediments 22, 2690–2705. https://doi.org/10.1007/s11368-022-03254-4

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Glob Chang Biol 24, 1405–1416. https://doi.org/10.1111/gcb.14020

de Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S., Rillig, M.C., 2019. Microplastics Can Change Soil Properties and Affect Plant Performance. Environ Sci Technol 53, 6044–6052. https://doi.org/10.1021/acs.est.9b01339

de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M.C., 2018. Impacts of Microplastics on the Soil Biophysical Environment. Environ Sci Technol 52, 9656–9665. https://doi.org/10.1021/acs.est.8b02212

Dorado, J., Zancada, M.-C., Almendros, G., López-Fando, C., 2003. Changes in soil properties and humic substances after long-term amendments with manure and crop residues in dryland farming systems. Journal of plant nutrition and soil science 166, 31–38. https://doi.org/10.1002/jpln.200390009

Duchafour, P., Jacquin, F., 1975. Comparaison des processus d'humification dans ies principaux types d'humus forestiers. Bullerin de l'dssociafion Fran~aise pour l'Etuak du Sol 1, 29–36.

Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ Sci Eur 28, 1–25. https://doi.org/10.1186/s12302-015-0069-y

Eivazi', F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol Biochem 20, 601–606. https://doi.org/10.1016/0038-0717(88)90141-1

Eldridge, D.J., Delgado-Baquerizo, M., Woodhouse, J.N., Neilan, B.A., 2016. Mammalian engineers drive soil microbial communities and ecosystem functions across a disturbance gradient. Journal of Animal Ecology 85, 1636–1646. https://doi.org/10.1111/1365-2656.12574

Fan, D. mei, Fan, K., Yu, C. ping, Lu, Y. ting, Wang, X. chang, 2017. Tea polyphenols dominate the short-term tea (Camellia sinensis) leaf litter decomposition. J Zhejiang Univ Sci B 18, 99–108. https://doi.org/10.1631/jzus.B1600044

Fassbender, A., C. Orr, J., G. Dickson, A., 2021. Technical note: Interpreting pH changes. Biogeosciences 18, 1407–1415. https://doi.org/10.5194/bg-18-1407-2021

Fei, Y., Huang, S., Zhang, H., Tong, Y., Wen, D., Xia, X., Wang, H., Luo, Y., Barceló, D., 2020. Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil. Science of the Total Environment 707. https://doi.org/10.1016/j.scitotenv.2019.135634

Fuller, S., Gautam, A., 2016. A Procedure for Measuring Microplastics using Pressurized Fluid Extraction. Environ Sci Technol 50, 5774–5780. https://doi.org/10.1021/acs.est.6b00816

Gao, M., Liu, Y., Dong, Y., Song, Z., 2021. Effect of polyethylene particles on dibutyl phthalate toxicity in lettuce (Lactuca sativa L.). J Hazard Mater 401, 1–11. https://doi.org/10.1016/j.jhazmat.2020.123422

García, C., Hernandez, T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun Soil Sci Plant Anal 28, 123–134. https://doi.org/10.1080/00103629709369777

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

Guo, Q.Q., Xiao, M.R., Ma, Y., Niu, H., Zhang, G.S., 2021. Polyester microfiber and natural organic matter impact microbial communities, carbon-degraded enzymes, and carbon accumulation in a clayey soil. J Hazard Mater 405. https://doi.org/10.1016/j.jhazmat.2020.124701

Hättenschwiler, S., Vitousek, P.M., 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol Evol 15, 238–243. https://doi.org/10.1016/S0169-5347(00)01861-9

Hossain, M.S., Rahman, G.K.M.M., Solaiman, A.R.M., Alam, M.S., Rahman, M.M., Mia, M.A.B., 2020. Estimating Electrical Conductivity for Soil Salinity Monitoring Using Various Soil-Water Ratios Depending on Soil Texture. Commun Soil Sci Plant Anal 51, 635–644. https://doi.org/10.1080/00103624.2020.1729378

Huang, D., Xu, Y., Lei, F., Yu, X., Ouyang, Z., Chen, Y., Jia, H., Guo, X., 2021. Degradation of polyethylene plastic in soil and effects on microbial community composition. J Hazard Mater 416, 1–9. https://doi.org/10.1016/j.jhazmat.2021.126173

Huang, Y., Zhao, Y., Wang, J., Zhang, M., Jia, W., Qin, X., 2019. LDPE microplastic films alter microbial community composition and enzymatic

activities in soil. Environmental Pollution 254, 1–10. https://doi.org/10.1016/j.envpol.2019.112983

Jorquera, A., Castillo, C., Murillo, V., Araya, J., Pinochet, J., Narváez, D., Pantoja-Gutiérrez, S., Urbina, M.A., 2022. Physical and anthropogenic drivers shaping the spatial distribution of microplastics in the marine sediments of Chilean fjords. Science of the Total Environment 814, 1–11. https://doi.org/10.1016/j.scitotenv.2021.152506

Li, H., Liu, L., 2022. Short-term effects of polyethene and polypropylene microplastics on soil phosphorus and nitrogen availability. Chemosphere 291, 1–9. https://doi.org/10.1016/j.chemosphere.2021.132984

Li, J., Xie, T., Zhu, H., Zhou, J., Li, C., Xiong, W., Xu, L., Wu, Y., He, Z., Li, X., 2021. Alkaline phosphatase activity mediates soil organic phosphorus mineralization in a subalpine forest ecosystem. Geoderma 404. https://doi.org/10.1016/j.geoderma.2021.115376

Liu, M., Ussiri, D.A.N., Lal, R., 2016. Soil Organic Carbon and Nitrogen Fractions under Different Land Uses and Tillage Practices. Commun Soil Sci Plant Anal 47, 1528–1541. https://doi.org/10.1080/00103624.2016.1194993

Liu, H., Yang, X., Liu, G., Liang, C., Xue, S., Chen, H., Ritsema, C.J., Geissen, V., 2017. Response of soil dissolved organic matter to microplastic addition in Chinese loess soil. Chemosphere 185, 907–917. https://doi.org/10.1016/j.chemosphere.2017.07.064

Meng, F., Yang, X., Riksen, M., Geissen, V., 2022. Effect of different polymers of microplastics on soil organic carbon and nitrogen – A mesocosm

experiment. Environ Res 204, 1–10. https://doi.org/10.1016/j.envres.2021.111938

Mingorance, M.D., Barahona, E., Fernández-Gálvez, J., 2007. Guidelines for improving organic carbon recovery by the wet oxidation method. Chemosphere 68, 409–413. https://doi.org/10.1016/j.chemosphere.2007.01.021

Miralles, I., Trasar-Cepeda, C., Soria, R., Ortega, R., Lucas-Borja, M.E., 2021. Environmental and ecological factors influencing soil functionality of biologically crusted soils by different lichen species in drylands. Science of the Total Environment 794, 1–14. https://doi.org/10.1016/j.scitotenv.2021.148491

Nannipieri, P., Ceccanti, B., Cervelli, S., Matarese, E., 1980. Extraction of Phosphatase, Urease, Proteases, Organic Carbon, and Nitrogen from Soil. Soil Microbiology and Biochemistry 44, 1011–1916. https://doi.org/10.2136/sssaj1980.03615995004400050028x

Nannipieri, P., Pedrazzini, F., Arcara, P.G., Piovanelli, C., 1979. Changes in aminoacids, enzyme activities, and biomasses during soil microbial growth. Soil Sci 127, 26–34.

Neculman, R., Rumpel, C., Matus, F., Godoy, R., Steffens, M., de la Luz Mora, M., 2013. Organic matter stabilization in two Andisols of contrasting age under temperate rain forest. Biol Fertil Soils 49, 681–689. https://doi.org/10.1007/s00374-012-0758-2

Nizzetto, L., Futter, M., Langaas, S., 2016. Are Agricultural Soils Dumps for Microplastics of Urban Origin? Environ Sci Technol. https://doi.org/10.1021/acs.est.6b04140

Olsen, S., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium bicarbonate, 939th ed. US Department of Agriculture.

Pascal, R. G. (1968). Les Composés phénoliques des végétaux. Dunod.

Penn, C.J., Camberato, J.J., 2019. A critical review on soil chemical processes that control how soil ph affects phosphorus availability to plants. Agriculture (Switzerland) 9, 1–18. https://doi.org/10.3390/agriculture9060120

Pinto-Poblete, A., Retamal-Salgado, J., López, M.D., Zapata, N., Sierra-Almeida, A., Schoebitz, M., 2022. Combined Effect of Microplastics and Cd Alters the Enzymatic Activity of Soil and the Productivity of Strawberry Plants. Plants 11, 1–15. https://doi.org/10.3390/plants11040536

Pinto-Poblete, A., Retamal-Salgado, J., Zapata, N., Sierra-Almeida, A., Schoebitz, M., 2023. Impact of polyethylene microplastics and copper nanoparticles: Responses of soil microbiological properties and strawberry growth. Applied Soil Ecology 184, 104773. https://doi.org/10.1016/j.apsoil.2022.104773

Poblete-Grant, P., Suazo-Hernández, J., Condron, L., Rumpel, C., Demanet, R., Malone, S.L., Mora, M. de L.L., 2020. Soil available P, soil organic carbon and aggregation as affected by long-term poultry manure application to Andisols under pastures in Southern Chile. Geoderma Regional 21, 1–10. https://doi.org/10.1016/j.geodrs.2020.e00271

Qi, Y., Ossowicki, A., Yang, X., Huerta Lwanga, E., Dini-Andreote, F., Geissen, V., Garbeva, P., 2020. Effects of plastic mulch film residues on wheat rhizosphere and soil properties. J Hazard Mater 387, 1–7. https://doi.org/10.1016/j.jhazmat.2019.121711

Redel, Y., Cartes, P., Demanet, R., Velásquez, G., Poblete-Grant, P., Bol, R., Mora, M.L., 2016. Assessment of phosphorus status influenced by Al and Fe compounds in volcanic grassland soils. J Soil Sci Plant Nutr 16, 490–506. https://doi.org/10.4067/S0718-95162016005000041

Ren, X., Tang, J., Liu, X., Liu, Q., 2020. Effects of microplastics on greenhouse gas emissions and the microbial community in fertilized soil. Environmental Pollution 256, 1–11. https://doi.org/10.1016/j.envpol.2019.113347

Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? Environ Sci Technol 46, 6453–6454. https://doi.org/10.1021/es302011r

Rillig, M.C., De Souza Machado, A.A., Lehmann, A., Klümper, U., 2019. Evolutionary implications of microplastics for soil biota. Environmental Chemistry 16, 3–7. https://doi.org/10.1071/EN18118

Rillig, M.C., Leifheit, E., Lehmann, J., 2021. Microplastic effects on carbon cycling processes in soils. PLoS Biol. https://doi.org/10.1371/JOURNAL.PBIO.3001130

Riveros, G., Urrutia, H., Araya, J., Zagal, E., Schoebitz, M., 2022. Microplastic pollution on the soil and its consequences on the nitrogen cycle: a review. Environmental Science and Pollution Research. https://doi.org/10.1007/s11356-021-17681-2

Rocha-Santos, T.A.P., Duarte, A.C., 2017. Characterization and Analysis of Microplastics. Elsevier.

Scheurer, M., Bigalke, M., 2018. Microplastics in Swiss Floodplain Soils. Environ Sci Technol 52, 3591–3598. https://doi.org/10.1021/acs.est.7b06003

Schmidt, M.A., Kreinberg, A.J., Gonzalez, J.M., Halvorson, J.J., French, E., Bollmann, A., Hagerman, A.E., 2013. Soil microbial communities respond differently to three chemically defined polyphenols. Plant Physiology and Biochemistry 72, 190–197. https://doi.org/10.1016/j.plaphy.2013.03.003

Shi, J., Sun, Y., Wang, X., Wang, J., 2022a. Microplastics reduce soil microbial network complexity and ecological deterministic selection. Environ Microbiol 24, 2157–2169. https://doi.org/10.1111/1462-2920.15955

Shi, J., Wang, J., Lv, J., Wang, Z., Peng, Y., Wang, X., 2022b. Microplastic presence significantly alters soil nitrogen transformation and decreases nitrogen bioavailability under contrasting temperatures. J Environ Manage 317, 1–12. https://doi.org/10.1016/j.jenvman.2022.115473

Soria, R., González-Pérez, J.A., de la Rosa, J.M., San Emeterio, L.M., Domene, M.A., Ortega, R., Miralles, I., 2022. Effects of technosols based on organic amendments addition for the recovery of the functionality of degraded quarry soils under semiarid Mediterranean climate: A field study. Science of the Total Environment 816, 1–13. https://doi.org/10.1016/j.scitotenv.2021.151572

Soria, Rocío, Ortega, R., Bastida, F., Miralles, I., 2021a. Role of organic amendment application on soil quality, functionality and greenhouse emission in a limestone quarry from semiarid ecosystems. Applied Soil Ecology 164, 1–13. https://doi.org/10.1016/j.apsoil.2021.103925

Soria, R., Rodríguez-Berbel, N., Ortega, R., Lucas-Borja, M.E., Miralles, I., 2021b. Soil amendments from recycled waste differently affect CO_2 soil emissions in restored mining soils under semiarid conditions. J Environ Manage 294, 1–14. https://doi.org/10.1016/j.jenvman.2021.112894

Staff, S.S., 2006. Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys; US Government Printing Office: Washington, DC, USA

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1

Tang, S., Lin, L., Wang, X., Sun, X., Yu, A., 2021. Adsorption of fulvic acid onto polyamide 6 microplastics: Influencing factors, kinetics modeling, site energy distribution and interaction mechanisms. Chemosphere 272, 1–11. https://doi.org/10.1016/j.chemosphere.2021.129638

Tapia-Torres, Y., Rodríguez-Torres, M.D., Elser, J.J., Islas, A., Souza, V., García-Oliva, F., Olmedo-Álvarez, G., 2016. How to live with phosphorus scarcity in soil and sediment: Lessons from bacteria. Appl Environ Microbiol 82, 4652–4662. https://doi.org/10.1128/AEM.00160-16

Thompson, R.C., Olson, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science (1979) 304, 838. https://doi.org/10.1126/science.1094559

Turner, B.L., Hopkins, D.W., Haygarth, P.M., Ostle, N., 2002. Glucosidase activity in pasture soils. Applied Soil Ecology 20, 152–157. https://doi.org/10.1016/S0929-1393(02)00020-3

USDA, 2010. Keys to Soil Taxonomy. A basic system of soil classification for making and interpreting soil surveys. USDA.

Visconti, F., de Paz, J.M., 2016. Electrical Conductivity Measurements in Agriculture: The Assessment of Soil Salinity. New Trends and Developments in Metrology 99–126. https://doi.org/10.5772/62741

Walkley, A., Black, I., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37, 29–38. https://doi.org/10.1097/00010694-193401000-00003

Wang, J., Huang, M., Wang, Q., Sun, Y., Zhao, Y., Huang, Y., 2020. LDPE microplastics significantly alter the temporal turnover of soil microbial communities. Science of the Total Environment 726, 1–8. https://doi.org/10.1016/j.scitotenv.2020.138682

Wang, X., Xing, Y., Lv, M., Zhang, T., Ya, H., Jiang, B., 2022. Recent advances on the effects of microplastics on elements cycling in the environment. Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2022.157884

Xu, B., Liu, F., Cryder, Z., Huang, D., Lu, Zhijiang, He, Y., Wang, H., Lu, Zhenmei, Brookes, P.C., Tang, C., Gan, J., Xu, J., 2020. Microplastics in the soil environment: Occurrence, risks, interactions and fate–A review. Crit Rev Environ Sci Technol 50, 2175–2222. https://doi.org/10.1080/10643389.2019.1694822

Ya, H., Xing, Y., Zhang, T., Lv, M., Jiang, B., 2022. LDPE microplastics affect soil microbial community and form a unique plastisphere on microplastics. Applied Soil Ecology, 180, 104623. https://doi.org/10.1016/j.apsoil.2022.104623

Yan, Y., Chen, Z., Zhu, F., Zhu, C., Wang, C., Gu, C., 2021. Effect of Polyvinyl Chloride Microplastics on Bacterial Community and Nutrient Status in Two Agricultural Soils. Bull Environ Contam Toxicol 107, 602–609. https://doi.org/10.1007/s00128-020-02900-2

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

Yu, H., Fan, P., Hou, J., Dang, Q., Cui, D., Xi, B., Tan, W., 2020. Inhibitory effect of microplastics on soil extracellular enzymatic activities by changing soil properties and direct adsorption: An investigation at the aggregate-fraction level. Environmental Pollution 267, 1–11. https://doi.org/10.1016/j.envpol.2020.115544

Yu, H., Qi, W., Cao, X., Hu, J., Li, Y., Peng, J., Hu, C., Qu, J., 2021. Microplastic residues in wetland ecosystems: Do they truly threaten the plant-microbe-soil system? Environ Int 156, 1–12. https://doi.org/10.1016/j.envint.2021.106708

Zagal, E., Rodríguez, N., Vidal, I., Quezada, L., 2002. Actividad microbiana en un suelo de origen volcanico bajo distinto manejo agronómico. Agricultura Técnica 62, 297–309. https://doi.org/10.4067/S0365-28072002000200012

Zhang, G.S., Liu, Y.F., 2018. The distribution of microplastics in soil aggregate fractions in southwestern China. Science of the Total Environment 642, 12–20. https://doi.org/10.1016/j.scitotenv.2018.06.004

Zhang, Z., Peng, W., Duan, C., Zhu, X., Wu, H., Zhang, X., Fang, L., 2022. Microplastics pollution from different plastic mulching years accentuate soil

microbial nutrient limitations. Gondwana Research 108, 91–101. https://doi.org/10.1016/j.gr.2021.07.028

Zhao, T., Lozano, Y.M., Rillig, M.C., 2021. Microplastics Increase Soil pH and Decrease Microbial Activities as a Function of Microplastic Shape, Polymer Type, and Exposure Time. Front Environ Sci 9, 1–14. https://doi.org/10.3389/fenvs.2021.675803

SUPPLEMENTARY INFORMATION

Effects of low-density polyethylene and polyamide microplastics on the microbiological and chemical characteristics of an Andisol

Soil Use and Management

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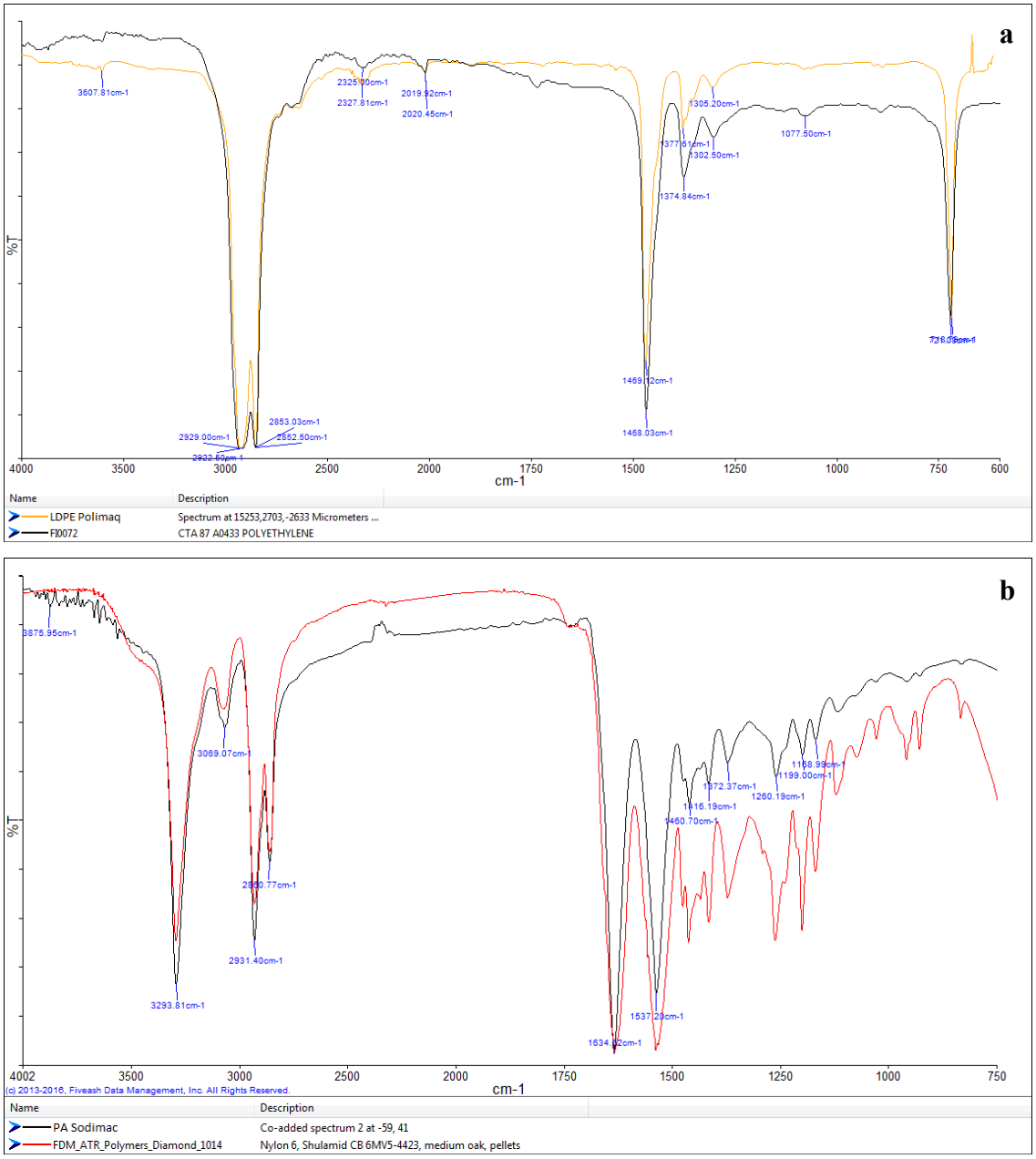

Figure S1. FTIR spectra of plastics used in the soil-MPs incubation and comparison with spectra from the equipment library. (a) LDPE; (b) PA.

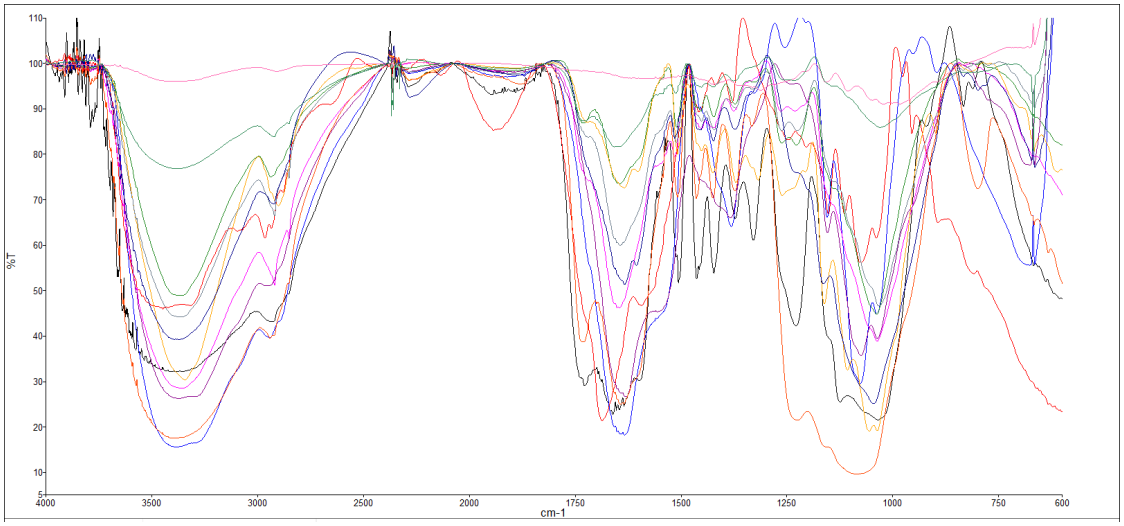

Figure S2. FTIR spectra of isolated particles from soil with lignocellulosic composition

a

	EC	pH	TOC	DOC	THE	HAs	FAs	HAs/FAs	CHO	POL	NH4+	NO2-	NO3-	AN	TP	AP	SBR	DHO	BGL	ACP	ALP
pH	-0.40																				
TOC	**-0.96***	0.59																			
DOC	-0.90	0.53	0.84																		
THE	0.72	0.32	-0.50	-0.60																	
HAs	0.92	-0.68	-0.93	**-0.97***	0.47																
FAs	-0.26	**0.99***	0.48	0.42	0.45	-0.58															
HAs/FAs	0.83	0.18	-0.66	-0.66	**0.97***	0.58	0.31														
CHO	-0.48	-0.61	0.25	0.28	-0.93	-0.15	-0.72	-0.89													
POL	0.26	-0.95	-0.51	-0.29	-0.49	0.50	**-0.96***	-0.31	0.70												
NH4+	0.88	-0.21	-0.88	-0.58	0.63	0.66	-0.09	0.79	-0.53	0.22											
NO2-	-0.49	**0.96***	0.63	0.71	0.14	-0.79	0.93	0.03	-0.48	-0.82	-0.19										
NO3-	-0.73	-0.23	0.63	0.40	-0.82	-0.40	-0.35	-0.90	0.83	0.23	-0.90	-0.20									
AN	0.88	-0.36	-0.92	-0.60	0.51	0.71	-0.25	0.69	-0.38	0.38	**0.99***	-0.33	-0.81								
TP	**-0.98***	0.25	0.89	0.89	-0.83	-0.87	0.11	-0.91	0.61	-0.08	-0.84	0.38	0.77	-0.81							
AP	-0.65	0.93	0.77	0.80	-0.02	-0.89	0.88	-0.15	-0.32	-0.79	-0.38	**0.98***	-0.01	-0.50	0.55						
SBR	0.81	0.16	-0.70	-0.51	0.86	0.51	0.29	0.94	-0.83	-0.19	0.91	0.10	**-0.99***	0.83	-0.85	-0.09					
DHO	**0.97***	-0.40	-0.89	**-0.97***	0.74	0.94	-0.28	0.81	-0.46	0.19	0.73	-0.56	-0.60	0.72	**-0.97***	-0.69	0.70				
BGL	-0.05	0.69	0.11	0.46	0.29	-0.44	0.71	0.32	-0.58	-0.49	0.37	0.81	-0.63	0.27	0.01	0.70	0.52	-0.25			
ACP	0.04	0.67	0.02	0.37	0.37	-0.35	0.70	0.41	-0.63	-0.48	0.45	0.76	-0.70	0.35	-0.09	0.64	0.61	-0.15	**0.99***		
ALP	-0.92	0.02	0.81	0.71	-0.88	-0.69	-0.11	**-0.97***	0.76	0.07	-0.92	0.12	0.93	-0.85	**0.95***	0.31	**-0.97***	-0.85	-0.30	-0.39	
URE	-0.81	0.62	0.77	**0.98***	-0.48	-0.95	0.52	-0.52	0.13	-0.36	-0.44	0.79	0.23	-0.48	0.80	0.85	-0.35	-0.92	0.62	0.53	0.57

b

	EC	pH	TOC	DOC	THE	HAs	FAs	HAs/FAs	CHO	POL	NH4+	NO2-	NO3-	AN	TP	AP	SBR	DHO	BGL	ACP	ALP
pH	0.27																				
TOC	-0.37	-0.49																			
DOC	-0.24	0.23	-0.23																		
THE	**0.60***	-0.01	0.29	**-0.53***																	
HAs	**0.60***	0.26	-0.35	-0.07	**0.64***																
FAs	0.29	-0.23	**0.66***	**-0.64***	**0.78***	0.02															
HAs/FAs	**0.82***	0.32	-0.34	-0.39	**0.80***	**0.82***	0.37														
CHO	0.20	0.39	0.28	-0.13	0.47	0.13	**0.51***	0.29													
POL	-0.21	0.27	0.02	0.45	-0.30	-0.18	-0.25	-0.31	0.04												
NH4+	**-0.50***	0.16	-0.01	0.01	-0.38	**-0.55***	-0.04	-0.33	0.16	0.04											
NO2-	0.07	0.41	-0.26	0.08	-0.28	-0.25	-0.17	-0.11	-0.12	0.06	0.08										
NO3-	**0.69***	0.27	-0.44	-0.43	0.47	**0.54***	0.17	**0.74***	-0.21	-0.20	-0.31	0.28									
AN	0.21	0.38	-0.40	-0.38	0.10	0.03	0.11	0.39	-0.06	-0.14	**0.53***	0.32	**0.64***								
TP	0.41	-0.08	0.32	-0.25	**0.58***	0.36	0.47	0.35	0.45	-0.06	**-0.53***	-0.27	0.08	-0.36							
AP	**0.67***	**0.54***	**-0.53***	-0.31	0.28	0.42	0.04	**0.64***	0.06	-0.19	-0.06	0.40	**0.72***	**0.59***	-0.09						
SBR	-0.13	-0.25	**0.79***	0.06	0.40	-0.04	**0.55***	-0.11	0.47	0.22	-0.17	-0.41	-0.42	**-0.52***	0.39	-0.47					
DHO	-0.39	-0.12	0.30	0.25	-0.33	-0.45	-0.06	-0.48	-0.17	-0.21	0.17	0.12	-0.37	-0.19	-0.38	-0.16	0.01				
BGL	0.25	0.34	-0.19	-0.02	0.28	0.29	0.14	0.41	0.49	**-0.56***	0.33	0.03	0.06	0.32	0.10	0.34	-0.18	0.07			
ACP	0.29	0.31	-0.35	-0.13	0.07	0.01	0.08	0.34	-0.02	**-0.50***	0.39	0.22	0.34	**0.62***	-0.31	**0.57***	-0.37	0.27	**0.57***		
ALP	-0.33	-0.05	**0.62***	-0.32	0.21	-0.27	0.49	-0.17	0.39	-0.36	0.27	0.00	-0.25	0.00	-0.10	-0.07	0.42	**0.50***	0.24	0.15	
URE	0.10	-0.08	0.32	-0.04	0.20	-0.36	**0.55***	0.03	0.37	-0.16	0.32	0.14	-0.09	0.18	0.21	-0.11	0.28	0.18	0.32	0.42	0.22

c

	EC	pH	TOC	DOC	THE	HAs	FAs	HAs/FAs	CHO	POL	NH4+	NO2-	NO3-	AN	TP	AP	SBR	DHO	BGL	ACP	ALP
pH	0.94																				
TOC	-0.04	-0.27																			
DOC	-0.33	-0.28	0.66																		
THE	0.90	**0.98***	-0.15	-0.10																	
HAs	-0.48	-0.60	0.87	0.85	-0.45																
FAs	0.86	**0.97***	-0.51	-0.45	0.91	-0.77															
HAs/FAs	0.80	**0.96***	-0.43	-0.22	**0.96***	-0.63	**0.97***														
CHO	0.61	0.78	-0.81	-0.66	0.68	-0.95	0.91	0.84													
POL	0.63	0.84	-0.24	0.15	0.90	-0.34	0.80	0.93	0.62												
NH4+	**-0.99***	-0.89	0.07	0.46	-0.83	0.54	-0.82	-0.73	-0.61	-0.51											
NO2-	0.86	0.91	-0.54	-0.65	0.82	-0.86	**0.97***	0.87	0.92	0.62	-0.87										
NO3-	**0.98***	0.86	0.14	-0.26	0.84	-0.33	0.74	0.69	0.45	0.53	**-0.97***	0.76									
AN	-0.14	-0.22	0.89	0.92	-0.05	0.91	-0.45	-0.26	-0.74	0.05	0.24	-0.59	-0.01								
TP	0.64	0.46	-0.24	-0.85	0.30	-0.64	0.50	0.27	0.49	-0.09	-0.76	0.69	0.64	-0.60							
AP	0.86	0.86	0.24	0.18	0.92	-0.09	0.70	0.78	0.35	0.81	-0.77	0.58	0.86	0.31	0.18						
SBR	0.16	0.16	0.70	0.88	0.34	0.67	-0.06	0.16	-0.40	0.46	-0.02	-0.25	0.23	0.91	-0.56	0.62					
DHO	-0.04	0.07	0.52	0.93	0.26	0.64	-0.10	0.15	-0.36	0.50	0.20	-0.33	0.00	0.84	-0.76	0.48	**0.95***				
BGL	-0.79	**-0.95***	0.53	0.35	-0.92	0.74	**-0.99***	-0.99	-0.91	-0.87	0.74	-0.92	-0.66	0.40	-0.36	-0.70	-0.01	-0.01			
ACP	-0.32	-0.18	0.45	**0.96***	0.01	0.69	-0.30	-0.05	-0.46	0.33	0.47	-0.53	-0.29	0.80	-0.92	0.21	0.84	**0.96***	0.17		
ALP	-0.35	-0.43	0.87	0.93	-0.27	**0.98***	-0.63	-0.46	-0.86	-0.14	0.44	-0.76	-0.22	**0.97***	-0.68	0.09	0.81	0.77	0.58	0.79	
URE	0.49	0.75	-0.78	-0.36	0.71	-0.79	0.87	0.88	0.93	0.79	-0.43	0.79	0.31	-0.55	0.15	0.40	-0.16	-0.04	-0.92	-0.12	-0.67

d

	EC	pH	TOC	DOC	THE	HAs	FAs	HAs/FAs	CHO	POL	NH4+	NO2-	NO3-	AN	TP	AP	SBR	DHO	BGL	ACP	ALP
pH	-0.44																				
TOC	-0.69	0.59																			
DOC	-0.16	0.94	0.55																		
THE	0.71	-0.70	-0.27	-0.42																	
HAs	-0.64	0.59	0.10	0.29	**-0.98***																
FAs	0.66	-0.74	-0.26	-0.48	**1.00***	**-0.98***															
HAs/FAs	0.81	-0.72	**-0.97***	-0.60	0.51	-0.35	0.50														
CHO	0.58	0.47	-0.15	0.69	0.05	-0.08	-0.03	0.15													
POL	-0.06	0.91	0.48	**1.00***	-0.37	0.25	-0.43	-0.52	0.76												
NH4+	0.58	-0.14	-0.88	-0.13	-0.09	0.23	-0.12	0.77	0.45	-0.06											
NO2-	0.10	-0.04	0.58	0.19	0.62	-0.75	0.62	-0.35	0.05	0.18	-0.74										
NO3-	0.20	-0.82	-0.05	-0.71	0.80	-0.79	0.85	0.26	-0.55	-0.71	-0.42	0.56									
AN	0.34	-0.89	-0.22	-0.76	0.85	-0.81	0.89	0.42	-0.48	-0.76	-0.26	0.47	**0.99***								
TP	0.93	-0.13	-0.67	0.12	0.40	-0.34	0.34	0.71	0.80	0.21	0.75	-0.11	-0.18	-0.04							
AP	0.37	**-0.99***	-0.60	**-0.97***	0.62	-0.50	0.67	0.70	-0.54	-0.95	0.16	-0.05	0.79	0.86	0.06						
SBR	0.26	0.75	0.09	0.88	-0.27	0.21	-0.35	-0.15	0.94	0.92	0.33	-0.03	-0.76	-0.74	0.56	-0.79					
DHO	-0.95	0.64	0.60	0.35	-0.89	0.83	-0.87	-0.78	-0.34	0.27	-0.36	-0.30	-0.51	-0.62	-0.76	-0.56	0.01				
BGL	-0.02	0.71	0.67	0.89	-0.01	-0.15	-0.06	-0.60	0.62	0.88	-0.41	0.62	-0.30	-0.38	0.10	-0.78	0.71	0.09			
ACP	0.50	0.31	-0.56	0.36	-0.26	0.33	-0.32	0.44	0.78	0.43	0.88	-0.58	-0.73	-0.60	0.78	-0.32	0.74	-0.19	0.06		
ALP	-0.77	-0.20	0.48	-0.41	-0.16	0.13	-0.09	-0.48	-0.94	-0.50	-0.70	0.14	0.45	0.33	-0.95	0.26	-0.79	0.53	-0.32	-0.88	
URE	0.71	0.25	-0.07	0.56	0.41	-0.47	0.34	0.17	0.91	0.63	0.23	0.41	-0.18	-0.13	0.78	-0.34	0.76	-0.58	0.68	0.51	-0.84

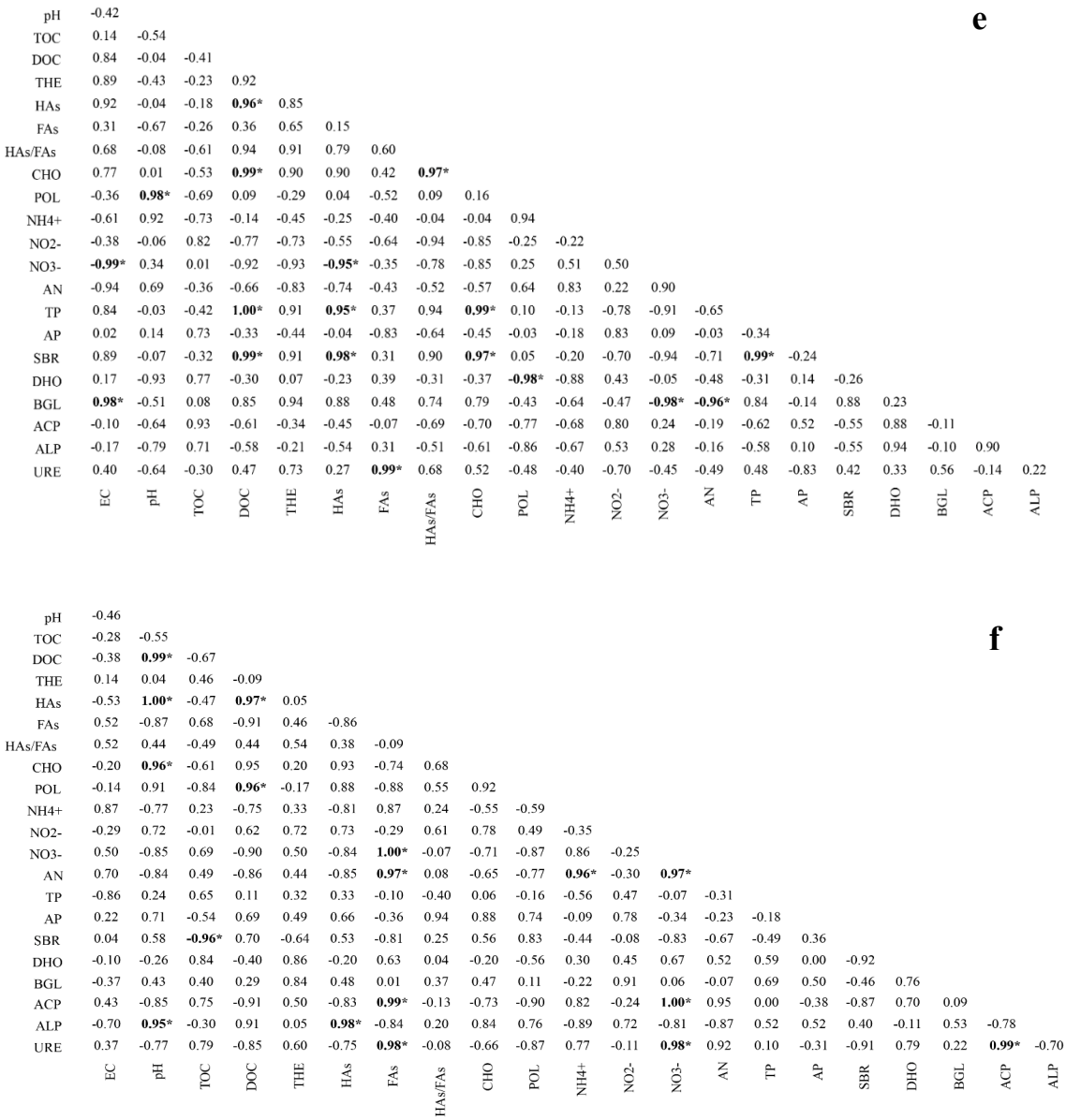

Figure S3. Significant Pearson correlations ($p < 0.05$) between chemical and microbiological properties. (a) Control soils unmodified with MPs, (b) Soil with MPs addition (LPDE 1 and 3%, and PA 1 and 3%), (c) soil with addition of LPDE 1%, (d) soil with addition of PA 1%, (e) soil with addition of LPDE 3%, (f) soil with addition of PA 3%. TOC: Total organic carbon; AP: available phosphorus; TP: total phosphorus; THE: total humic extract; HAs: humic acids; FAs: fulvic acids; HAs/FAs: humification degree; CHO: carbohydrates; POL: polyphenols; BGL: β-glucosidase; ALP: Alkaline phosphatase; ACP: Acid phosphatase; URE: Urease; DHO: Dehydrogenase; DOC: Dissolved organic carbon; SBR: Soil

basal respiration; NH_4^+: Ammonium; NO_2^-: Nitrite; NO_3^-: Nitrate; pH: pH; EC: electrical conductivity; AN: Available nitrogen . Asterisk (*) and bold indicate significant at $p < 0.05$ probability level.

Table S1. Count of MPs particles at 6 weeks of soil incubation

Treatment	Mean (MPs g^{-1} soil)
LDPE 1%	109 ± 29,1
PA 1%	1077 ± 487,5
LDPE 3%	265 ± 101,7
PA 3%	1504 ± 257,0

Average of four replicates (average ± standard deviation)

At the end of the experiment, the MPs were counted according to the protocol of Barili et al. (2023) with slight modifications. Briefly, 2 g of soil were collected and treated with Fenton's reagent for 1 h at 0 °C in order to remove all organic components. The treated soil was then filtered and dried for 24 h at 60 °C. Subsequently, the soil was placed in a 50 mL tube and resuspended with 40 mL of a NaCl solution (density of 1.23 g cm^{-3}) and mixed for 20 min. Afterward, the tubes were kept vertical for 48 h to separate the MPs from the soil particles. Finally, the supernatant was filtered through a Whatmann nº 40 filter, then the filters were dried at 60 °C always kept in a glass Petri dish until optical inspection. Visual sorting was performed under a stereomicroscope Euromex Stereo Blue (Euromex, The Netherlands) with a magnification range of between 0.7 X, and 4.5 X. Particles were isolated using fine metal tweezers and counted.

Barili, S., Bernetti, A., Sannino, C., Montegiove, N., et al. 2023. Impact of PVC microplastics on soil chemical and microbiological parameters. Environmental Research, 229, 115891. https://doi.org/10.1016/j.envres.2023.115891

CAPITULO 3: Microplastic Pollution in Andisol: Effects on Soil Microbiology, Nitrogen Cycling, and *Raphanus sativus* L. Growth.

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Abstract

Microplastics (MPs) alter soil properties and plant physiology and constitute significant risks to crop health and food quality. This study assessed the effects of MPs (polyamide-PA, low-density polyethylene-LDPE, and polypropylene-PP) on Andisol and Raphanus sativus L. as model plant. Plant characteristics, including growth, chlorophyll content, oxidative stress, antioxidant capacity, and bioactive compound profiles were evaluated. In addition, the effects on soil nutrients, microbiological properties, and nitrogen-cycling gene abundance were studied, revealing alterations in both the soil and

plants. Soil pH increased up to 4.3 %, whereas dissolved organic carbon and polyphenol levels decreased, particularly in the PA (26 %) and PP (29 %) treatments. LDPE and PP increased the β-glucosidase activity (14 % and 23 % respectively) and basal soil respiration (25 % and 26 % respectively). Nitrogen-cycling genes nifH, amoA, and nirS were notably more abundant in PP-treated soils, with increases of 79 %, 76 %, and 62 %, respectively. In R. sativus, PA and LDPE increased SPAD values by 9.9 % and increased biomass in rootlets (155 % in PA, 60 % in LDPE) and radishes (125 % in PP). Oxidative stress levels in radishes increased by 63 % and 73 % after LDPE and PP exposure, respectively. MPs altered glucosinolate profiles in both leaves and rootlets. Furthermore, PA and LDPE modified the anthocyanin profiles in leaves, whereas PP altered the profile in radish. This study highlights the impact of MPs on soil health and plant physiology, identifying polyphenols and microbial biomass carbon as key predictors of R. sativus responses.

Keywords: Nitrogen-cycling genes; bioactive compounds; plant defence mechanisms; soil pollution; oxidative stress; microcosms.

1. INTRODUCTION

Microplastics (MPs), defined as plastic particles measuring between 100 nm and 5 mm, have become ubiquitous environmental pollutants with the potential to affect various ecosystems, including terrestrial environments in wich plants grow (Barnes et al., 2009; De Souza Machado et al., 2018a; Thompson et al., 2004). MPs are either intentionally manufactured to be small (primary MPs) or derived from the breakdown of larger plastic debris, such as mulch film and household waste (Riveros et al., 2022). As plastic waste continues to accumulate, understanding its implications has become increasingly important (Galloway et al., 2017). Recent studies have shown that MPs alter soil properties, plant physiology, and ecosystem dynamics (De Souza Machado et al., 2019; Rillig et al., 2019). MPs enter soil via different pathways, such as wind,

rainfall, surface runoff, and atmospheric deposition (Zhang et al., 2020). Once in the soil, MPs serve as a habitat for microorganisms (called plastisphere), altering microbial communities and their ecology (Yi et al., 2021). Furthermore, MPs accumulate over time, modifying the soil physicochemical properties of the soil and interacting with other pollutants, which affects soil health, biodiversity, and ecosystem functions (Nizzetto et al., 2016).

MPs also alter nutrient contents, such as carbon (C), nitrogen (N), and phosphorus (P), affecting microbial nutrient availability (Fu et al., 2024; Li et al., 2024). Moreover, this alteration impacts CO_2 emissions, potentially leading to a lack of soil nutrient (Gao et al., 2021). MPs can directly alter biogeochemical cycles because certain MPs incorporate N during polymer synthesis; this N can be released into the soil and potentially alter soil N cycle. MPs can also indirectly affect the soil microbiota and enzymes involved in N-cycle reactions, further affecting N transformations in the ecosystem (Riveros et al., 2022). MPs can inhibit soil enzyme activity, which is crucial for nutrient cycling and overall soil health. Studies have shown that MPs can reduce the activity of urease, an enzyme important for N cycling (Yu et al., 2020). Alterations in microbial communities associated with the N cycle can impair the functional capacity of N-cycling genes, leading to disrupted N cycling in soil ecosystems (Wang et al., 2024a; Wang et al., 2022b). Studies have suggested that addition of MPs can alter the abundance of *amoA* genes. Gao et al. (2021) found that MPs decreased the abundance of ammonia-oxidizing bacteria (AOB) *amoA* genes without affecting ammonia-oxidizing archaea (AOA) *amoA* genes, potentially inhibiting N_2O emissions by reducing ammonia-oxidizing bacteria during nitrification of the soil. MPs in the soil can also influence the abundance of the *nifH* gene, which is crucial for N fixation. Studies have shown that adding MPs can enhance the abundance of the *nifH* gene depending on the incubation period, potentially altering N-fixing microorganisms in the soil (Rong et al., 2021). This disruption can have drastic consequences for soil fertility and plant growth, as N is a critical nutrient for plant development (Wang et al., 2024a).

Studies have indicated that MPs contamination can have cascading effects for plant water uptake, nutrient availability, absorption, and overall growth, ultimately leading to lower crop yield (Cusworth et al., 2024; De Souza Machado et al., 2019, 2018; Wang et al., 2024a). The effects of MPs on plants have garnered significant scrutiny, although studies remain limited because of the many variables present in the published research, leading to controversial results. The effects of MPs on plants depend on numerous factors, such as polymer type, shape, concentration, and photodegradation (De Souza Machado et al., 2019; Lozano et al., 2024, 2021; Shah et al., 2023). Regarding shoot, leaves, root and/or bulb biomass (fresh weight), some studies have shown increases in *Daucus carota*, *Allium fistulosum*, and *Glycine max* (De Souza Machado et al., 2019; Lozano et al., 2021; Shah et al., 2023). However, these parameters were reduced in *Lactuca sativa*, *Solanum lycopersicum and Fragaria x ananassa* (Nuamzanei et al., 2024; Pinto-Poblete et al., 2023a; Wang et al., 2024b). Moreover, the effects on biomass depend on whether the belowground or aboveground plant parts have been studied (De Souza Machado et al., 2019; Tong et al., 2023a).

In addition to altering morphology, it has been shown that plants grown in both soils and substrates contaminated with MPs exhibit altered biochemistry, particularly concerning oxidative stress. MPs induce cellular stress by promoting reactive oxygen species (ROS) accumulation, causing damage from oxidation, as evidenced by the increased malondialdehyde (MDA) content in tissues (López et al., 2022; Nuamzanei et al., 2024; Wang et al., 2024b; Wang et al., 2024c). Cellular stress caused by MPs triggers a cascade of responses in plants, such as disturbances in antioxidant enzyme activities that counteract ROS, including peroxidase (POD), ascorbate peroxidase (APX), and superoxide dismutase (SOD) (Ranauda et al., 2024; Wang et al., 2024c). The importance of studying the effects on plants is not only ecological, but also nutritional, as it is crucial to evaluate the impact on plants that grow in contact with this widespread

contaminant, which will later be consumed by humans. A widely consumed plant, the common radish (*Raphanus sativus* L.), is a crop species with a subterranean edible part known for its nutrients and bioactive compounds, such as glucosinolates and anthocyanins, which have health-promoting effects in humans (López et al., 2022). Few studies have been conducted on this species grown in soils contaminated with MPs. Botyanszká et al. (2022) studied the growth of *R. sativus* in a silty loamy soil (Luvisol) contaminated with MPs (PS, PVC, and HDPE) and found an increase in the fresh weight of the aboveground and underground zones, although these values were not statistically significant. In contrast Cui et al. (2022) observed a decrease in both shoot and root fresh weights of *R. sativus* grown in soils contaminated with PVC MPs. Furthermore, in the roots, PA and PVC MPs increased the MDA content, whereas PP decreased SOD activity and increased POD activity (Cui et al., 2022). Regarding the effect of MPs on bioactive compounds, López et al. (2022) studied *R. sativus* sprouts grown in coconut fiber and observed a decrease in total glucosinolate content due to the effect of HDPE MPs. Additionally, they found that MPs increased the total anthocyanin content, antioxidant capacity (ORAC), and MDA content in sprouts.

The study of bioactive phytochemicals in plant foods is highly relevant because of their health-promoting properties. Therefore, the present study aimed to elucidate the effects of MPs on biomass, stress, and phytochemical content in mature *R. sativus* plants grown in an Andisol. Soil predictors were also determined to identify changes in *R. sativus* grown in soils polluted with MPs. To our knowledge, this is the first study using *R. sativus* grown in Andisol. The importance of studying the Andisols lies in their high carbon storage capacity attributed to their abundance of short-range order minerals, including allophane and imogolite-type materials (Neculman et al., 2013). Andisols also play an important role in Chilean agriculture, covering approximately 50 % of national land area for cereal production (Poblete-Grant et al., 2020; Soil Survey Staff, 2022).

2. MATERIALS AND METHODS

2.1. Study location and experimental design.

Soil was collected from a site near Chillán city, which is characterized by the absence of recent anthropogenic activity (Lat. 36°31'59.4"S; Long. 71°55'35.7"W). The soil corresponded to the Andisol and Diguillín series (Typic Melanoxerand) (Zagal et al., 2002). In total, 15 kg of soil was collected using a metal shovel (excluding the O horizon) and transported to the laboratory in metal bucket. The soil was air-dried and sieved through a 2 mm mesh. The initial chemical properties of the soil and the absence of MPs were analyzed and reported in our previous study (Riveros et al., 2024).

2.2. MPs materials and potted plant microcosm experiments.

LDPE films, PP films, and PA ropes were purchased from agricultural supply stores (LDPE in Polimaq Ltd., Concepción, Chile; PA and PP in Sodimac S.A.). LDPE and PP films were crushed using an Ultra-turrax® homogenizer (Ultra-TurraxT25, IKA, USA) to obtain particles of the size range of MPs (0.25 – 3.5 mm and 0.1 – 4 mm respectively). PA particles were obtained by manually cutting the PA ropes using scissors to obtain particles of 2–5 mm. To confirm the polymer type, the MPs were analysed using a microscope and FTIR spectrometer system (Spotlight 400, Perkin Elmer) following the methodology of Riveros et al. (2024) (Figure S1). Once the polymer was confirmed, MPs particles (PA, LDPE, and PP) were UV-sterilized (254 nm) for an hour to reduce microbial contamination and then combined with soil using a metal trowel at 2 % doses (dry weight), which can be regarded as an environmentally relevant concentration (Fuller and Gautam, 2016). Clean MPs-free soil was used as control. Each microcosm was performed in a 1000 mL sterilized clay pot with 400 g of air-dried soil. A totally randomized design was used with five repetitions per treatment. The experiment was pre-incubated for eight weeks, and then autoclaved river sand was added in a 2:1 ratio (soil: sand).

Seeds of *R. sativus cv. sparkler* of the same size were surface-sterilized by soaking in 2.5 g L^{-1} of NaClO under aeration for 2 h, and then immersed in distilled water for 24 h. For germination, each seed was placed 5 mm deep in the soil in seed starting trays. The soil used in this study was the same as that used in the experiment. After 2 weeks of a 16/8 h photoperiod in a plant growth chamber (21 °C), four homogeneous and uniformly grown seedlings of *R. sativus* were planted in each clay pot. After 12 weeks, the plants and soil were separated to perform soil and plant analyses. Throughout the pre-incubation and plant growth period, pots received water every two days to maintain soil moisture at 60 % by adjusting weight loss due to evapotranspiration and were maintained with a 16/8 h photoperiod in a plant growth chamber (21 °C) at Universidad de Concepción, Concepción, Chile.

2.3. Soil microcosm analysis

2.3.1. Soil chemical parameters

Once the experiment was completed, the soil samples were subjected to air-drying to determine their chemical composition. The pH of the soil was determined using a soil:water suspension (1:2.5) and a digital pH meter (Model edge® HI2020, Hanna Instruments, USA). Dissolved organic carbon (DOC) were measured with a TOC analyser (TOC-L, Shimadzu, Kyoto, Japan).

Available phosphorus (AP), nitrate (NO_3^-), and ammonium (NH_4^+) were determined according to Sadzawka et al. (2006) and available N (AN) was calculated as the sum of NH_4^+ and NO_3^-. Carbohydrate content (CH) was determined via cold extraction on a soil:water suspension of 1:10 for 1 h while agitated using the anthrone–sulfuric acid method (Brink et al., 1960). Polyphenol (PO) content was measured in the same extract using Folin-Ciocalteu reagent with the method of Folin–Denis (Ribéreau-Gayon Pascal, 1968). CH (625 nm) and PO (750 nm) absorbance were measured using a spectrophotometer (Epoch BioTek, USA).

2.3.2. Enzymatic and microbial activity assays

Soil basal respiration (BR) was determined using the closed-jar incubation method (Alef and Nannipieri, 1995). The soil (25 g) was placed in an incubation flask with a test tube containing NaOH (0.2 M) (7.5 mL). The flasks were incubated under a tight seal at 22°C for 10 days. NaOH (1 mL) was then removed and 2 mL of $BaCl_2$ (1 M) was added. The solution was finally titrated with HCl (0.1 M) until reached the endpoint at the equivalence point (pH = 8.3), with phenolphthalein as an acid-based indicator. The results are presented in μg CO_2 g^{-1} h^{-1}. The concentration of microbial biomass carbon (MBC) was determined based on the chloroform-fumigation extraction method (Tong et al., 2023b).

Dehydrogenase activity (DHA) was analysed with 1 g of the reduction of 0.2 mL of iodonitrotetrazolium chloride (INT 0.4 %) reduced to iodonitrotetrazolium formazan (INTF) at 22 °C (García et al., 1997). The INTF produced was extracted with a 1:1 (v/v) mixture of ethanol and $HCON(CH_3)_2$ (dimethylformamide), and spectrophotometric measurements were performed at 490 nm. Activity quantification used a calibration curve made with data obtained by incubating INTF standards under the aforementioned conditions and expressed as μg INTF g^{-1} of soil. Urease activity (UR) was determined as described by Nannipieri et al. (1980) with slight adjustments. Briefly, urea was used as the substrate, and phosphate (0.1 M) was used as a buffer (phosphate buffer pH 7). Aliquots (2 mL of buffer and 0.5 mL substrate) were added to a 0.5 g soil sample and incubated for 90 min at 30°C. Ammonium (NH_4^+) was determined using the colorimetric method at 660 nm, as described by Kandeler and Gerber (1988a) and Kandeler and Gerber (1988b). Enzymatic activity was expressed as μmol N-NH_4^+ g^{-1} h^{-1}. β-Glucosidase activity (BG) was analysed following the method described by Eivazi' and Tabatabai (1988). The method involved extraction and determination of p-nitrophenol after 0.5 g of soil was incubated with buffered p-nitrophenyl-β-D-glucopyranoside solution (2.5 mL) for 1 h at 37°C. Acid phosphatase (MUB buffer, pH 6.5) activity was determined as

described by Tabatabai and Bremner (1969). This method uses colorimetric estimation of p-nitrophenol released by phosphatase activity during soil incubation with buffered sodium p-nitrophenyl phosphate solution for 1 h at 37°C. β-Glucosidase and acid phosphatase activities were quantified by reference to a curve of calibration built from p-nitrophenol determined at 398 nm and indicated as μmol PNF g^{-1} soil h^{-1}.

2.3.3. Soil DNA extraction and the abundance of functional genes related to the nitrogen cycle.

Total soil DNA was isolated from 200 mg of microcosm soil using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany), following the manufacturer´s instructions. DNA concentration and quality were determined using a BioTek Epoch Spectrophotometer (BioTek Co., Vermont, USA) and agarose electrophoresis.

The bacterial 16S rRNA gene and functional marker genes involved in nitrogen fixation (*nif*H), bacterial ammonia oxidation (*amoA*), bacterial nitrite reduction (*nirS*), and nitrous oxide reduction (*nosZ*) were determined via real-time quantitative PCR (qPCR) with primers and thermal conditions (Table S1) and following methodologies of Keshri et al. (2015) and Wan et al. (2023) with modifications. PCR reactions were performed in 15 μL containing 20 ng of DNA, 1X × KAPA SYBR FAST Universal 2X qPCR Master Mix (Kapa Biosystems, Massachusetts, USA), 530 nM of each primer, and filled with molecular-grade water. Dissociation analysis was performed after all amplifications were completed to detect primer dimers and nonspecific amplicons. All real-time PCR reactions were performed in duplicate on the Agilent AriaMx Real-Time PCR System (Agilent Technologies, California, USA) in accordance with MIQE guidelines (Bustin et al., 2009). The quantification cycle (Cq) and data were extracted using the Agilent AriaMx software, and the number of gene copies per gram of soil in each sample was calculated by comparing the gene copies of the standard curve.

To obtain standard qPCR assay curves, bacterial 16S rRNA and functional marker genes were amplified from soil DNA using the primers listed Table S1. PCR products were analyzed by agarose gel electrophoresis and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). The purified PCR products were cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) and transformed into *Escherichia coli* JM109 competent cells (Promega, Madison, WI, USA). Single clones of *E. coli* were isolated and verified by sequencing using T7 and SP6 promoter primers. The selected sequences were submitted to the NCBI database (GenBank accession numbers: 16S rRNA in PQ464602, *nifH* in PQ474304, *amoA* in PQ474305, *nirS* in PQ474306, and *nosZ* in PQ474307). Positive clones from solid plates were cultured overnight in 25 mL of LB broth containing 100 mg L^{-1} ampicillin at 37°C. Plasmid extraction and purification using the E.Z.N.A. Plasmid Mini Kit I (Omega, Norcross, GA, US), copy numbers were calculated using Equation 1, assuming an average molecular mass of 660 Da for 1 bp of double-stranded DNA:

1) $$\text{Copy number (copies } \mu\text{L}^{-1}) = \frac{6.02\times10^{23}\times C\times10^{-9}}{660\times L}$$

Where L is the length of the standard in base pairs and C is the concentration of the standard expressed in ng μL^{-1}. Serial tenfold dilutions (10^{-1} – 10^{-9}) of plasmids harboring the targeted gene inserts were used as qPCR standards. The melting curve always exhibited a single peak. The calibration curves indicated a correlation coefficient of $R^2 > 0.99$, and the efficiency ranged from 95 to 106 % in all assays. PCR efficiency was calculated using Equation 2:

2) $$\text{Efficiency} = \left(10^{(-1/\text{Slope})}\right) - 1$$

The constant and slope were determined using the regression equation (y = mx + c) calculated on the graph, which plotted Cq against the copy number of the standard gene (16S rRNA, *nifH*, *amoA*, *nirS*, and *nosZ*). The gene copy numbers of each target gene were expressed per gram of soil, and the values were log-transformed.

2.4. *R. sativus* analysis

2.4.1. Determining of chlorophyll content and plant growth

The Relative chlorophyll content was measured using a SPAD-502 Plus relative chlorophyll meter (Konica Minolta. Inc., Tokyo). SPAD values were obtained from each leaf (3x per leaf), and average values from all leaves were calculated. One day after the chlorophyll content was examined, the *R. sativus* plants were removed from the pots, washed with distilled water, and dried with a paper towel. The plants were then separated into leaves, radish, and rootlets using a scalpel, and the fresh weights of the leaves, radish (thickened and edible roots), and rootlets were immediately recorded using an electronic scale (RADWAG, model WTB 2000, Radom, Poland). The harvested plants were immediately frozen in liquid nitrogen and stored at -80°C for later analysis.

2.4.2. Measurement of biomarkers associated with oxidative stress in *R. sativus*.

To determine the antioxidant activity response in plants under MPs stress, enzyme activity was measured in the leaves, radish, and rootlets of *R. sativus*. For antioxidant activity: superoxide dismutase (E.C. 1.15.1.1, SOD), glutathione reductase (E.C. 1.6.4.2, GR), ascorbate peroxidase (E.C. 1.11.1.11, APX), and peroxidase (POX) levels were measured spectrophotometrically according to (Palma et al., 2014). Briefly, 100 mg of frozen tissue was mixed with a buffer containing 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM 2-mercaptoethanol, and 20 % (w/w) PVPP. The resulting extracts were used for

enzymatic reactions, and enzyme activity was measured as absorbance change over time, as previously described (Palma et al., 2014). Using bovine albumin as the standard, the total soluble protein content was measured at 595 nm following the Bradford method (1976). The protein concentration was calculated and expressed as mg g^{-1} fresh weight.

Lipid peroxidation was determined using the thiobarbituric acid (TBA) reaction by measuring malondialdehyde (MDA) equivalents as per the method of Heath and Packer (1968) and Hodges et al. (1999). Briefly, leaves, radishes, and rootlets were homogenized with liquid nitrogen, 100 mg of pulverized tissue was mixed with trichloroacetic acid (TCA; 0.1 % w/v), and samples were centrifuged at 12000 x g at 4°C for 10 min. Chromagen was formed by mixing 0.5 mL the supernatant (0.5 mL with TCA 20 % (w/v) and TBA 0.5 % (w/v). The samples were heated at 95°C for 20 min and centrifuged at 12000 x g at 4°C for 10 min. The absorbance of the supernatants was measured for readings (532, 600, and 440 nm). The 600 nm reading was used to discount the nonspecific turbidity of the sample, and the 440 nm readings were included in order to discount the sugars proportional to the sucrose concentration being measured and could approximate the total soluble sugars present in the samples. The controls for each sample were prepared without TBA and were calculated according to Hodges et al. (1999). MDA concentrations were expressed as nmol MDA mg^{-1} FW.

2.4.3. Quantification of glucosinolates and anthocyanins in leaves, radish and roots

The bioactive compounds (glucosinolates and anthocyanins) from leaves, radish and roots were extracted as described by López et al. (2022). Glucosinolates were extracted from frozen samples (100 mg) with 1 mL of methanol 70 % (v/v). The samples were then heated for 20 min at 70°C in a heating bath and shaken every 5 min. The extraction was halted by adding the reaction mixture to ice water for 5 min. Finally, the extracts were centrifuged (5

min at 16,000 *g*). The supernatants were collected and filtered (0.45 µm PVDF). All samples were stored at −20°C before analysis. To extract anthocyanins, each sample (0.5 g) was poured into 5 mL of 50:49:1 (methanol: water: formic acid), stirred or vortexed for 5 min, left in an ultrasonic bath for 1h, and maintained at 4°C overnight. The samples were then centrifuged for 10 min at 10,000 rpm and filtered through a 0.22 µm PVDF syringe filter. The extracts were then placed in amber vials for chromatographic analyses. Analytical grade solvents were used (Merck, Germany).

Glucosinolates were identified by their retention times, UV–Vis spectra, and fragmentation patterns (MSn and M) using HPLC-DAD-ESIMS^n, as described by Baenas et al. (2016). Chromatograms were recorded at 227 nm, while intact glucosinolates were quantified using glucoerucin and glucobrassicin as external standards for aliphatic and indolic glucosinolates (Sigma-Aldrich, USA). The analysis was performed in triplicate, and the results were expressed as mg 100 g^{-1} DW. For anthocyanin analysis, peak identification was performed under pre-established conditions for these compounds in radish sprouts (Baenas et al., 2015). Extracted samples were quantified using a Hitachi HPLC-DAD system (Merck, Germany) under identical chromatographic conditions. Chromatograms were recorded at 520 nm, and cyanidin 3-O-glucoside was used as the external standard (Sigma-Aldrich, USA). The analysis was performed in triplicate, and the results were expressed in mg 100 g^{-1} DW.

2.4.4. Antioxidant capacity in plant-parts

The oxygen radical absorbance capacity (ORAC-FL) assay was used to measure the free radical scavenging activity, as described by López et al. (2018). The antioxidant capacity of ORAC-FL was measured by counting fluorescence variations after 120 min of the reaction with the radical. The assay was performed using microplates (96-well) using a Synergy H1 hybrid multi-mode microplate reader (Biotek, US), and six replicates were performed. The results were expressed as µmol Trolox 100 g^{-1} dry weight.

2.5. Statistical analysis

Significant differences in soil and plant characteristic between the different MPs treatments and control soils were analyzed by permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) ($p > 0.05$), using permutation tests to obtain P values. A sample similarity matrix was constructed using the Euclidean Distance to test the effects of MPs treatments for each individual variable. Pairwise test comparisons were then performed with a multivariate t-test analogue, and probability levels were estimated by permutation (Eldridge et al., 2016), using a total of 999 permutations. A 95% probability threshold was used as a marker to consider significant differences.

Principal component analysis (PCA) and Pearson correlation test (r) were performed to explore the relationships between the different variables studied (soil and plant) for each MPs treatment and control without MPs addition. For PERMANOVA and PCA, we used the statistical package PRIMER + PERMANOVA (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) for Windows. Pearson correlations were performed using the Statgraphics Centurion XVIII-X64 software (StatPoint Technologies, Inc., Warrenton, VA, USA).

A distance-based linear model (DistLM) was used to determine the relative importance of each soil property in the *studied R. sativus parameters*. For the DistLM routine, “marginal” tests of the relationship between an independent variable and the response variable (*R. sativus* parameters) were performed to identify the independent variables that explained variability among different treatments. Subsequent “sequential” tests of the individual variables were performed to evaluate their significant contribution to the explained variance of the response variable. The Akaike Information Criterion (AIC) (Akaike, 1974) was adopted to select the best model, and a step-by-step procedure was used to build the model. For PERMANOVA, PCA, and DistLM analyses, we used the statistical package PRIMER + PERMANOVA (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) for Windows.

3. RESULTS

3.1. Effects of MPs on soil parameters.

The results of the PERMANOVA analysis revealed significant differences ($p < 0.05$) in soil samples from the soil-plant system with MPs incubation compared to the treatment without MPs (Table 1). The presence of MPs led to significant changes in soil chemical properties, including pH and C. All MPs treatments increased the pH values between 3.7 % and 4.3 %. According to C nutrients, soils with PA showed a decrease of 26 % in DOC content, and soils with PP showed a decrease of 29 % in polyphenol concentration compared to the control treatment. Regarding NO_3^+ and AN, a decrease was observed in treatments with MPs; however, this decrease was not statistically significant. AP did not vary among the treatments.

Table 1. Chemical properties of the control soil and soils with MPs addition.

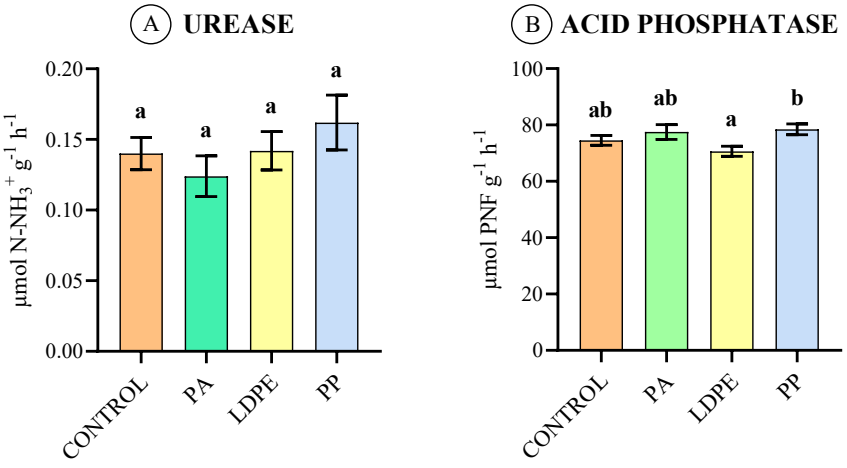
	CONTROL	PA	LDPE	PP
pH	5.56 ± 0.130 a	5.79 ± 0.119 b	5.80 ± 0.110 b	5.77 ± 0.072
DTC (g-C kg^{-1})	0.74 ± 0.137 a	0.76 ± 0.304 a	0.92 ± 0.415 a	0.92 ± 0.492
DOC (g-C kg^{-1})	0.65 ± 0.129 a	0.48 ± 0.064 b	0.84 ± 0.413 ab	0.84 ± 0.494
CH (mg-C kg^{-1})	8.59 ± 1.039 a	8.15 ± 0.434 a	8.78 ± 0.982 a	8.37 ± 0.385
PO (mg kg^{-1})	22.46 ± 4.121 a	19.46 ± 5.625 ab	17.54 ± 5.381 ab	15.95 ± 4.676
NO_3^- (mg kg^{-1})	4.83 ± 3.524 a	2.33 ± 0.947 a	3.33 ± 1.402 a	2.95 ± 0.838
NH_4^+ (mg kg^{-1})	3.46 ± 0.934 a	3.50 ± 0.552 a	3.46 ± 0.789 a	3.96 ± 0.643
AN (mg kg^{-1})	8.27 ± 2.723 a	5.85 ± 0.818 a	6.77 ± 0.999 a	6.91 ± 1.043
AP (mg kg^{-1})	26.72 ± 0.507 a	26.16 ± 0.513 a	26.54 ± 0.783 a	26.82 ± 0.630

Note: Average of five replicates (average ± standard deviation). Different letters within the same row indicate significant differences among treatments according to PERMANOVA analyses ($p < 0.05$). CONTROL: Control soil without MPs added; PA: Soil with 2 % (w/w) PA-MPs added; LDPE: Soil with 2 % (w/w) LDPE-MPs added; PP: Soil with 2 % (w/w) PP-MPs added.

Abbreviations: PA, polyamide; LDPE, low-density polyethylene; PP, polypropylene; AN, available nitrogen; AP, available phosphorus; CH,

carbohydrates; DOC, dissolved organic carbon; DTC, dissolved total carbon; NH_4^+, Ammonium; NO_3^-, Nitrate; PO, polyphenols.

After MPs incubation, some microbiological properties in the soil microcosms were altered compared to the MPs-free control (Figure 1) (PERMANOVA; $p < 0.05$). The enzymatic activity of acid phosphatase in soils containing MPs was not significantly different from that in the control soil; however, PP had 11 % greater activity than the LDPE treatment (Figure 1B). The β-glucosidase activity (Figure 1C), LDPE and PP increased the enzymatic activity by 14 % and 23 %, respectively, compared to the control treatment. Compared to the control, the BR values increased in all MPs treatments (Figure 1E); however, only LDPE and PP were statistically significant (25 % and 26 % higher CO_2 production, respectively). Urease, dehydrogenase, and microbial biomass carbon did not show statistically significant changes after microcosm-MP incubation (Figure 1 A, D, F).



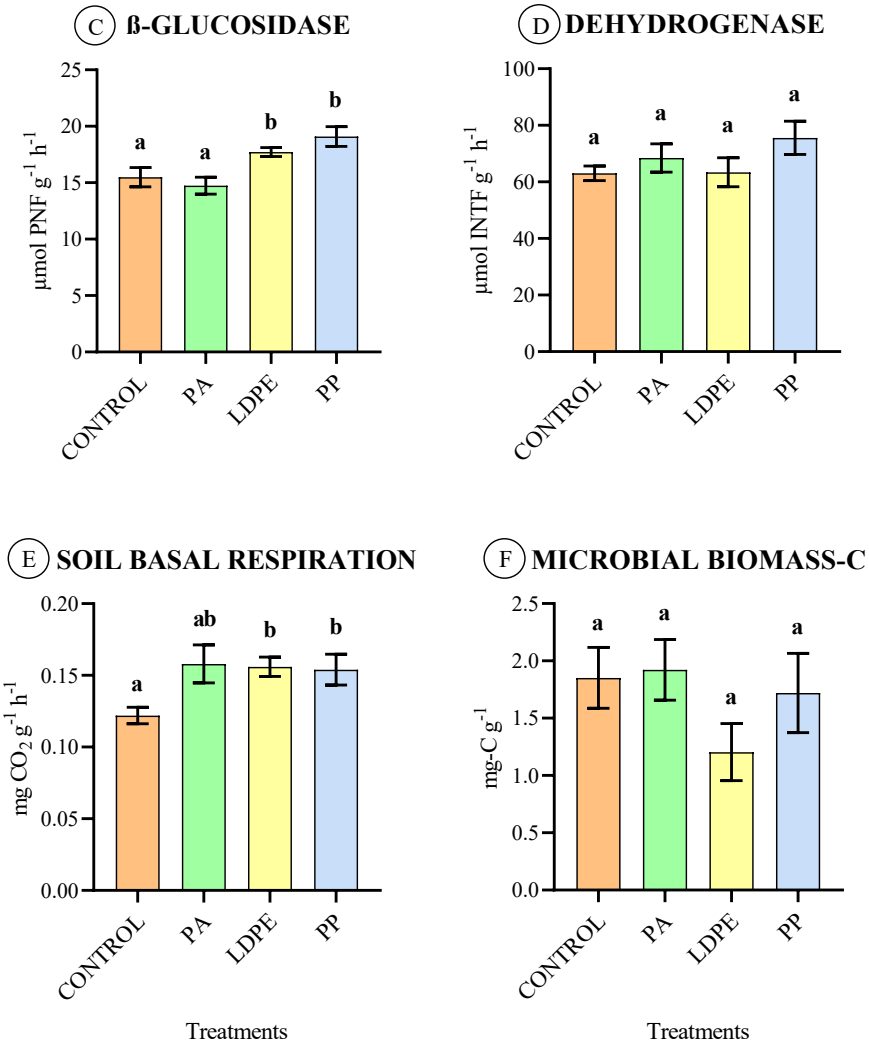


Fig 1. Microbiological properties of control soil and soil with MPs addition. (a) Urease; (b) Acid phosphatase; (c) β-glucosidase; (d) Dehydrogenase; (e) Soil basal respiration; (f) Microbial biomass-C. CONTROL: Control soil without MPs; PA: Soil with 2 % (w/w) PA-MPs; LDPE: Soil with 2 % (w/w) LDPE-MPs; PP: Soil with 2 % (w/w) PP-MPs). Different letters indicate significant differences among treatments according to PERMANOVA analyses ($p < 0.05$). Values are expressed as mean ± standard error.

The qPCR results showed that MPs exposure had no significant effect on the abundance of 16S rRNA and *nosZ* genes. Nevertheless, compared with the control, the abundance of *nifH, amoA, and nirS* was significantly increased by

PP (79 %, 76 %, and 62 %, respectively). Regarding the *nirS* gene, LDPE treatment also increased its abundance by 56 % ($p < 0.05$; Figure 2).

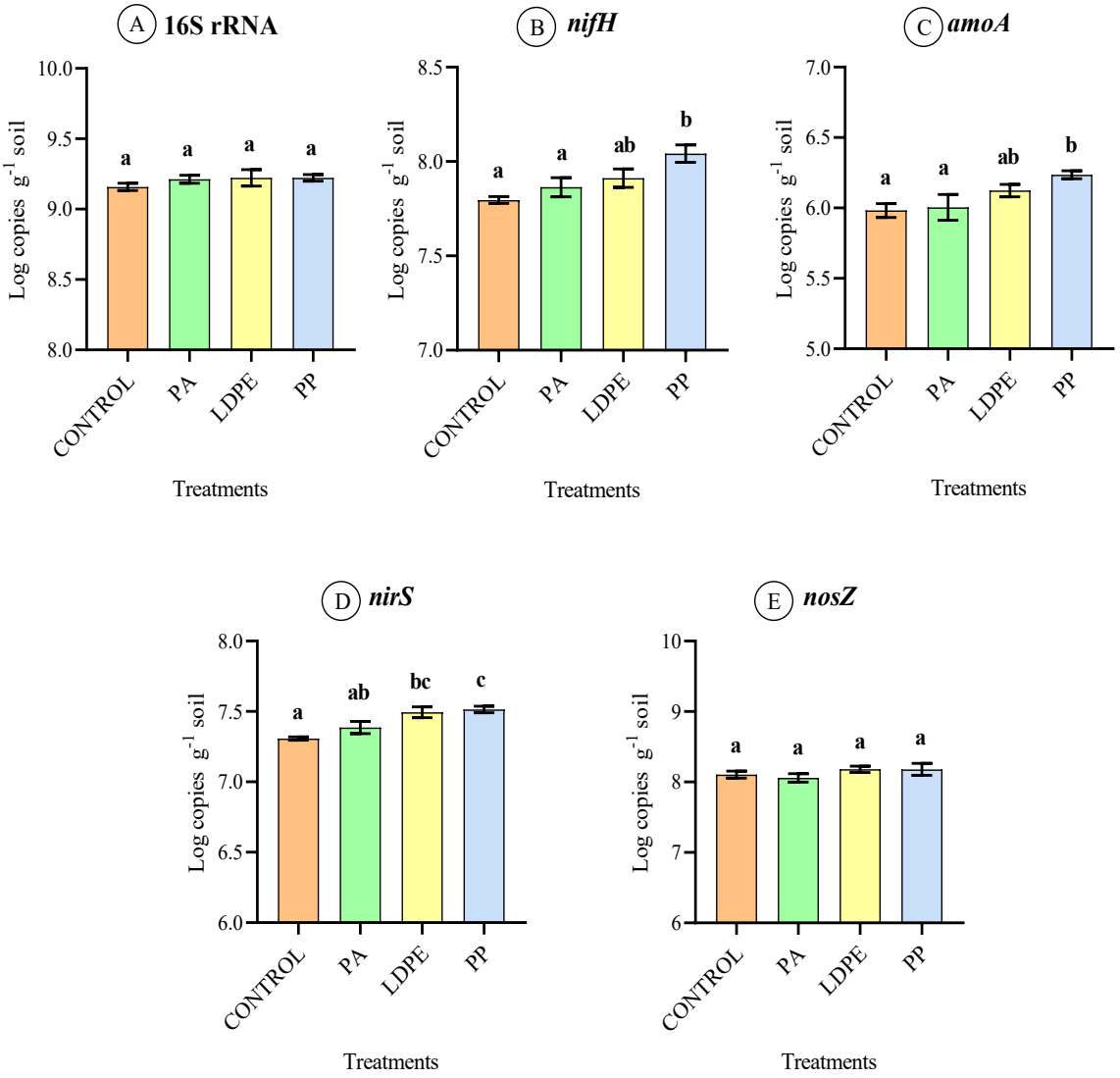


Fig. 2. Total abundance of 16S rRNA (A), *nifH* (B), *amoA* (C), *nirS* (D), and *nosZ* (E) genes in control soil and soils with MPs addition. CONTROL: Control soil without MPs added; PA: Soil with 2% (w/w) of PA-MPs added; LDPE: Soil with 2% (w/w) of LDPE-MPs added; PP: Soil with 2% (w/w) of PP-MPs added. Different lowercase letters indicate significant differences among treatments according to PERMANOVA analyses ($p < 0.05$). Values are expressed as mean with standard error.

3.2. Effects of MPs on *R. sativus* parameters.

After 12 weeks of plant growth, the morphological properties of plants grown with MPs were altered compared to those of plants grown in the control soil (Figure 3). Adding MPs produced a leaf biomass increase compared with the control treatment, but only LDPE and PP were statistically significant (28 % and 36 % increases, respectively). The weight of radish biomass (part of the thickened and edible root) increased in all treatments; however, due to the great variability, this was only statistically significant in plants grown in PP (125 % increase). The biomass of rootlets increased in soil with PA (155 %) and LDPE (60 %) compared with the control treatment. In addition, the biomass of the entire plant significantly increased in all treatments compared with that of the control (PA: 56 %, LDPE: 47 %, and PP: 48 % increase). The relative chlorophyll content (SPAD value) was 9.9 % higher in plants grown in PA and LDPE than in the control (Figure 4). The analysis of soluble protein content revealed differential responses between plant parts and treatments. Leaf protein levels were not significantly different between the treatments and control (Figure S2A). In radish tissues, protein content increased by 220 % and 92 % in the PA and PP treatments versus the control (Figure S2B). Conversely, in rootlets, protein levels decreased by 29 % in the PA treatment compared with the control (Figure S2C).

R. sativus grown in MPs showed higher MDA content in the thickened root (radish), versus the control. However, this increase was not statistically significant in the soils with PA. For treatments with LDPE and PP, the MDA content increases in radishes were 63% and 73%, respectively (Figure 5B) ($p < 0.05$). No significant changes were observed in either the leaves or rootlets compared to the control plants (Figure 5A, 5C). The values from the ORAC assay (antioxidant capacity assay) of plants grown in soil with MPs showed significant differences in both leaves and radishes when compared to control plants (Figure 5D and 5E). In the leaves, a decrease in the amount of Trolox between 22 % and 33 % was observed depending on the MPs used. However,

in the case of radish, increases of 35 % and 53 % in the amount of Trolox were observed with the use of PA and PP, respectively.

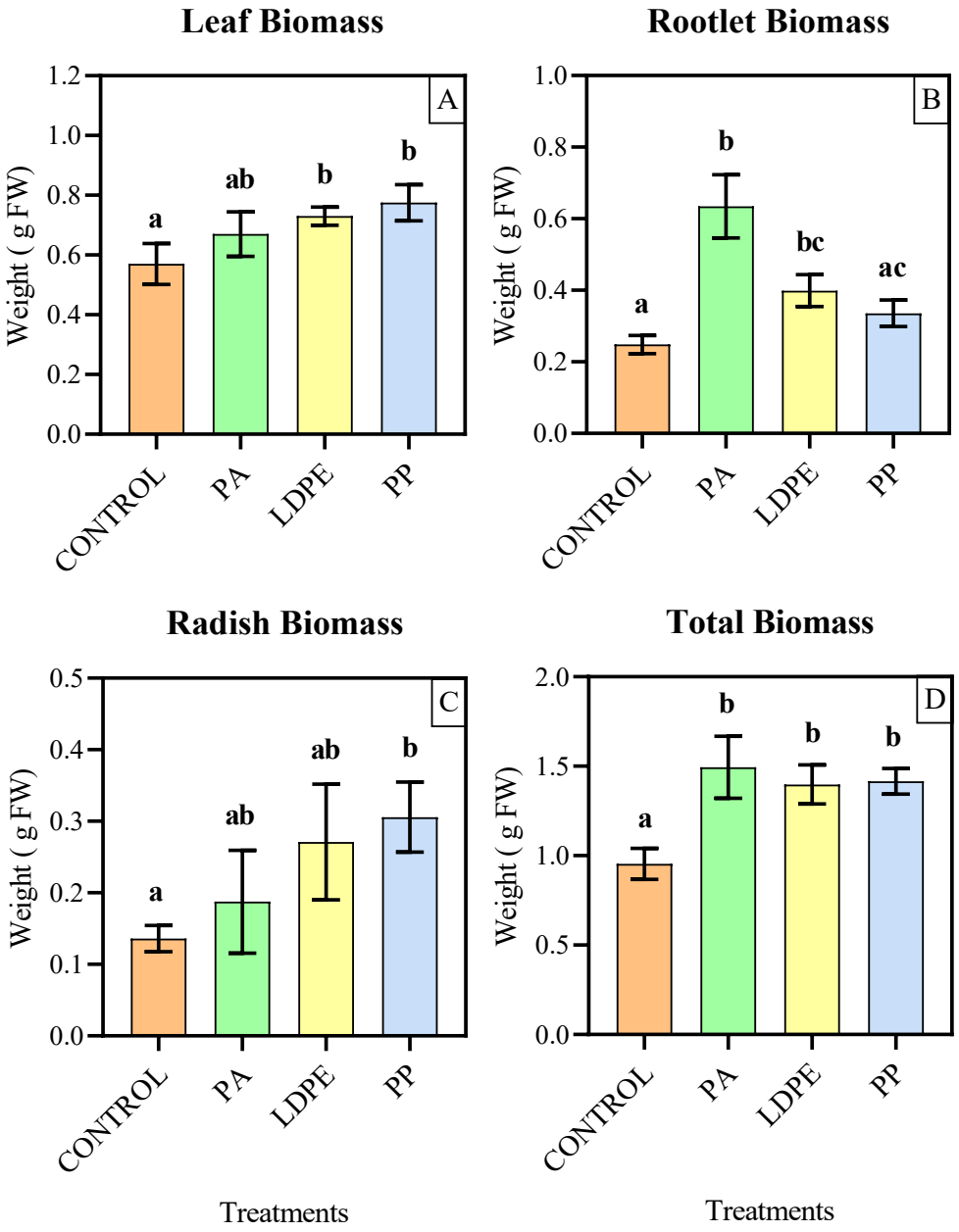


Fig.3. Biomass in *R. sativus* grown in control and soils with MPs addition. (A) Leaf biomass, (B) rootlets biomass, (C) radish biomass, and (D) total biomass. CONTROL: Control soil without MPs addition; PA: Soil with 2 % (w/w) PA-MPs added; LDPE: Soil with 2 % (w/w) LDPE-MPs added; PP: Soil with 2 % (w/w)

PP-MPs added. Different letters indicate significant differences among treatments according to PERMANOVA analyses ($p < 0.05$). Values are expressed as mean with standard error.

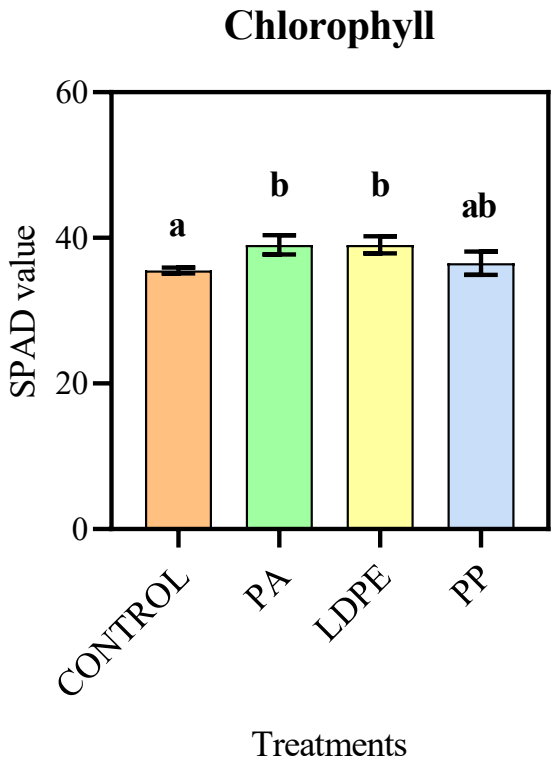


Fig.4. Chlorophyll content (SPAD Value) in *R. sativus* grown in control soil and soils with MPs addition. CONTROL: Control soil without MPs addition; PA: Soil with 2 % (w/w) of PA-MPs added; LDPE: Soil with 2 % (w/w) of LDPE-MPs added; PP: Soil with 2 % (w/w) of PP-MPs added. Different letters indicate significant differences among treatments according to PERMANOVA analyses ($p < 0.05$). Values are expressed as mean with standard error.

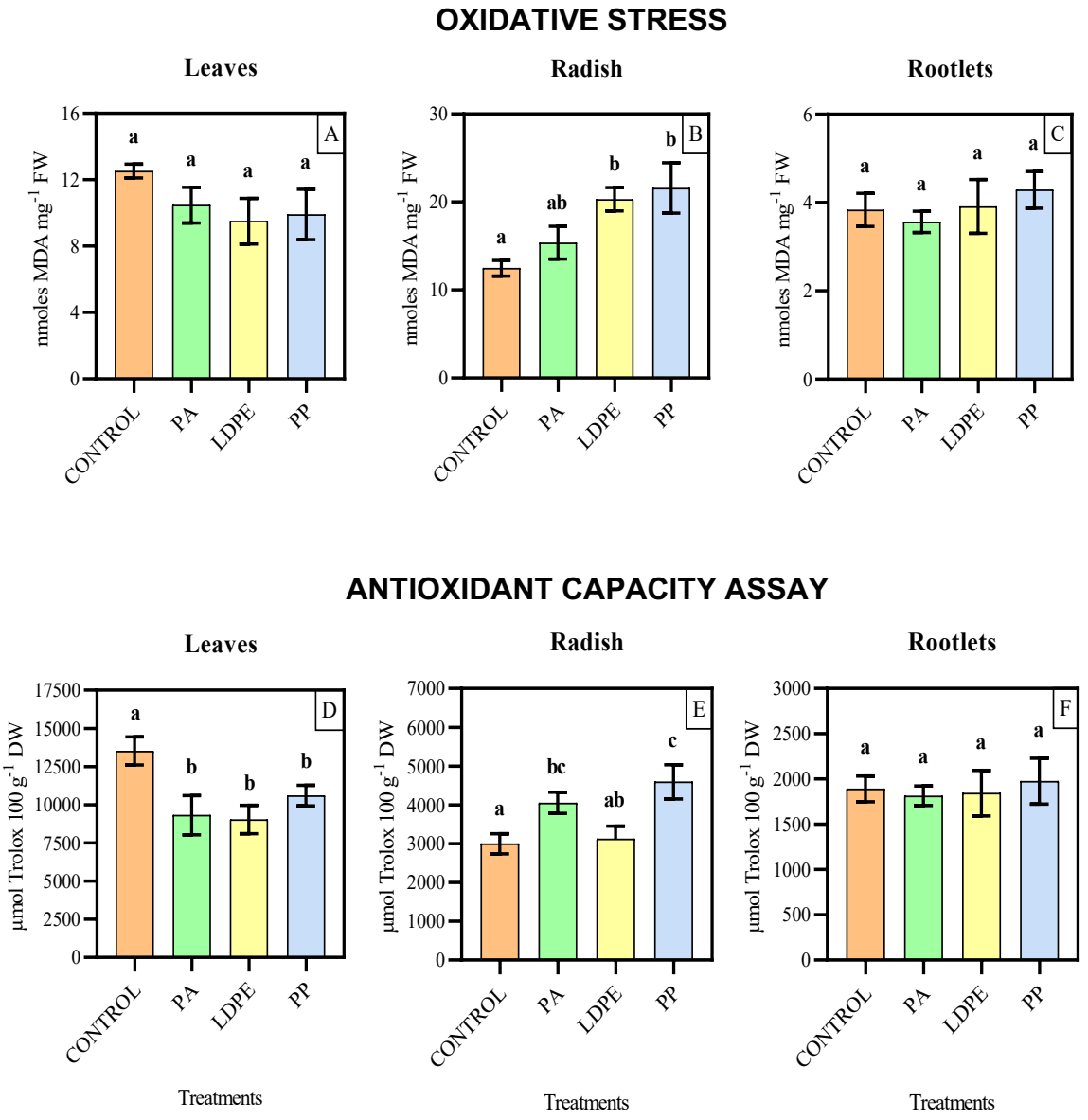


Fig.5. Oxidative stress (MDA content; A, B, and C) and antioxidant capacity assay (ORAC; D, E, and F) in *R. sativus* grown in control soil and soils with MPs addition. (A) Leaf biomass; (B) Rootlet biomass; (C) Radish biomass; (D) Total biomass. CONTROL: Control soil without MPs addition; PA: Soil with 2 % (w/w) of PA-MPs added; LDPE: Soil with 2 % (w/w) of LDPE-MPs added; PP: Soil with 2 % (w/w) of PP-MPs added. Different letters indicate significant differences among treatments according to PERMANOVA analyses ($p < 0.05$). Values are expressed as mean with standard error.

An enzymatic antioxidant response was observed in *R. sativus* grown in soils containing MPs (Table 2). However, statistically significant differences compared to the control were evident only in the leaves and rootlets ($p < 0.05$). In the leaves, SOD activity increased by 41 % in plants grown in LDPE compared to the control. In rootlets, PP exposure led to a 22 % reduction in SOD activity, whereas PA exposure caused a 70 % reduction in POX enzymatic activity.

Table 2. Enzyme activities in *R. sativus* plant parts grown in control soil and soils with MPs addition

	CONTROL	PA	LDPE	PP
LEAVES				
SOD (U $mg^{-1}min^{-1}$)	3.01 ± 0.250 a	2.94 ± 0.700 a	4.24 ± 0.888 b	3.14 ± 0.9
APX (mM Asc mg $prot^{-1}h^{-1}$)	0.52 ± 0.235 a	0.74 ± 0.222 a	0.58 ± 0.309 a	0.82 ± 0.2
GR (mM NADH mg $prot^{-1}h^{-1}$)	2.26 ± 0.538 a	2.37 ± 0.067 a	2.46 ± 0.398 a	2.44 ± 0.2
POX (mM H_2O_2 mg $prot^{-1}$ h^{-1})	0.11 ± 0.032 a	0.14 ± 0.029 a	0.11 ± 0.024 a	0.13 ± 0.0
RADISH				
SOD (U $mg^{-1}min^{-1}$)	0.11 ± 0.062 a	0.13 ± 0.018 a	0.13 ± 0.062 a	0.16 ± 0.0
APX (mM Asc mg $prot^{-1}h^{-1}$)	2.55 ± 1.114 a	1.51 ± 0.586 a	2.60 ± 0.941 a	1.83 ± 0.5
GR (mM NADH mg $prot^{-1}h^{-1}$)	2.84 ± 0.823 a	2.19 ± 0.897 a	3.04 ± 1.471 a	2.49 ± 1.1
POX (mM H_2O_2 mg $prot^{-1}$ h^{-1})	2.15 ± 0.885 a	2.59 ± 1.644 a	2.50 ± 1.088 a	1.43 ± 0.4
ROOTLETS				
SOD (U $mg^{-1}min^{-1}$)	2.12 ± 0.241 a	2.08 ± 0.302 a	1.93 ± 0.159 a	1.65 ± 0.1
APX (mM Asc mg $prot^{-1}h^{-1}$)	0.33 ± 0.112 a	0.33 ± 0.078 a	0.33 ± 0.112 a	0.40 ± 0.1
GR (mM NADH mg $prot^{-1}h^{-1}$)	0.87 ± 0.079 a	0.78 ± 0.094 a	0.81 ± 0.071 a	0.76 ± 0.1
POX (mM H_2O_2 mg $prot^{-1}$ h^{-1})	1.37 ± 0.587 a	0.40 ± 0.224 b	1.56 ± 0.928 a	2.22 ± 0.9

Note: Average of five replicates (average ± standard deviation). Different letters within the same row indicate significant differences among treatments according to PERMANOVA analyses ($p < 0.05$). CONTROL: Control soil without MPs added; PA: Soil with 2 % (w/w) of PA-MPs added; LDPE: Soil with 2 % (w/w) of LDPE-MPs added; PP: Soil with 2 % (w/w) of PP-MPs added. Abbreviations: PA, polyamide; LDPE, low density polyethylene; PP, polypropylene; SOD, superoxide dismutase; APX, ascorbate peroxidase; GR, glutathione reductase; POX, peroxidase.

R. sativus grown in soil polluted with MPs did not showed significantly alterations of the total content of glucosinolates compared to the control in leaves, radishes nor rootlets (Table 3). However, some individual glucosinolate contents showed significant changes compared with the control treatment *(p* < 0.05) (Table 3). In leaves, GRE (4-methylsulphinyl-3-butenyl) increased in all treatments with MPs (PA 58 %, LDPE 60 %, and PP 39 %). In the case of radish, no significant differences were observed in any glucosinolate content between the MPs treatments and the control. However, when comparing treatments with PA and PP, the latter had 42 % more total glucosinolates than treatment with PA *(p* < 0.05). In rootlets, dehydroerucin (DH-EU) decreased by 55 % in the PA treatment compared with the control, but methoxyglucobrassicin (MGB) and glucobrassicin (GBS) drastically increased with LDPE (105%) and PP (60%) treatments compared with the control *(p* < 0.05).

R. sativus grown in soil with MPs showed significant alterations in anthocyanin content, depending on the type of MPs and the part of the plant studied (Table 4). Total leaf anthocyanin content decreased in the PA treatment compared with the control (46 %) (*p* < 0.05), suggesting PA MPs suppress anthocyanin biosynthesis. Specific anthocyanins, such as 6-Cy and 5-Cy, were decreased by PA treatment (50 % and 70 %, respectively). LDPE treatment decreased the anthocyanin content compared to that in the control, particularly in 2-Cy (76 %). In the case of 3-Cy, a decrease was observed in all treatments with MPs; however, the differences among the treatments were not statistically significant because of high variability. For radish, opposite effects were observed (Table 4). There was no significant difference in total anthocyanin content between treatments; however, individual anthocyanins such as 5-Cy, 7-Cy, and 8-Cy showed an increase under PP treatment (105 %, 146 %, and 91 %, respectively) compared to the control.

Table 3. Glucosinolate content (mg 100 g^{-1} DW) in *R. sativus* plant parts after different MPs treatments

	GRE	DH-EU	GBS	MGB	TOTAL
GSL Leaves					
CONTROL	9.51 ± 1.676 a	N.D.	65.19 ± 21.433 a	53.05 ± 17.253 a	127.75 ± 20.209 a
PA	14.98 ± 3.559 b	N.D.	57.46 ± 9.886 a	41.83 ± 19.365 a	114.27 ± 25.324 a
LDPE	15.21 ± 3.476 b	N.D.	41.26 ± 22.744 a	51.54 ± 24.585 a	108.01 ± 40.933 a
PP	13.21 ± 1.724 b	N.D.	80.01 ± 37.733 a	56.79 ± 28.017 a	150.00 ± 40.201 a
GSL Radish					
CONTROL	32.62 ± 26.179 ab	40.93 ± 17.534 ab	55.94 ± 29.447 ab	28.83 ± 19.905 ab	158.32 ± 66.740 ab
PA	22.85 ± 15.792 ab	28.69 ± 11.339 a	59.87 ± 14.569 a	33.78 ± 17.467 a	145.19 ± 40.668 a
LDPE	11.78 ± 7.493 a	45.92 ± 31.887 ab	69.20 ± 40.962 ab	28.65 ± 24.750 a	155.55 ± 87.543 ab
PP	23.69 ± 5.746 b	57.18 ± 17.780 b	93.24 ± 22.421 b	32.14 ± 18.747 a	206.25 ± 40.874 b
GSL Rootlets					
CONTROL	N.D.	35.39 ± 11.329 a	16.37 ± 5.712 a	25.82 ± 6.450 a	77.58 ± 19.023 a
PA	N.D.	16.07 ± 10.794 b	30.98 ± 23.391 ab	23.94 ± 10.549 a	71.00 ± 40.370 a
LDPE	N.D.	33.50 ± 12.681 a	33.59 ± 12.969 b	32.89 ± 15.421 ab	99.97 ± 38.403 a
PP	N.D.	38.73 ± 15.246 a	24.38 ± 9.126 ab	41.28 ± 9.129 b	104.40 ± 18.044 a

Note: Statistical comparisons among treatments were performed separately for each compound and each plant part, with values reported as the mean of five replicates (mean ± standard deviation). Different letters within each column denote significant differences among treatments based on PERMANOVA analysis ($p < 0.05$). CONTROL: Control soil without MPs added; PA: Soil with 2 % (w/w) of PA-MPs added; LDPE: Soil with 2 % (w/w) of LDPE-MPs added; PP: Soil with 2 % (w/w) of PP-MPs added.

Abbreviations: N.D, not detected; PA, polyamide; LDPE, low-density polyethylene; PP, polypropylene; GRE, glucoraphenin; GBS, glucobrassicin; MGB, methoxyglucobrassicin; DH-EU, dehydroeuricin.

Table 4. Anthocyanin content (mg 100 g^{-1} DW) in *R. sativus* plant parts after different MPs treatments.

	1-Cy	2-Cy	3-Cy	4-Cy	5-Cy	6-Cy	7-Cy	8-Cy	Total
Leaves anthocyanin									
CONTROL	N.D.	2.10 ± 1.149 a	2.23 ± 1.345 a	0.75 ± 0.506 a	1.75 ± 0.366 a	1.88 ± 1.164 a	N.D.	0.43 ± 0.357 a	9.14 ± 3.537 a
PA	N.D.	1.45 ± 0.073 a	0.88 ± 0.438 a	0.69 ± 0.391 a	0.87 ± 0.180 b	0.56 ± 0.218 b	N.D.	0.51 ± 0.219 a	4.95 ± 0.933 b
LDPE	N.D.	0.50 ± 0.067 b	0.84 ± 0.196 a	1.09 ± 0.433 a	1.45 ± 1.247 ab	0.97 ± 0.531 ab	N.D.	0.53 ± 0.183 a	5.39 ± 1.491 ab
PP	N.D.	1.00 ± 0.626 ab	0.90 ± 0.54 a	0.78 ± 0.41 a	1.45 ± 1.174 ab	1.40 ± 0.818 a	N.D.	0.31 ± 0.212 a	5.84 ± 2.332 ab
Radish anthocyanin									
CONTROL	0.65 ± 0.381 a	2.60 ± 0.826 a	47.88 ± 5.610 a	N.D.	2.23 ± 1.212 a	N.D.	3.59 ± 1.093 a	1.93 ± 0.688 a	60.11 ± 8.508 a
PA	1.02 ± 0.534 a	2.62 ± 0.890 a	53.51 ± 23.503 a	N.D.	2.41 ± 0.741 a	N.D.	4.04 ± 1.116 a	2.54 ± 0.864 ab	67.59 ± 28.008 a
LDPE	0.69 ± 0.594 a	2.43 ± 0.912 a	41.06 ± 18.155 a	N.D.	2.09 ± 0.628 a	N.D.	3.96 ± 0.887 a	1.67 ± 0.764 a	53.34 ± 21.364 a
PP	0.88 ± 0.359 a	2.49 ± 0.787 a	52.00 ± 10.841 a	N.D.	4.57 ± 1.429 b	N.D.	8.82 ± 3.462 b	3.69 ± 1.292 b	74.34 ± 11.777 a

Note: Statistical comparisons among treatments were performed separately for each compound and each plant part, with values reported as the mean of five replicates (mean ± standard deviation). Different letters within each column denote significant differences among treatments based on PERMANOVA analysis ($p < 0.05$). CONTROL: Control soil without MPs added; PA: Soil with 2 % (w/w) of PA-MPs added; LDPE: Soil with 2 % (w/w) of LDPE-MPs added; PP: Soil with 2 % (w/w) of PP-MPs added.

Abbreviations: N.D, not detected; PA, polyamide; LDPE, low-density polyethylene; PP, polypropylene; 1-Cy, CY-3-O-(SI)soph-5-O-glu; 2-Cy, CY-3-O-(FE)soph-5-O-(MA)shop; 3-Cy, CY-3-O-(FE-SI)soph-5-O-soph + CY-3-O-(pCoA)soph-5-O-(MA)glu; 4-Cy, CY-3-O-(pCoA)soph-5-O-(MA)glu + CY-3-O-(FE-SI)diglu-5-O-glu; 5-Cy,CY-3-O-(SI)soph-5-O-(MA)glu + CY-3-O-(FE)soph-5-O-(MA)glu; 6-Cy, CY-3-O-(pCoA-SI)soph-5-O-(MA)soph or CY-3-O-(diFE)soph-5-O-(MA)soph; 7-Cy, CY-3-O-(pCoA-SI)soph-5-O-(MA)glu + CY-3-O-(pCoA-SI)soph-5-O-(MA)soph or Cy-3-O-(pCoA-SI)soph-5-O-(MA)soph + Cy-3-O-(FE-SI)soph-5-O-(MA)glu; 8-Cy, Cy-3-0-(pCoA-SI)soph-5-O-(MA)glu.

3.3. Relationships between soil and *R. sativus* characteristics

PCA biplots were constructed by integrating all variables evaluated, separating the soil and plant characteristics (Supplementary Figure S3). The PCA biplot for the first two principal components was calculated from microbiological and chemical soil properties (Figure S3 A) explained 40.7 % of the total variability, with PC1 and PC2 explaining 23.8 % and 16.9 % of the variance, respectively. The microbiological and chemical eigenvectors clearly differentiated the soil samples by varying treatments (Figure S3 A). The PA, LDPE, and PP treatments and the control soil formed independent clusters in different factor space regions. The horizontal axis (PC1) distinguishes soil without MPs (left cluster) from soils with the addition of PA and LDPE (center clusters), followed by PP (right cluster). The soil properties with the largest positive loadings on PC1 (*nirS, nifH* and *amoA* genes abundances) mainly influenced scores for soils with PP and LDPE. In contrast, variables with more negative loadings (PO and MBC) mainly influenced control and PA scores. The PCA biplot for the initial two main components calculated using the *R. sativum* leaf characteristics (Figure S3 B) explained 41.8% of the total variability, with PC1 and PC2 explaining 27.2% and 14.6% of the variance, respectively. Eigenvectors differentiated the control samples by treatment (Figure S3 B). The horizontal axis (PC1) differentiated between leaves from the control treatment (left cluster) and leaves from the MPs treatments (center clusters). Leaf characteristics with the largest positive loadings on PC1 (GRE, Apx, and GR) mainly influenced the scores for soils with MPs, whereas variables with higher negative loadings (total anthocyanins and ORAC) mainly influenced the scores of the control. A PCA biplot of the first two main components calculated from the radishes of *R. sativum* (Figure S3 C) explained 47.8% of the total variability, with PC1 and PC2 explaining 28.6% and 19.2% of overall variance, respectively. Eigenvectors differentiated the PP treatment from the other treatments (Figure S3 C). The horizontal axis (PC1) differentiated radishes from plants grown in the PP treatment (right cluster) from radishes from plants grown in soils with PA, LDPE, and the control (center-left clusters). The radish characteristics with the

highest negative loadings on PC1 (ORAC and some anthocyanins) mostly influenced PP treatment scores. The PCA biplot for the first two main components was constructed using the rootlet characteristics of *R. sativum* (Figure S3 D) explained 50.7% of overall variability, with PC1 and PC2 explaining 36.1% and 14.6% of the variance, respectively. Eigenvectors differentiated rootlets in the PA treatment compared to those in the other treatments (Figure S3 D). The rootlet characteristic that had the greatest influence on the PA treatment scores was fresh weight (W).

Pearson's correlation (*r*) analysis (Figure S4) revealed interactions between soil properties. Soil properties showed strong positive correlations ($p < 0.05$) (Figure S4A), with the highest NH_4^+ and AN ($r = 0.94$), and DOC and DTC ($r = 0.87$). Notably, several positive correlations were also found among gene abundance, including *nifH* and *nirS* ($r = 0.87$), *nifH* and *amoA* ($r = 0.83$), and *nirS* and *amoA* ($r = 0.83$). In addition, MPs pollution has tissue-specific responses in *R. sativus* (leaves, radish, and rootlets). According to the correlation analysis of the leaves (Figure S4B), several interesting positive correlations were observed ($p < 0.05$), such as the SPAD value and GRE ($r = 0.62$), 3-Cy and ORAC ($r = 0.70$), and total anthocyanins and ORAC ($r = 0.69$). A strong correlation was also observed between total anthocyanin and 3-Cy anthocyanin content ($r = 0.90$). Analysis of the correlations in *R. sativus* radishes (Figure S4C), certain compounds showed a positive relationship with the total content of bioactive compounds. For instance, GBS was strongly positively correlated with total glucosinolate content ($r = 0.84$), and 3-Cy with total anthocyanin content ($r = 0.97$). In particular, 3-Cy had a highly positive correlation with the total anthocyanin content in both leaves and radishes, suggesting its important role in the anthocyanin content within plant tissues. Finally, in rootlets (Figure S4D), an interesting positive correlation was evident between MGB content and POX activity ($r = 0.68$).

DistLM analysis indicated that PO, MBC, *nifH,* and *nirS* significantly influenced *R. sativus* characteristics when the soil properties were analyzed independently of plant characteristics (Supplementary Table S2) ($p < 0.05$). In contrast, UR, BG, ACP, CH, BR, pH, DTC, DOC, NO3, NH4, AN, AP, 16S, *amoA*, and *nosZ* did not significantly affect plant characteristics. The best model for predicting changes in *R. sativus* from soil with was composed of PO and MBC MPs (R^2 = 0.36928, AICs = 112.09; Table S2).

Distance-based redundancy analysis (dbRDA) for soil properties indicated that the percentage variation explained by dbRDA1 was 89.5% out of the fitted model and 33.1% out of the total variation, whereas the total variation explained by dbRDA2 was 10.5% out of the fitted model and 3.9% out of the total variation (Figure 6). The dbRDA1 axis clearly discriminated between the *R. sativus* responses of the control for LDPE and PP. The results revealed that MBC and PO had a strong influence on *R. sativus*, especially in plants grown in PP and LDPE-amended soils. The model clearly separated the control groups from the clusters formed by LDPE and PP treatments (Figure 6).

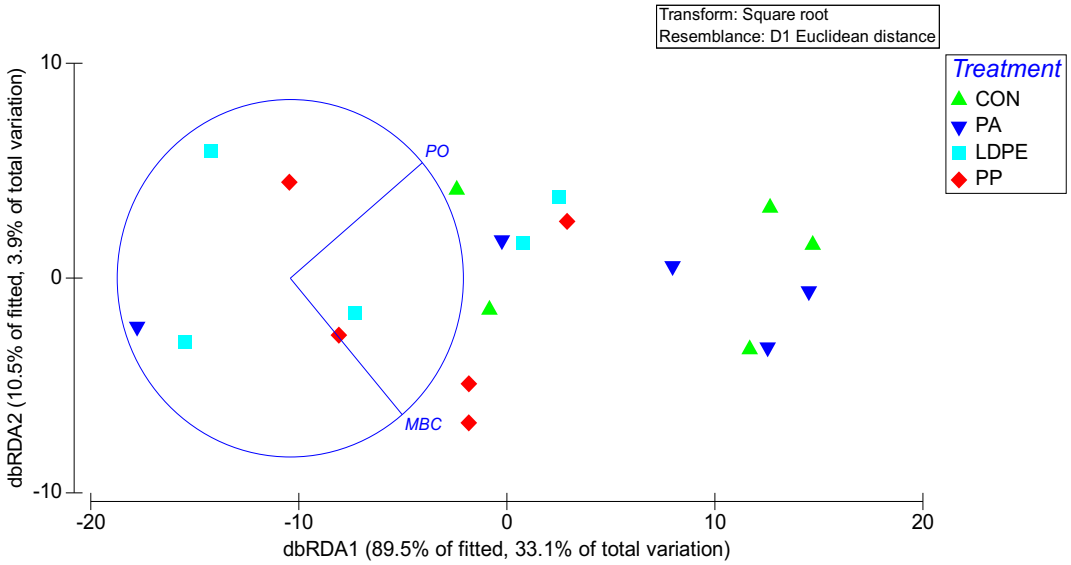


Fig. 6. Distance-based Redundancy analysis (dbRDA) bi-plot of different treatments of soil polluted with MPs and control soil. MBC; microbial biomass-C; PO, polyphenol content.

4. DISCUSSION

4.1. Changes in soil parameters driven by MPs addition.

Our study results show that MPs can alter the microbiological and chemical properties of Andisol over a short period of time. Soil pH increased in all MPs treatments, which agrees with other studies conducted in an Andisol where MPs of similar size were used (Riveros et al., 2024). The “master variable” for soil chemistry is soil pH, given its effect on numerous chemical reactions and nutrient availability (Miralles et al., 2021; Penn and Camberato, 2019)

The DOC levels decreased in the PA treatments (2 % w/w). This aligns the results of Riveros et al., (2024), who found that DOC decreased in soils incubated with 3 % w/w PA. Studies conducted with lower doses of PA (1 % or 0.5 % w/w) have not shown any statistically significant changes (Riveros et al., 2024; Shi et al., 2022). PO tended to decrease in MPs treatments; however, this decrease was statistically significant only in the PP treatment. This result agrees with Riveros et al. (2024), which indicated that polyphenols are negatively affected by MPs pollution. Polyphenols play a key role in soil by serving as substrates for microbial respiration (Schmidt et al., 2013). Both carbon sources (PO and DOC) were affected by the MPs contamination. Upon entering the soil, MPs destroy their structure along with the physical protection of soil aggregates, changing the biodegradation of litter and soil organic matter and causing rapid soil nutrient depletion, ultimately affecting carbon pools such as DOC content, which microorganisms use as an immediate source of C (Liu et al., 2016; Yu et al., 2020; Zhang et al., 2022b).

The present study showed that MPs addition altered some microbiological properties, including enzymatic activities, soil basal respiration, and the abundance of N cycle genes in the short term when *R. sativus* was present. First, LDPE decreased acid phosphatase activity. However, Fei et al. (2020) and (Pinto-Poblete et al., 2023) indicated that acid phosphatase activity was

enhanced in soil polluted with PVC and HDPE. Phosphatase activity can vary depending on several factors. For ex. Ya et al. (2022) demonstrated that phosphatase activity increases in soil polluted with LDPE on day 7 of soil incubation; however, on day 35, activity fell compared to the soil without MPs addition. Zhao et al. (2021) showed that variations in acid phosphatase activity depend on the period, polymer type, and shape. These variations underscore the need for further research into the effects of MPs on phosphatase activity. bPhosphatase enzymes facilitate the breakdown of phosphate in soil, transforming organic phosphorus into inorganic phosphorus, which is essential for plants, microorganisms, and ecosystem functions (Wang et al., 2022b) The addition of β-glucosidase MPs (LDPE and PP) increased their activity; however, Riveros et al. (2024) and Shah et al. (2023) reported different results in treatment with PA (1 % and 3 % w/w), PS (10 % w/w), LDPE (3 % and 10 % w/w), and PVC (10 % w/w), which decreased β-glucosidase activity. β-Glucosidase helps degrade cellulose in soils and regulates an important energy source supply for microorganisms. Turner et al. (2002) and Zhao et al. (2021) showed that alterations in β-glucosidase activity depend on the polymer type and exposure time of MPs incubations. The urease and dehydrogenase activities were not affected by MPs addition. This agrees with Pinto-Poblete et al. (2023, 2022) and Riveros et al. (2024), who also studied MPs incubations in an Andisol, and found no MPs pollution impact. However, studies on other soil orders have described altered activities of both enzymes with PS, PVC, LDPE, and PP (Fan et al., 2022; Fei et al., 2020; Shah et al., 2023; Ya et al., 2022). Allophane is a predominant clay in Andisols and can immobilize enzymes (Floody et al., 2009). This capacity of allophane probably inhibits urease and dehydrogenase activity; however, additional research is required to validate this hypothesis. MPs addition did not affect MBC, but increased BR. These changes were not aligned with (Pinto-Poblete et al., 2023, 2022) and Shi et al. (2022) who used lower doses of MPs (0.2 g MPs kg^{-1} soil and 0.5% w/w, respectively). This is because high MPs doses can add C and serve as nutrients that increase microbial activity (De Souza Machado et al., 2019; Shi et al., 2022).

In this study, qPCR showed that MPs pollution had no effect on the bacterial 16S rRNA gene copy number, suggesting that MPs pollution did not statistically affect bacterial abundance in the soil, which aligns with the results obtained in the MBC analysis. Rong et al. (2023) studied 16S rRNA gene abundance in soils after adding of PE, PA, PES, or PVC MPs (2 % w/w), finding that MPs addition affected the 16S rRNA gene abundance in fluvo-aquic soil but not black soil, which indicates that effects on the abundance of bacteria in soils is mainly due to the soil type studied. The majority of functional genes participating in N cycling were not significantly altered by the addition of PA or LDPE MPs. However, in soils contaminated with PP, a statistically significant increase in the abundance of *amoA, nifH,* and *nirS* genes was observed compared that to in the control soil. An increase in the abundance of the *nifH* and *nirS* genes with the addition of PP was also reported by Kim et al. (2023). *NifH* is a nitrogen fixation-related functional gene that encodes a nitrogenase reductase subunit that fixes N_2 to ammonia (Shen et al., 2022; Sun et al., 2023, 2022). The abundance of *amoA* was also higher in PP. This did not agree with Kim et al. (2023) who observed no changes in gene abundance when PP was added. However, Rong et al. (2021) demonstrated a stimulatory effect of PA, PES, and PVC on AOB *amoA* abundance in soils. These differences may be because in our study, the AOA *amoA* genes (archaeal) were not quantified, which in some agricultural soils have been found to be up to 3000 times more than the AOB *amoA* genes (bacteria) (Leininger et al., 2006). The *amoA* gene is a nitrification-related functional gene that encodes ammonia monooxygenase, which oxidizes ammonia to hydroxylamine (NH_2OH) (Kim et al., 2023; Rong et al., 2021). Moreover, *nirS* is a denitrification-related functional gene that encodes a nitrite reductase that converts NO_2^- into NO (Sun et al., 2023, 2022). The stimulatory effect of the addition of PP was also demonstrated by Kim et al. (2023) who suggested an increase in the abundance of bacteria capable of reducing nitrite (Rong et al., 2023). The *nosZ* gene*,* which encodes nitrous oxide reductase (N_2O to N_2) (denitrification), was not affected by MPs addition which is consistent with the findings of Kim et al. (2023). To the best of our knowledge,

this is the first study regarding the effect of MPs on nitrogen cycling functional gene abundance in an Andisol and complements the few studies carried out worldwide in soils. It is important to study various soil types because it has been demonstrated that various soil ecosystems respond differently to MPs with respect to nitrogen cycling in soil (Rong et al., 2023). The results regarding the addition of PP and its effect on nitrogen-related genes confirmed that MPs could alter nitrogen fixation, nitrification, and denitrification. However, in our study, the changes in microbial composition were not statistically reflected in the N composition of the soil, indicating that microbial relationships, enzymatic reactions, and soil nutrients are related in a complex manner, which requires more in-depth studies.

4.2. Changes in *R. sativus* induced by MPs addition

In the present study, morphological and physiological alterations were observed in *R. sativus* plants grown in soil contaminated with MPs. The increase in leaves, radish and rootlets biomass evidenced by the action of certain MPs, and the overall biomass increase with all MPs, is not new; previous authors have demonstrated biomass increases in plants grown in soils contaminated with MPs (*Allium fistulosum, Glycine max* L., and *R. sativus* L.) (Botyanszká et al., 2022; De Souza Machado et al., 2019; Shah et al., 2023). The increase in biomass may be attributed to indirect factors that change soil properties, such as MPs decreasing soil bulk density, which reduces penetration resistance for plant roots and therefore enhances water and nutrient uptake (De Souza Machado et al., 2018b). Direct factors included a noticeable increase in the relative chlorophyll content, especially in plants grown under the PA and LDPE treatments. Chlorophyll content is related to growth, and it has been demonstrated that plants with higher chlorophyll content exhibit greater development than those with lower levels of this pigment (Del Ángel-Hernández et al., 2017).

In our study, more soluble proteins were observed in radishes grown in PA and PP, consistent with Zhang et al. (2023), who also reported greater soluble proteins in *Zea mays* along with enhanced chlorophyll content and biomass. Soluble proteins serve as the primary osmotic adjustment substances in plants, indicating the impact of external stress and are crucial in maintaining the stability of cellular enzyme systems (Li et al., 2023). Conversely, soluble proteins decreased in the rootlets of plants grown in PA, which may be attributed to the translocation of proteins from rootlets to the radish via the phloem (Paultre et al., 2016). However, more in-depth research is necessary to investigate this hypothesis thoroughly.

In this study, we analyzed malondialdehyde accumulation in various plant parts, including leaves, rootlets, and radish. A significant MDA content increase was noted only in radish, specifically in LDPE and PP. These results align with previous findings that MPs generally cause an increase in MDA content in *R. sativus* (Cui et al., 2022; López et al., 2022), as well as in other plant species (Ma et al., 2022; Wu et al., 2024). Malondialdehyde, a byproduct of lipid peroxidation, serves as a marker for cell damage in plants under stress (Sahasa et al., 2023). Oxidative stress generally causes ROS accumulation Wang et al. (2022) and plant cells raise their antioxidant enzyme activity levels to remove excess internal ROS to avoid toxic damage (Guo et al., 2022). Cellular protection mechanisms against stress, such as the antioxidant capacity of tissues and activity of stress-response enzymes in plants, have been studied. ORAC analysis revealed that MPs triggered higher antioxidant capacity in the edible root (radish) of the plant. However, there was no enzymatic response in the radish compared with that in the control. In the rootlets, decreases in POX (PA treatment) and SOD (PP treatment) were evident. These results are consistent with those of Cui et al. (2022), who observed a decrease in SOD in the roots of *R. sativus* under PP treatment. Studies on other species, such as *Brassica chinensis* L. (Zhang et al., 2022a) and herbaceous ornamental plants, showed that SOD, POD, and CAT were stimulated by MP-related stress (Guo et

al., 2022). Enzymatic responses are species dependent, and MPs can create oxidative stress in a dose- and size-dependent manner (Wang et al., 2022a).

The total glucosinolate content showed no significant alterations in MPs treatment compared with the control in leaves, radishes, or rootlets. However, specific glucosinolates were affected. In leaves, a significant increase in the GRE was observed across all MP treatments, indicating a specific response to MPs exposure. In the case of radishes distinct glucosinolate profiles were observed between radishes grown under the PP and radishes in LDPE and PA treatments. Specifically, PP treatment resulted in higher levels of dehydroerucin and glucobrassicin, indicating that certain glucosinolates were more responsive to PP than to PA or LDPE exposure. In rootlets, dehydroeuricin decreased in the PA treatment in contrast to the control, but glucobrassicin and methoxyglucobrassicin content increased under LDPE and PP. This suggests that MPs, particularly PP and LDPE, can modulate glucosinolate biosynthesis in rootlets, potentially triggering stress response. This is consistent with previous studies on *Brassica oleracea* var. *sabauda* plants exposed to PS and PE MPs that showed a slight increase in glucosinolate content in response to MPs exposure (Liese et al., 2024). Overall, the MPs tended to alter the glucosinolate profile, leading to an increase in specific glucosinolates in the rootlets of *R. sativus* compared with the control. Several glucosinolates are involved in the regulation of water uptake through aquoporins, changing the root hydraulic characteristics (Martínez-Ballesta et al., 2015, 2014) which is coincident with our work. Additionally, glucosinolates secreted by roots modify all reactions in the rhizosphere, interacting with microorganisms and alter microbial community structure (Chroston et al., 2024; Nicolas-Espinosa et al., 2023). Furthermore, our study showed an increase in indole glucosinolates in rootlets (methoxyglucobrassicin and glucobrassicin), and it has been shown that some of these glucosinolates, such as glucobrassicin, break down into indole-3-acetonitrile, a precursor of the auxin indole-3-acetic acid (IAA), a phytohormone involved in plant development and growth (Hornbacher et al., 2022). This could

explain the increase in biomass of *R. sativus*. However, more studies measuring this phytohormone are needed to confirm this. The results showed the impact of MPs on the nutritional and bioactive compounds of this crop, which could have implications for food quality and human health. Additional studies are needed to understand the mechanisms underlying these changes and their long-term implications for food safety and agricultural productivity.

MPs pollution has varying effects on anthocyanin content depending on the plant part analyzed and the MPs tested. In leaves, specific anthocyanins, such as 5-Cy and 6-Cy, showed decreased content under PA treatment, leading to a general decrease in total anthocyanin content compared with the control. Conversely, in the radishes, this trend was completely different; the total anthocyanin content did not show significant differences across treatments. However, individual anthocyanins such as 5-Cy, 7-Cy, and 8-Cy exhibited substantial increases in the PP treatment compared to the control. These findings partially align with López et al. (2022), who also observed an increase in anthocyanin content in *R. sativus*, although they analysed whole sprouts without separating plant organs. Interestingly, the data suggest that MPs effects are both treatment- and tissue-specific, while PA and LDPE treatments led to lower anthocyanin content in leaves, and PP treatment resulted in an increase in radishes. This demonstrates a tissue-specific response to MPs, likely driven by direct physical contact and upregulation of certain anthocyanins in radishes, as a protective mechanism against oxidative stress. Anthocyanins are crucial in neutralizing reactive oxygen species, and their increased in response to MPs exposure highlights their critical role in plant defence against oxidative damage (Lee et al., 2022). Enhanced concentrations of these anthocyanins, particularly in edible parts, could have implications for the nutritional quality of plants. Understanding such interactions is key for assessing the broader implications of MPs contamination in agroecosystems and developing strategies to mitigate their impact on human health and food quality. Further molecular studies are required to elucidate the mechanisms underlying these changes.

4.3. MPs' effect on the interrelationships of soil and R. sativus characteristics

Overall, the PCA highlighted treatment-specific effects on soil variables, with nitrogen cycling and carbon dynamics being the primary factors affected by different MPs. Separate clustering of the control group underscores the significant alterations in soil chemical and microbial properties caused by MPs. These changes have been widely documented in previous studies across various soil types, demonstrating the broad impact of MPs on soil balance and ecosystems (Li et al., 2024; Riveros et al., 2024; Yan et al., 2021). Significant relationships between nutrient dynamics and soil microbial properties were identified using PCA and Pearson correlation analysis. Treatments of PP and LDPE were associated with N-related microbial processes, as indicated by positive loadings of PC1 for the genes *nirS, nifH*, and *amoA*. The strong Pearson correlation among functional genes *nifH, nirS*, and *amoA* suggests an interconnected nitrogen cycle influenced by MPs, highlighting co-regulation of nitrogen fixation and nitrification processes. This suggests that these MPs have the potential to alter nitrogen cycle pathways, thereby affecting plant growth. Several authors have described changes in soil properties playing a key role in the nitrogen cycle (Riveros et al., 2022; Rong et al., 2021; Sun et al., 2022). These alterations indicate potential long-term soil health and plant growth impacts in farm systems contaminated with MPs.

The results of the PCA and Pearson correlation analyses highlighted the significant impact of MPs on various physiological and biochemical parameters of *R. sativus*, demonstrating tissue-specific responses in leaves, radishes, and rootlets. This aligns with the literature, which stated that *R. sativus* exhibits tissue-specific responses to MPs exposure, with distinct effects observed in the roots and leaves (Cui et al., 2022). The PCA biplot for leaf characteristics explained 41.8 % of the total variability, with PC1 differentiating control samples from MP-treated samples. The positive loadings of glucoraphenin, ascorbate

peroxidase, and glutathione reductase activities in the MPs treatments suggest that these glucosinolates and, to a lesser extent, these antioxidant enzymes are upregulated in response to MPs pollution. The correlations between 3-Cy, total anthocyanins, and ORAC suggest that anthocyanins play a key role in antioxidant defense in leaves, with 3-Cy contributing significantly to total anthocyanin levels in leaves. López et al. (2022) demonstrated an increase in anthocyanins and glucosinolates in broccoli and radish exposed to MPs, providing evidence that anthocyanins, such as antioxidant enzymes, are essential for plants' defence against oxidative stress by neutralizing ROS. Correlation analysis indicated a positive relationship between the SPAD (relative chlorophyll content) and glucoraphenin. This behavior was also observed in *B. oleracea* plants under drought stress, in which the aromatic glucosinolate glucosinalbin showed a strong positive correlation with SPAD values (Ben Ammar et al., 2023). This indicates that higher chlorophyll levels may be associated with higher production of certain glucosinolates under stress. Nevertheless, further research is required to confirm this hypothesis.

In radish characteristics, PCA explained 47.8 % of the total variability, with the PP treatment separating spatially from the other treatments along PC1. The correlation analysis revealed that 3-Cy was one of the critical anthocyanins in the tissues of radishes, as its content was significantly positively correlated with the total anthocyanin content, suggesting that it plays a key role in oxidative stress defence (López et al., 2022). For rootlet characteristics, PCA explained 51 % of the total variability, with fresh weight contributing most significantly to the separation of MPs treatment. Contrary to expectations, this study showed an increase in rootlets biomass (fresh weight) due to soil contamination with PA and LDPE MPs. However, this increase may be attributed to indirect factors that change soil properties, such as lower soil bulk density caused by MPs, which decreases soil penetration resistance, allowing for improved root growth and enhanced water and nutrient uptake (De Souza Machado et al., 2018b). Alternatively, it may be due to the observed increase in indole glucosinolates in

rootlets (methoxyglucobrassicin and glucobrassicin) and their role in the synthesis of the phytohormone IAA, which participates in plant development and growth (Hornbacher et al., 2022). Finally, the correlation between methoxyglucobrassicin and POX activity in rootlets suggested that MPs exposure may trigger oxidative stress in root tissues, with methoxyglucobrassicin production potentially playing a protective role against oxidative damage. This indicates that glucosinolates in rootlets are involved in plant defence mechanisms against MP-induced stress, as shown by López et al. (2022). PCA and correlation results emphasize the complex interaction between MPs and plant metabolism, with potential implications for crop quality and nutritional value. Further research is needed to understand long-term impacts of MPs on plant productivity and health in agricultural systems.

DistLM analyses offer valuable insights into how soil properties affect the physiological and biochemical characteristics of *R. sativus* in soils polluted by MPs. DistLM results showed that the soil properties PO, MBC, *nifH*, and *nirS* gene abundance were predictor variables of *R. sativus* grown in soil with MPs. Interestingly, the importance of PO can be attributed to its role as a plant-derived compound that significantly influences soil dynamics by working as a substrate for microbial respiration or by selectively promoting certain microbial communities (Schmidt et al., 2013). Phenolic compounds can directly influence decomposer communities' activity and composition, thereby affecting decomposition rates and nutrient cycling, particularly nitrogen cycling, through their ability to bind to proteins (Fan et al., 2017; Hättenschwiler and Vitousek, 2000). Prior studies have also shown that MPs alter soil bacterial networks' structure, and the functional groups involved in soil N cycling processes (Rong et al., 2021). Further studies on the links between polyphenols and the abundance of N-cycle-related genes will help to fill important gaps.

dbRDA identified MBC and PO as primary predictors in the influence of MPs-polluted soil on *R. sativus*. Changes in MBC reflect shifts in microbial

activity and community structure, influencing nutrient availability and plant-microbe interactions. In addition, prior studies indicated that MBC was directly related to soil organic carbon and plant biomass (Wang et al., 2021). Additionally, PO may indicate a plant response to oxidative stress, especially in *R. sativus*, which has been found to have higher concentrations of anthocyanins and polyphenols than other Brassica species (Tomas et al., 2021). These results show the importance of studying PO in more depth, not only in soil but also in studying total polyphenols in plants and their exudation into the soil. These findings stress the importance of considering soil-plant interactions when evaluating the ecological impacts of MPs pollution in agricultural systems. More studies are needed to understand the long-term effects of these soil-plant interactions on plant health and productivity.

5. CONCLUSION

This study provides evidence of the significant effects of MPs on both Andisol properties *and R. sativus* characteristics. MPs alter key soil chemical and microbiological properties increasing soil pH while causing a decrease in both DOC and PO contents, particularly in PA- and PP-treated soils. The addition of MPs increased microbial activity, as evidenced by elevated soil respiration and β-glucosidase activity in the LDPE and PP treatments. However, no significant changes were observed in urease or dehydrogenase activity. Importantly, the abundance of nitrogen-cycling genes, including *nifH*, *amoA*, and *nirS*, was significantly higher in PP-contaminated soils, indicating that MPs influence critical processes in the nitrogen cycle, potentially affecting nutrient availability for plants. In *R. sativus*, MPs resulted in considerable increases in total biomass, especially in rootlets and radishes, with radish biomass increasing by up to 125 % in PP-treated soils. Chlorophyll content (SPAD values) also increased in plants grown in soils with PA and LDPE, indicating enhanced photosynthesis and growth. However, this biomass gain was accompanied by a higher oxidative stress, as indicated by elevated MDA content in radishes under

LDPE and PP treatments. Despite oxidative stress, the antioxidant capacity (ORAC values) increased in radishes, although enzyme activities (SOD and POX) showed varying and tissue-specific responses. For example, SOD activity increased in leaves under LDPE treatment, whereas POX activity was reduced in rootlets under PA treatment. Furthermore, the study revealed that MPs have a complex, tissue-specific impact on bioactive compounds, such as glucosinolates and anthocyanins. The total glucosinolate content remained unchanged; however, specific glucosinolates increased (glucoraphenin increased in leaves across all MPs treatments and indole glucosinolates in rootlets). Anthocyanin content responded differently across tissues, decreasing in leaves but increasing in radishes under PP treatment. These findings show that MPs alter plant growth, stress responses and change phytochemical and nutritional composition, potentially influencing their health benefits for humans. PO and MBC have emerged as key predictors of *R. sativus* responses to MPs pollution, with potential long-term effects on plant-microbe interactions and soil fertility. These findings highlight the need for more long-term studies to better comprehend the broader implications of MPs in agroecosystems, particularly their potential impact on crop productivity, food safety, and human health. Addressing MP contamination in soil is critical for ensuring sustainable agricultural practices and safeguarding the nutritional quality of crops.

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CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article.

CONSENT FOR PUBLICATION

All the authors have read the manuscript and have agreed to submit it in its current form for consideration for publication.

REFERENCES

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans Automat Contr 19, 716–723. https://doi.org/10.1109/TAC.1974.1100705

Alef, K., Nannipieri, P., 1995. Methods in Applied Soil Microbiology and Biochemistry: Bioremediation of soil, Methods in Applied Soil Microbiology and Biochemistry. https://doi.org/10.1016/B978-0-12-513840-6.X5014-9

Anderson, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences 58, 626–639. https://doi.org/10.1139/cjfas-58-3-626

Baenas, N., Ferreres, F., García-Viguera, C., Moreno, D.A., 2015. Radish sprouts-Characterization and elicitation of novel varieties rich in anthocyanins. Food Research International 69, 305–312. https://doi.org/10.1016/j.foodres.2015.01.009

Baenas, N., Villaño, D., García-Viguera, C., Moreno, D.A., 2016. Optimizing elicitation and seed priming to enrich broccoli and radish sprouts in glucosinolates. Food Chem 204, 314–319. https://doi.org/10.1016/j.foodchem.2016.02.144

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philosophical

Transactions of the Royal Society B: Biological Sciences 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Ben Ammar, H., Arena, D., Treccarichi, S., Di Bella, M.C., Marghali, S., Ficcadenti, N., Lo Scalzo, R., Branca, F., 2023. The Effect of Water Stress on the Glucosinolate Content and Profile: A Comparative Study on Roots and Leaves of Brassica oleracea L. Crops. Agronomy 13. https://doi.org/10.3390/agronomy13020579

Botyanszká, L., Šurda, P., Vitková, J., Lichner, Igaz, D., 2022. Effect of microplastics on silty loam soil properties and radish growth. Journal of Hydrology and Hydromechanics 70, 321–329. https://doi.org/10.2478/johh-2022-0018

Brink, R.H., Dubach, P., Lynch, D.L., 1960. Measurement of carbohydrates in soil hydrolyzates with anthrone. Soil Sci 89, 157–166. https://doi.org/10.1097/00010694-196003000-00006

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clin Chem 55, 611–622. https://doi.org/10.1373/CLINCHEM.2008.112797

Chroston, E.C.M., Bziuk, N., Stauber, E.J., Ravindran, B.M., Hielscher, A., Smalla, K., Wittstock, U., 2024. Plant glucosinolate biosynthesis and breakdown pathways shape the rhizosphere bacterial/archaeal community. Plant Cell Environ 47, 2127–2145. https://doi.org/10.1111/PCE.14870

Cui, M., Yu, S., Yu, Y., Chen, X., Li, J., 2022. Responses of cherry radish to different types of microplastics in the presence of oxytetracycline. Plant Physiology and Biochemistry 191, 1–9. https://doi.org/10.1016/j.plaphy.2022.09.012

Cusworth, S.J., Davies, W.J., McAinsh, M.R., Stevens, C.J., 2024. A nationwide assessment of microplastic abundance in agricultural soils: The influence of plastic crop covers within the United Kingdom. Plants People Planet 6, 304–314. https://doi.org/10.1002/ppp3.10430

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Glob Chang Biol 24, 1405–1416. https://doi.org/10.1111/gcb.14020

De Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S., Rillig, M.C., 2019. Microplastics Can Change Soil Properties and Affect Plant Performance. Environ Sci Technol 53, 6044–6052. https://doi.org/10.1021/acs.est.9b01339

De Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M.C., 2018. Impacts of Microplastics on the Soil Biophysical Environment. Environ Sci Technol 52, 9656–9665. https://doi.org/10.1021/acs.est.8b02212

Del Ángel-Hernández, M., Zermeño-Gonzalez, A., Isain Melendres-Alvarez, A., Gabriel Campos-Magaña, S., Cadena-Zapata, M., Arturo Del Bosque-Villarreal, G., 2017. Núm.5 30 de junio-13 de agosto, Revista Mexicana de Ciencias Agrícolas.

Eivazi', F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol Biochem 20, 601–606. https://doi.org/10.1016/0038-0717(88)90141-1

Eldridge, D.J., Delgado-Baquerizo, M., Woodhouse, J.N., Neilan, B.A., 2016. Mammalian engineers drive soil microbial communities and ecosystem functions across a disturbance gradient. Journal of Animal Ecology 85, 1636–1646. https://doi.org/10.1111/1365-2656.12574

Fan, D. mei, Fan, K., Yu, C. ping, Lu, Y. ting, Wang, X. chang, 2017. Tea polyphenols dominate the short-term tea (Camellia sinensis) leaf litter decomposition. J Zhejiang Univ Sci B 18, 99–108. https://doi.org/10.1631/jzus.B1600044

Fan, P., Tan, W., Yu, H., 2022. Effects of different concentrations and types of microplastics on bacteria and fungi in alkaline soil. Ecotoxicol Environ Saf 229. https://doi.org/10.1016/j.ecoenv.2021.113045

Fei, Y., Huang, S., Zhang, H., Tong, Y., Wen, D., Xia, X., Wang, H., Luo, Y., Barceló, D., 2020. Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil. Science of the Total Environment 707. https://doi.org/10.1016/j.scitotenv.2019.135634

Floody, M.C., Theng, B.K.G., Reyes, P., Mora, M.L., 2009. Natural nanoclays: applications and future trends – a Chilean perspective. Clay Miner 44, 161–176. https://doi.org/10.1180/CLAYMIN.2009.044.2.161

Fu, B., Zhou, W., Chen, Y., Wu, Y., Gan, W., She, N., Ma, Y., 2024. A bibliometric perspective on the occurrence and migration of microplastics in soils amended with sewage sludge. Water Environment Research 96. https://doi.org/10.1002/wer.11054

Fuller, S., Gautam, A., 2016. A Procedure for Measuring Microplastics using Pressurized Fluid Extraction. Environ Sci Technol 50, 5774–5780. https://doi.org/10.1021/acs.est.6b00816

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat Ecol Evol. https://doi.org/10.1038/s41559-017-0116

Gao, B., Yao, H., Li, Y., Zhu, Y., 2021. Microplastic Addition Alters the Microbial Community Structure and Stimulates Soil Carbon Dioxide Emissions in Vegetable-Growing Soil. Environ Toxicol Chem 40, 352–365. https://doi.org/10.1002/etc.4916

García, C., Hernandez, T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun Soil Sci Plant Anal 28, 123–134. https://doi.org/10.1080/00103629709369777

Guo, M., Zhao, F., Tian, L., Ni, K., Lu, Y., Borah, P., 2022. Effects of polystyrene microplastics on the seed germination of herbaceous ornamental plants. Science of the Total Environment 809. https://doi.org/10.1016/j.scitotenv.2021.151100

Hättenschwiler, S., Vitousek, P.M., 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol Evol 15, 238–243.

Heath' And, R.L., Packer, L., 1968. Photoperoxidation in isolated Chloroplasts I. Kinetics and Stoichiometry of Fatty Acid Peroxidation, ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS.

Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611.

Hornbacher, J., Horst-Niessen, I., Herrfurth, C., Feussner, I., Papenbrock, J., 2022. First experimental evidence suggests use of glucobrassicin as source of auxin in drought-stressed Arabidopsis thaliana. Front Plant Sci 13, 1025969. https://doi.org/10.3389/FPLS.2022.1025969/BIBTEX

Kandeler, E., Gerber, H., 1988a. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fertil Soils 6, 68–72. https://doi.org/10.1007/BF00257924

Kandeler, E., Gerber, H., 1988b. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fertil Soils 6, 68–72. https://doi.org/10.1007/BF00257924

Keshri, J., Yousuf, B., Mishra, A., Jha, B., 2015. The abundance of functional genes, cbbL, nifH, amoA and apsA, and bacterial community structure of intertidal soil from Arabian Sea. Microbiol Res 175, 57–66. https://doi.org/10.1016/J.MICRES.2015.02.007

Kim, K., Song, I.G., Yoon, H., Park, J.W., 2023. Sub-micron microplastics affect nitrogen cycling by altering microbial abundance and activities in a soil-legume system. J Hazard Mater 460. https://doi.org/10.1016/j.jhazmat.2023.132504

Lee, T.Y., Kim, L., Kim, D., An, S., An, Y.J., 2022. Microplastics from shoe sole fragments cause oxidative stress in a plant (Vigna radiata) and impair soil environment. J Hazard Mater 429, 128306. https://doi.org/10.1016/J.JHAZMAT.2022.128306

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809. https://doi.org/10.1038/nature04983

Li, G., Tang, Y., Son, Y., Zhao, Xiaoxun, Iqbal, B., Khan, K.Y., Guo, R., Yin, W., Zhao, Xin, Du, D., 2024. Divergent responses in microbial metabolic limitations and carbon use efficiency to variably sized polystyrene microplastics in soil. Land Degrad Dev 35, 2658–2671. https://doi.org/10.1002/ldr.5090

Li, R., Tu, C., Li, L., Wang, X., Yang, J., Feng, Y., Zhu, X., Fan, Q., Luo, Y., 2023. Visual tracking of label-free microplastics in wheat seedlings and their effects on crop growth and physiology. J Hazard Mater 456, 131675. https://doi.org/10.1016/J.JHAZMAT.2023.131675

Liese, B., Stock, N.L., Düwel, J., Pilger, C., Huser, T., Müller, C., 2024. Uptake of microplastics and impacts on plant traits of savoy cabbage. Ecotoxicol Environ Saf 272. https://doi.org/10.1016/j.ecoenv.2024.116086

Liu, M., Ussiri, D.A.N., Lal, R., 2016. Soil Organic Carbon and Nitrogen Fractions under Different Land Uses and Tillage Practices. Commun Soil Sci Plant Anal 47, 1528–1541. https://doi.org/10.1080/00103624.2016.1194993

López, M.D., Baenas, N., Retamal-Salgado, J., Zapata, N., Moreno, D.A., 2018. Underutilized Native Biobío Berries: Opportunities for Foods and Trade. Nat Prod Commun 13, 1681–1684.

López, M.D., Toro, M.T., Riveros, G., Illanes, M., Noriega, F., Schoebitz, M., García-Viguera, C., Moreno, D.A., 2022. Brassica sprouts exposed to microplastics: Effects on phytochemical constituents. Science of the Total Environment 823. https://doi.org/10.1016/j.scitotenv.2022.153796

Lozano, Y.M., Gordillo-Rocha, H., Waldman, W.R., Rillig, M.C., 2024. Photodegradation modifies microplastic effects on soil properties and plant performance. Journal of Applied Ecology 61, 13–24. https://doi.org/10.1111/1365-2664.14514

Lozano, Y.M., Lehnert, T., Linck, L.T., Lehmann, A., Rillig, M.C., 2021. Microplastic Shape, Polymer Type, and Concentration Affect Soil Properties and Plant Biomass. Front Plant Sci 12. https://doi.org/10.3389/fpls.2021.616645

Ma, J., Aqeel, M., Khalid, N., Nazir, A., Alzuaibr, F.M., Al-Mushhin, A.A.M., Hakami, O., Iqbal, M.F., Chen, F., Alamri, S., Hashem, M., Noman, A., 2022. Effects of microplastics on growth and metabolism of rice (Oryza sativa L.). Chemosphere 307. https://doi.org/10.1016/j.chemosphere.2022.135749

Martínez-Ballesta, M., Moreno-Fernández, D.A., Castejón, D., Ochando, C., Morandini, P.A., Carvajal, M., 2015. The impact of the absence of aliphatic glucosinolates on water transport under salt stress in Arabidopsis thaliana. Front Plant Sci 6, 138057. https://doi.org/10.3389/FPLS.2015.00524/BIBTEX

Martínez-Ballesta, M.D.C., Muries, B., Moreno, D.Á., Dominguez-Perles, R., García-Viguera, C., Carvajal, M., 2014. Involvement of a glucosinolate (sinigrin) in the regulation of water transport in Brassica oleracea grown under salt stress. Physiol Plant 150, 145–160. https://doi.org/10.1111/PPL.12082

Miralles, I., Trasar-Cepeda, C., Soria, R., Ortega, R., Lucas-Borja, M.E., 2021. Environmental and ecological factors influencing soil functionality of biologically crusted soils by different lichen species in drylands. Science of the Total Environment 794, 1–14. https://doi.org/10.1016/j.scitotenv.2021.148491

Nannipieri, P., Ceccanti, B., Cervelli, S., Matarese, E., 1980. Extraction of Phosphatase, Urease, Proteases, Organic Carbon, and Nitrogen from Soil. Soil Microbiology and Biochemistry 44, 1011–1916. https://doi.org/10.2136/sssaj1980.03615995004400050028x

Neculman, R., Rumpel, C., Matus, F., Godoy, R., Steffens, M., de la Luz Mora, M., 2013. Organic matter stabilization in two Andisols of contrasting age under temperate rain forest. Biol Fertil Soils 49, 681–689. https://doi.org/10.1007/s00374-012-0758-2

Nicolas-Espinosa, J., Garcia-Ibañez, P., Lopez-Zaplana, A., Yepes-Molina, L., Albaladejo-Marico, L., Carvajal, M., 2023. Confronting Secondary Metabolites with Water Uptake and Transport in Plants under Abiotic Stress. International Journal of Molecular Sciences 2023, Vol. 24, Page 2826 24, 2826. https://doi.org/10.3390/IJMS24032826

Nizzetto, L., Futter, M., Langaas, S., 2016. Are Agricultural Soils Dumps for Microplastics of Urban Origin? Environ Sci Technol. https://doi.org/10.1021/acs.est.6b04140

Nuamzanei, Changmai, U., SK, S., Kumar, N., Borah, B., Chikkaputtaiah, C., Saikia, R., Phukan, T., 2024. Impact of polyvinyl chloride (PVC) microplastic on growth, photosynthesis and nutrient uptake of Solanum lycopersicum L. (Tomato). Environmental Pollution 349, 123994. https://doi.org/10.1016/j.envpol.2024.123994

Palma, F., López-Gómez, M., Tejera, N.A., Lluch, C., 2014. Involvement of abscisic acid in the response of Medicago sativa plants in symbiosis with Sinorhizobium meliloti to salinity. Plant Science 223, 16–24. https://doi.org/10.1016/j.plantsci.2014.02.005

Paultre, D.S.G., Gustin, M.P., Molnar, A., Oparka, K.J., 2016. Lost in Transit: Long-Distance Trafficking and Phloem Unloading of Protein Signals in Arabidopsis Homografts. Plant Cell 28, 2016–2025. https://doi.org/10.1105/TPC.16.00249

Penn, C.J., Camberato, J.J., 2019. A critical review on soil chemical processes that control how soil ph affects phosphorus availability to plants. Agriculture (Switzerland) 9, 1–18. https://doi.org/10.3390/agriculture9060120

Pinto-Poblete, A., Retamal-Salgado, J., López, M.D., Zapata, N., Sierra-Almeida, A., Schoebitz, M., 2022. Combined Effect of Microplastics and Cd Alters the

Enzymatic Activity of Soil and the Productivity of Strawberry Plants. Plants 11, 1–15. https://doi.org/10.3390/plants11040536

Pinto-Poblete, A., Retamal-Salgado, J., Zapata, N., Sierra-Almeida, A., Schoebitz, M., 2023. Impact of polyethylene microplastics and copper nanoparticles: Responses of soil microbiological properties and strawberry growth. Applied Soil Ecology 184, 104773. https://doi.org/10.1016/j.apsoil.2022.104773

Poblete-Grant, P., Suazo-Hernández, J., Condron, L., Rumpel, C., Demanet, R., Malone, S.L., Mora, M. de L.L., 2020. Soil available P, soil organic carbon and aggregation as affected by long-term poultry manure application to Andisols under pastures in Southern Chile. Geoderma Regional 21, 1–10. https://doi.org/10.1016/j.geodrs.2020.e00271

Ranauda, M.A., Zuzolo, D., Maisto, M., Tartaglia, M., Scarano, P., Prigioniero, A., Sciarrillo, R., Guarino, C., 2024. Microplastics affect soil-plant system: Implications for rhizosphere biology and fitness of sage (Salvia officinalis L.). Environmental Pollution 346. https://doi.org/10.1016/j.envpol.2024.123656

Ribéreau-Gayon Pascal, 1968. Les Composés phénoliques des végétaux. Dunod, Paris.

Rillig, M.C., Lehmann, A., de Souza Machado, A.A., Yang, G., 2019. Microplastic effects on plants. New Phytologist. https://doi.org/10.1111/nph.15794

Riveros, G., Soria, R., Villafuerte, A., Gómez, P., Ortega, R., Miralles, I., Schoebitz, M., 2024. Effects of low-density polyethylene and polyamide microplastics on the microbiological and chemical characteristics of an Andisol. Soil Use Manag 40. https://doi.org/10.1111/sum.12991

Riveros, G., Urrutia, H., Araya, J., Zagal, E., Schoebitz, M., 2022. Microplastic pollution on the soil and its consequences on the nitrogen cycle: a review. Environmental Science and Pollution Research 29, 7997–8011. https://doi.org/10.1007/s11356-021-17681-2

Rong, L., Wang, Y., Meidl, P., Wang, L., Sun, H., 2023. Microplastics affect soybean rhizosphere microbial composition and function during vegetative and reproductive stages. Ecotoxicol Environ Saf 252. https://doi.org/10.1016/j.ecoenv.2023.114577

Rong, L., Zhao, Longfei, Zhao, Leicheng, Cheng, Z., Yao, Y., Yuan, C., Wang, L., Sun, H., 2021. LDPE microplastics affect soil microbial communities and nitrogen cycling. Science of the Total Environment 773. https://doi.org/10.1016/j.scitotenv.2021.145640

Sadzawka, A., Carrasco, M.A., Grez, R., Mora, M., Flores, H., Neaman, A., 2006. Métodos de análisis recomendados para los suelos de Chile. Revision 2006, Serie Actas INIA N34. ed. Instituto de Investigaciones Agropecuarias.

Sahasa, R.G.K., Dhevagi, P., Poornima, R., Ramya, A., Karthikeyan, S., Priyatharshini, S., 2023. Dose-dependent toxicity of polyethylene microplastics (PE-MPs) on physiological and biochemical response of blackgram and its associated rhizospheric soil properties. Environ Sci Pollut Res Int 30, 119168–119186. https://doi.org/10.1007/s11356-023-30550-4

Schmidt, M.A., Kreinberg, A.J., Gonzalez, J.M., Halvorson, J.J., French, E., Bollmann, A., Hagerman, A.E., 2013. Soil microbial communities respond differently to three chemically defined polyphenols. Plant Physiology and Biochemistry 72, 190–197. https://doi.org/10.1016/j.plaphy.2013.03.003

Shah, T., Ali, A., Haider, G., Asad, M., Munsif, F., 2023. Microplastics alter soil enzyme activities and microbial community structure without negatively affecting plant growth in an agroecosystem. Chemosphere 322. https://doi.org/10.1016/j.chemosphere.2023.138188

Shen, M., Song, B., Zhou, C., Almatrafi, E., Hu, T., Zeng, G., Zhang, Y., 2022. Recent advances in impacts of microplastics on nitrogen cycling in the environment: A review. Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2021.152740

Shi, J., Sun, Y., Wang, X., Wang, J., 2022. Microplastics reduce soil microbial network complexity and ecological deterministic selection. Environ Microbiol 24, 2157–2169. https://doi.org/10.1111/1462-2920.15955

Soil Survey Staff, 2022. Keys to Soil Taxonomy.

Sun, X., Tao, R., Xu, D., Qu, M., Zheng, M., Zhang, M., Mei, Y., 2023. Role of polyamide microplastic in altering microbial consortium and carbon and nitrogen cycles in a simulated agricultural soil microcosm. Chemosphere 312. https://doi.org/10.1016/j.chemosphere.2022.137155

Sun, X., Zhang, X., Xia, Y., Tao, R., Zhang, M., Mei, Y., Qu, M., 2022. Simulation of the effects of microplastics on the microbial community structure and nitrogen cycle of paddy soil. Science of the Total Environment 818. https://doi.org/10.1016/j.scitotenv.2021.151768

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem 1, 301–307. https://doi.org/10.1016/0038-0717(69)90012-1

Thompson, R.C., Olson, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science (1979) 304, 838. https://doi.org/10.1126/science.1094559

Tomas, M., Zhang, L., Zengin, G., Rocchetti, G., Capanoglu, E., Lucini, L., 2021. Metabolomic insight into the profile, in vitro bioaccessibility and bioactive properties of polyphenols and glucosinolates from four Brassicaceae microgreens. Food Research International 140. https://doi.org/10.1016/j.foodres.2020.110039

Tong, Yaoyao, Ding, J., Xiao, M., Shahbaz, M., Zhu, Z., Chen, M., Kuzyakov, Y., Deng, Y., Chen, J., Ge, T., 2023. Microplastics affect activity and spatial distribution of C, N, and P hydrolases in rice rhizosphere. Soil Ecology Letters 5. https://doi.org/10.1007/s42832-022-0138-2

Tong, Yongpeng, Long, Y., Yang, Z., 2023. Effects of warming and isolation from precipitation on the soil carbon, nitrogen, and phosphorus, and their stoichiometries in an alpine meadow in the Qinghai–Tibet Plateau: A greenhouse warming study. Front Ecol Evol 11. https://doi.org/10.3389/fevo.2023.1149240

Turner, B.L., Hopkins, D.W., Haygarth, P.M., Ostle, N., 2002. Glucosidase activity in pasture soils. Applied Soil Ecology 20, 152–157. https://doi.org/10.1016/S0929-1393(02)00020-3

Wan, S., Lin, Y., Ye, G., Fan, J., Hu, H.W., Zheng, Y., Jin, S.S., Duan, C., He, J.Z., 2023. Long-term manure amendment reduces nitrous oxide emissions through decreasing the abundance ratio of amoA and nosZ genes in an Ultisol. Applied Soil Ecology 184, 104771. https://doi.org/10.1016/J.APSOIL.2022.104771

Wang, C., Qu, L., Yang, L., Liu, D., Morrissey, E., Miao, R., Liu, Z., Wang, Q., Fang, Y., Bai, E., 2021. Large-scale importance of microbial carbon use efficiency and necromass to soil organic carbon. Glob Chang Biol 27, 2039–2048. https://doi.org/10.1111/gcb.15550

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

Wang, Weixuan, Xie, Y., Li, H., Dong, H., Li, B., Guo, Y., Wang, Y., Guo, X., Yin, T., Liu, X., Zhou, W., 2024b. Responses of lettuce (Lactuca sativa L.) growth and soil properties to conventional non-biodegradable and new biodegradable microplastics. Environmental Pollution 341. https://doi.org/10.1016/j.envpol.2023.122897

Wang, Wenfeng, Zhang, Z., Gao, J., Wu, H., 2024a. The impacts of microplastics on the cycling of carbon and nitrogen in terrestrial soil ecosystems: Progress and prospects. Science of the Total Environment 915. https://doi.org/10.1016/j.scitotenv.2024.169977

Wang, X., Gao, W., Cai, K., Pan, W., Tu, Z., 2024c. Response of Tobacco (Nicotiana tabacum L.) Growth to Soil Microplastic Pollution. Pol J Environ Stud. https://doi.org/10.15244/pjoes/182894

Wang, X., Xing, Y., Lv, M., Zhang, T., Ya, H., Jiang, B., 2022b. Recent advances on the effects of microplastics on elements cycling in the environment. Science of the Total Environment 849, 1–13. https://doi.org/10.1016/j.scitotenv.2022.157884

Wu, H., He, B., Chen, B., Liu, A., 2024. Influence of polyethylene microplastics on Brassica rapa: Toxicity mechanism investigation. Emerg Contam 10. https://doi.org/10.1016/j.emcon.2024.100380

Ya, H., Xing, Y., Zhang, T., Lv, M., Jiang, B., 2022. LDPE microplastics affect soil microbial community and form a unique plastisphere on microplastics. Applied Soil Ecology 180, 1–11. https://doi.org/10.1016/j.apsoil.2022.104623

Yan, Y., Chen, Z., Zhu, F., Zhu, C., Wang, C., Gu, C., 2021. Effect of Polyvinyl Chloride Microplastics on Bacterial Community and Nutrient Status in Two Agricultural Soils. Bull Environ Contam Toxicol 107, 602–609. https://doi.org/10.1007/s00128-020-02900-2

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

Yu, H., Fan, P., Hou, J., Dang, Q., Cui, D., Xi, B., Tan, W., 2020. Inhibitory effect of microplastics on soil extracellular enzymatic activities by changing soil properties and direct adsorption: An investigation at the aggregate-fraction level. Environmental Pollution 267, 1–11. https://doi.org/10.1016/j.envpol.2020.115544

Zagal, E., Rodríguez, N., Vidal, I., Quezada, L., 2002. Actividad microbiana en un suelo de origen volcanico bajo distinto manejo agronómico. Agricultura Técnica 62, 297–309. https://doi.org/10.4067/S0365-28072002000200012

Zhang, B., Yang, X., Chen, L., Chao, J., Teng, J., Wang, Q., 2020. Microplastics in soils: a review of possible sources, analytical methods and ecological impacts. Journal of Chemical Technology and Biotechnology. https://doi.org/10.1002/jctb.6334

Zhang, K., Gao, N., Li, Y., Dou, S., Liu, Z., Chen, Y., Ma, C., Zhang, H., 2023. Responses of maize (Zea mays L.) seedlings growth and physiological traits triggered by polyvinyl chloride microplastics is dominated by soil available nitrogen. Ecotoxicol Environ Saf 252, 114618. https://doi.org/10.1016/J.ECOENV.2023.114618

Zhang, Z., Li, Y., Qiu, T., Duan, C., Chen, L., Zhao, S., Zhang, X., Fang, L., 2022a. Microplastics addition reduced the toxicity and uptake of cadmium to Brassica chinensis L. Science of the Total Environment 852. https://doi.org/10.1016/j.scitotenv.2022.158353

Zhang, Z., Peng, W., Duan, C., Zhu, X., Wu, H., Zhang, X., Fang, L., 2022b. Microplastics pollution from different plastic mulching years accentuate soil microbial nutrient limitations. Gondwana Research 108, 91–101. https://doi.org/10.1016/j.gr.2021.07.028

Zhao, T., Lozano, Y.M., Rillig, M.C., 2021. Microplastics Increase Soil pH and Decrease Microbial Activities as a Function of Microplastic Shape, Polymer Type, and Exposure Time. Front Environ Sci 9. https://doi.org/10.3389/fenvs.2021.675803

SUPPLEMENTARY INFORMATION

Microplastic Pollution in Andisol: Effects on Soil Microbiology, Nitrogen Cycling, and Raphanus sativus L. Growth.

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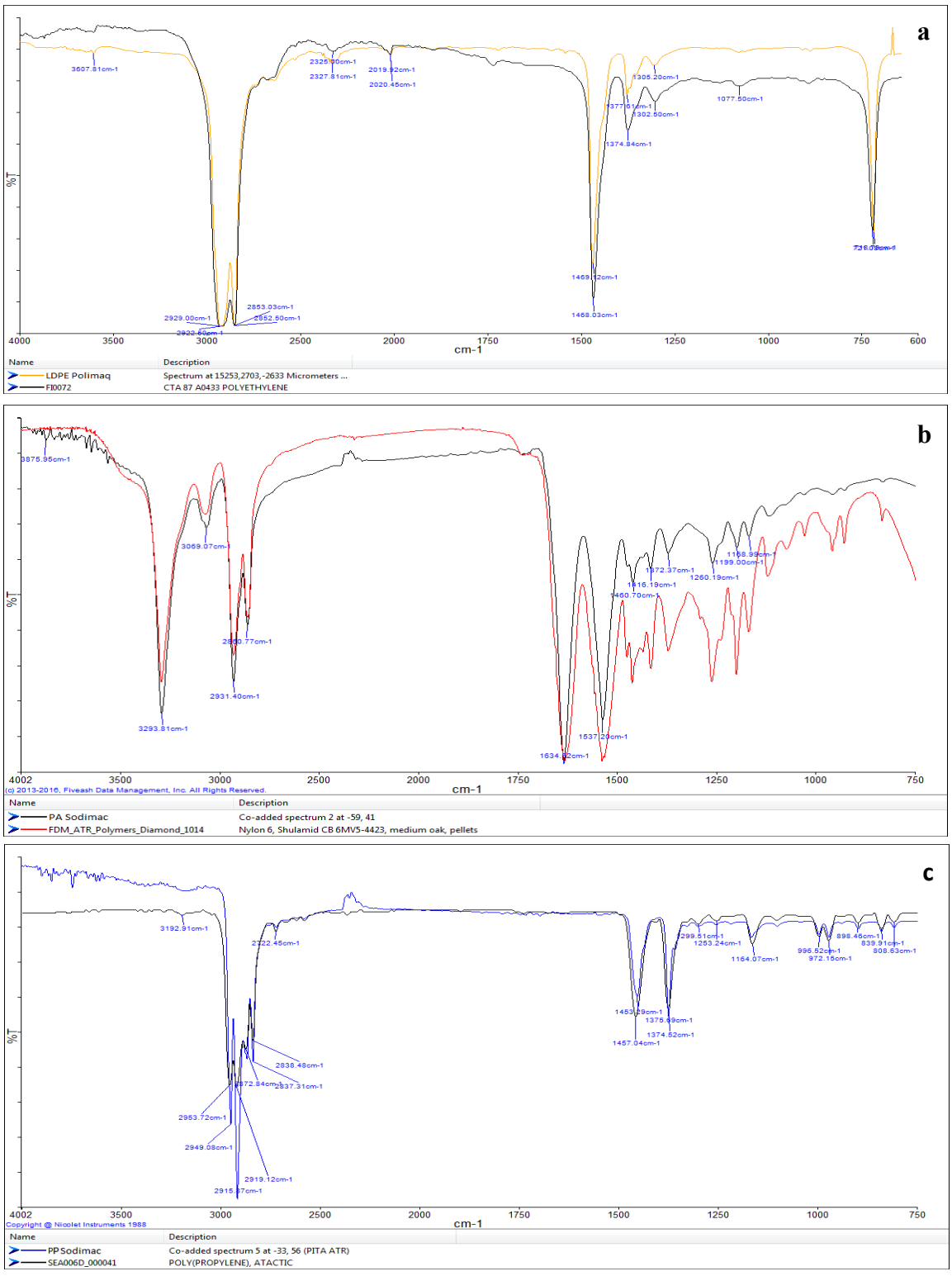


Figure S1. FTIR spectra of plastics used in the soil-MPs incubation and comparison with spectra from the equipment library. (a) LDPE; (b) PA; (c) PP

Table S1. Primers and thermal cycling conditions for qPCR reactions

Target gene	Primer Name	Primer sequence (5´- 3´)	Reaction condition	Reference
16S RNA	16S-F 16S-rtR	AGAGTTTGATCMTGGCTCAG GCTGCCTCCCGTAGGAGT	95°C for 3 min; 35 cycles (95°C for 5 s, 60°C for 10 s and, 72°C for 20 s).	(Keshri et al., 2013)
nifH	nifHpolF nifHpolR	TGCGAYCCSAARGCBGACTC ATSGCCATCATYTCRCCGGA	95°C for 3 min; 35 cycles (95°C for 5 s, 60°C for 10 s and, 72°C for 20 s).	(Wan et al., 2023)
amoA (AOB)	amoA1F amoA2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	95°C for 3 min; 35 cycles (95°C for 5 s, 60°C for 10 s and, 72°C for 20 s).	(Rong et al., 2021)
nirS	nirS4F nirS6R	TTCRTCAAGACSCAYCCGAA CGTTGAACTTRCCGGT	95°C for 3 min; 35 cycles (95°C for 10 s, 60°C for 10 s and, 72°C for 20 s).	(Yang et al., 2018)
nosZ	nosZClado1F nosZClado1R	CGCRACGGCAASAAGGTSMSSG CAKRTGCAKSGCRTGGCAGAA	95°C for 3 min; 35 cycles (95°C for 10 s, 60°C for 10 s and, 72°C for 30 s).	(Kim et al., 2020)

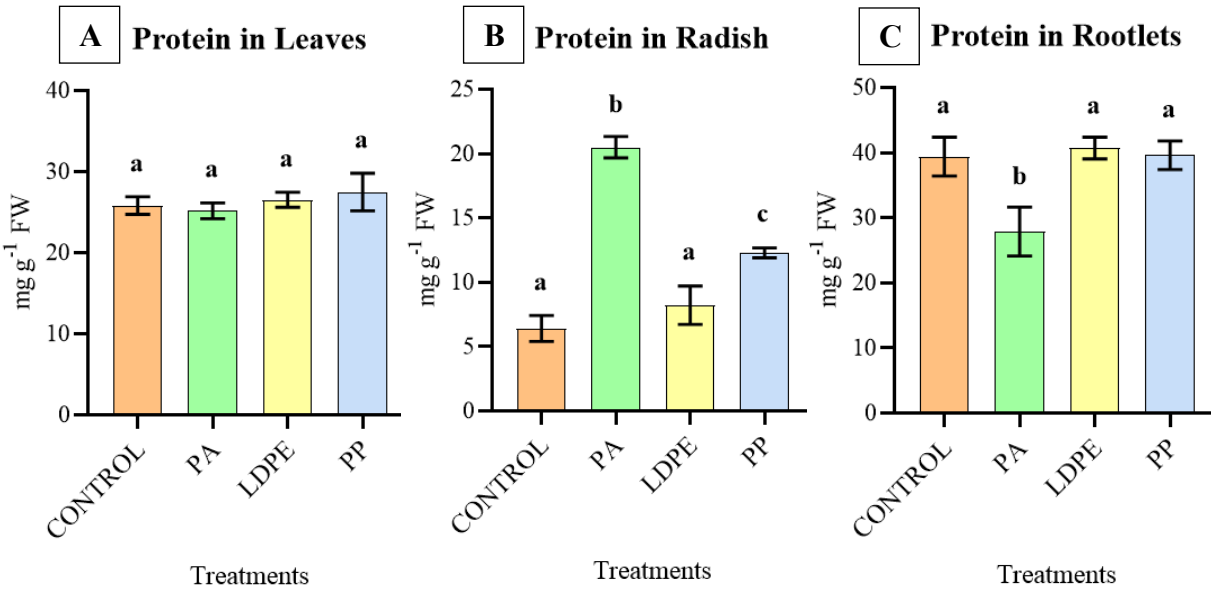


Figure S2. Protein content in (A) leaves, (B) radish, and (C) rootlets of *R. sativus* in the control soil and soil with MPs addition. CONTROL: Control soil without MPs added; PA: Soil with 2 % (w/w) of PA-MPs added; LDPE: Soil with 2 % (w/w) of LDPE-MPs added; PP: Soil with 2 % (w/w) of PP-MPs added. Different lowercase letters indicate significant differences among treatments according to PERMANOVA analyses ($p < 0.05$). Values are expressed as mean with standard error.

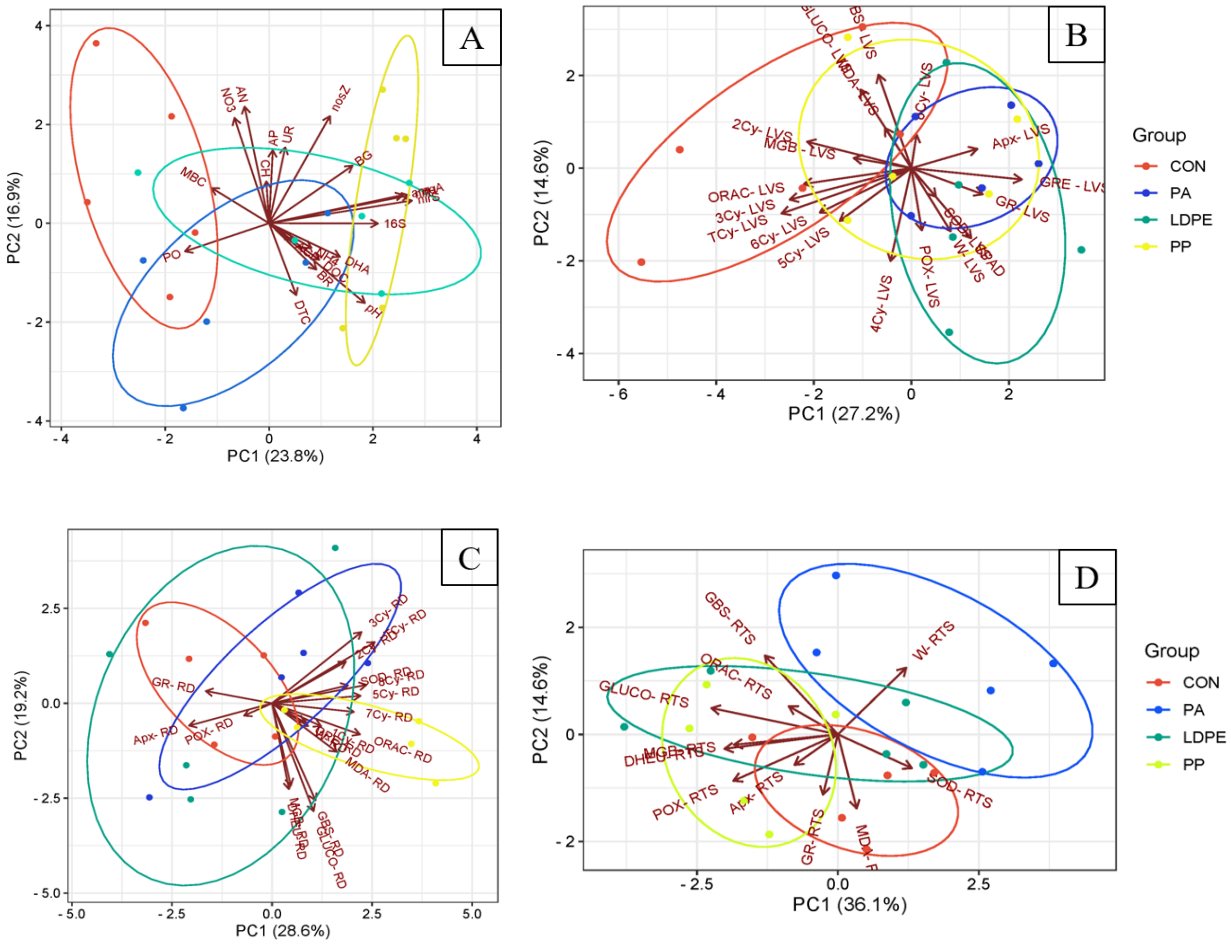
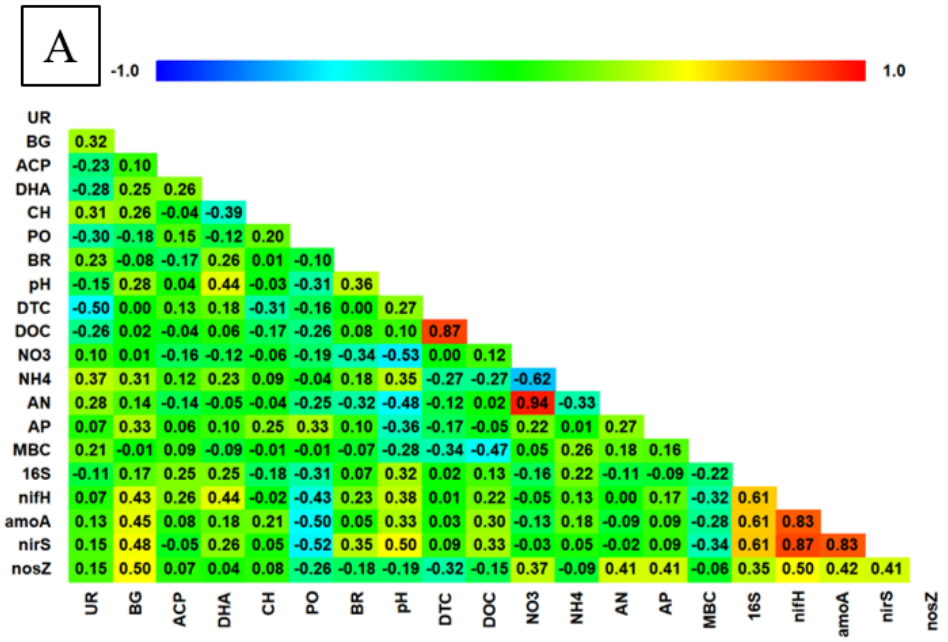


Figure S3. Principal component analysis of the different soil treatments and their soil or plant part characteristics. A: Soil; B: leaves, C: radish, and D: rootlets. CON: Soil without MPs addition; PA: Soil with 2% PA–MPs added (w/w); LDPE: Soil with 2% LDPE–MPs added (w/w); PP: Soil with 2% PP–MPs added (w/w).

Abbreviations: LVS, leaves; RD, radish; RTS, rootlets; ACP, acid phosphatase; AN, Available nitrogen; AP, available phosphorus; UR, Urease; BG, β-glucosidase; CH, carbohydrates; DHA, Dehydrogenase; DOC, Dissolved organic carbon; DTC, Dissolved total carbon; NH4, Ammonium; NO3, Nitrate; pH, pH; PO, polyphenols; BR, Soil basal respiration; MBC, Microbial biomass C; 16S, Gen 16S RNA; nifH, Gen *nifH*; amoA, gen *amoA*; nirS, Gen *nirS*; nosZ, Gen *nosZ.* GLUCO, Total glucosinolates; GRE, Glucoraphanin; GBS, Glucobrassicin; MGB, Methoxyglucobrassicin; DH-EU, Dehydroeuricin; TCy,

Total anthocyanin; 1-Cy, CY-3-O-(SI)soph-5-O-glu; 2-Cy, CY-3-O-(FE)soph-5-O-(MA)shop; 3-Cy, CY-3-O-(FE-SI)soph-5-O-soph + CY-3-O-(pCoA)soph-5-O-(MA)glu; 4-Cy, CY-3-O-(pCoA)soph-5-O-(MA)glu + CY-3-O-(FE-SI)diglu-5-O-glu; 5-Cy, CY-3-O-(SI)soph-5-O-(MA)glu + CY-3-O-(FE)soph-5-O-(MA)glu; 6-Cy, CY-3-O-(pCoA-SI)soph-5-O-(MA)soph or CY-3-O-(diFE)soph-5-O-(MA)soph; 7-Cy, CY-3-O-(pCoA-SI)soph-5-O-(MA)glu + CY-3-O-(pCoA-SI)soph-5-O-(MA)soph or Cy-3-O-(pCoA-SI)soph-5-O-(MA)soph + Cy-3-O-(FE-SI)soph-5-O-(MA)glu; 8-Cy, Cy-3-0-(pCoA-SI)soph-5-O-(MA)glu; SOD, Superoxide dismutase; APX, Ascorbate peroxidase; GR, glutathione reductase; POX, Peroxidase; ORAC, ORAC; MDA, MDA; W, Fresh weight.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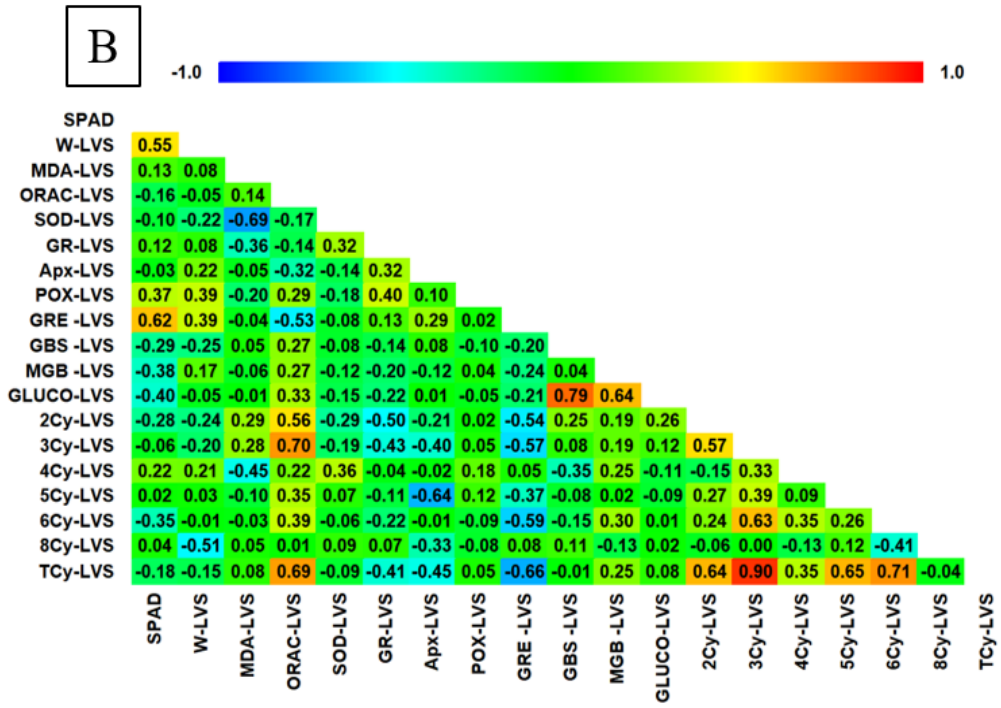
UR
BG 0.32
ACP -0.23 0.10
DHA -0.28 0.25 0.26
CH 0.31 0.26 -0.04 -0.39
PO -0.30 -0.18 0.15 -0.12 0.20
BR 0.23 -0.08 -0.17 0.26 0.01 -0.10
pH -0.15 0.28 0.04 0.44 -0.03 -0.31 0.36
DTC -0.50 0.00 0.13 0.18 -0.31 -0.16 0.00 0.27
DOC -0.26 0.02 -0.04 0.06 -0.17 -0.26 0.08 0.10 0.87
NO3 0.10 0.01 -0.16 -0.12 -0.06 -0.19 -0.34 -0.53 0.00 0.12
NH4 0.37 0.31 0.12 0.23 0.09 -0.04 0.18 0.35 -0.27 -0.27 -0.62
AN 0.28 0.14 -0.14 -0.05 -0.04 -0.25 -0.32 -0.48 -0.12 0.02 0.94 -0.33
AP 0.07 0.33 0.06 0.10 0.25 0.33 0.10 -0.36 -0.17 -0.05 0.22 0.01 0.27
MBC 0.21 -0.01 0.09 -0.09 -0.01 -0.01 -0.07 -0.28 -0.34 -0.47 0.05 0.26 0.18 0.16
16S -0.11 0.17 0.25 0.25 -0.18 -0.31 0.07 0.32 0.02 0.13 -0.16 0.22 -0.11 -0.09 -0.22
nifH 0.07 0.43 0.26 0.44 -0.02 -0.43 0.23 0.38 0.01 0.22 -0.05 0.13 0.00 0.17 -0.32 0.61
amoA 0.13 0.45 0.08 0.18 0.21 -0.50 0.05 0.33 0.03 0.30 -0.13 0.18 -0.09 0.09 -0.28 0.61 0.83
nirS 0.15 0.48 -0.05 0.26 0.05 -0.52 0.35 0.50 0.09 0.33 -0.03 0.05 -0.02 0.09 -0.34 0.61 0.87 0.83
nosZ 0.15 0.50 0.07 0.04 0.08 -0.26 -0.18 -0.19 -0.32 -0.15 0.37 -0.09 0.41 0.41 -0.06 0.35 0.50 0.42 0.41
UR BG ACP DHA CH PO BR pH DTC DOC NO3 NH4 AN AP MBC 16S nifH amoA nirS nosZ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MDA-LVS 0.13 0.08
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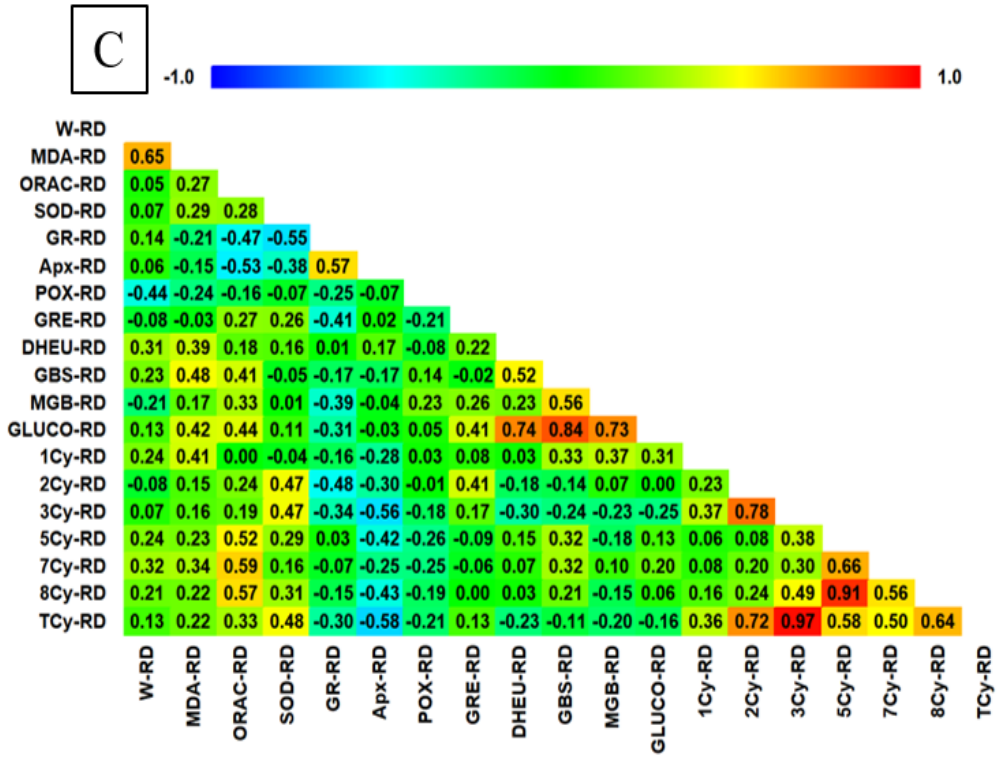
SOD-LVS -0.10 -0.22 -0.69 -0.17
GR-LVS 0.12 0.08 -0.36 -0.14 0.32
Apx-LVS -0.03 0.22 -0.05 -0.32 -0.14 0.32
POX-LVS 0.37 0.39 -0.20 0.29 -0.18 0.40 0.10
GRE -LVS 0.62 0.39 -0.04 -0.53 -0.08 0.13 0.29 0.02
GBS -LVS -0.29 -0.25 0.05 0.27 -0.08 -0.14 0.08 -0.10 -0.20
MGB -LVS -0.38 0.17 -0.06 0.27 -0.12 -0.20 -0.12 0.04 -0.24 0.04
GLUCO-LVS -0.40 -0.05 -0.01 0.33 -0.15 -0.22 0.01 -0.05 -0.21 0.79 0.64
2Cy-LVS -0.28 -0.24 0.29 0.56 -0.29 -0.50 -0.21 0.02 -0.54 0.25 0.19 0.26
3Cy-LVS -0.06 -0.20 0.28 0.70 -0.19 -0.43 -0.40 0.05 -0.57 0.08 0.19 0.12 0.57
4Cy-LVS 0.22 0.21 -0.45 0.22 0.36 -0.04 -0.02 0.18 0.05 -0.35 0.25 -0.11 -0.15 0.33
5Cy-LVS 0.02 0.03 -0.10 0.35 0.07 -0.11 -0.64 0.12 -0.37 -0.08 0.02 -0.09 0.27 0.39 0.09
6Cy-LVS -0.35 -0.01 -0.03 0.39 -0.06 -0.22 -0.01 -0.09 -0.59 -0.15 0.30 0.01 0.24 0.63 0.35 0.26
8Cy-LVS 0.04 -0.51 0.05 0.01 0.09 0.07 -0.33 -0.08 0.08 0.11 -0.13 0.02 -0.06 0.00 -0.13 0.12 -0.41
TCy-LVS -0.18 -0.15 0.08 0.69 -0.09 -0.41 -0.45 0.05 -0.66 -0.01 0.25 0.08 0.64 0.90 0.35 0.65 0.71 -0.04
SPAD W-LVS MDA-LVS ORAC-LVS SOD-LVS GR-LVS Apx-LVS POX-LVS GRE -LVS GBS -LVS MGB -LVS GLUCO-LVS 2Cy-LVS 3Cy-LVS 4Cy-LVS 5Cy-LVS 6Cy-LVS 8Cy-LVS TCy-LVS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ORAC-RD 0.05 0.27
SOD-RD 0.07 0.29 0.28
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Apx-RD 0.06 -0.15 -0.53 -0.38 0.57
POX-RD -0.44 -0.24 -0.16 -0.07 -0.25 -0.07
GRE-RD -0.08 -0.03 0.27 0.26 -0.41 0.02 -0.21
DHEU-RD 0.31 0.39 0.18 0.16 0.01 0.17 -0.08 0.22
GBS-RD 0.23 0.48 0.41 -0.05 -0.17 -0.17 0.14 -0.02 0.52
MGB-RD -0.21 0.17 0.33 0.01 -0.39 -0.04 0.23 0.26 0.23 0.56
GLUCO-RD 0.13 0.42 0.44 0.11 -0.31 -0.03 0.05 0.41 0.74 0.84 0.73
1Cy-RD 0.24 0.41 0.00 -0.04 -0.16 -0.28 0.03 0.08 0.03 0.33 0.37 0.31
2Cy-RD -0.08 0.15 0.24 0.47 -0.48 -0.30 -0.01 0.41 -0.18 -0.14 0.07 0.00 0.23
3Cy-RD 0.07 0.16 0.19 0.47 -0.34 -0.56 -0.18 0.17 -0.30 -0.24 -0.23 -0.25 0.37 0.78
5Cy-RD 0.24 0.23 0.52 0.29 0.03 -0.42 -0.26 -0.09 0.15 0.32 -0.18 0.13 0.06 0.08 0.38
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8Cy-RD 0.21 0.22 0.57 0.31 -0.15 -0.43 -0.19 0.00 0.03 0.21 -0.15 0.06 0.16 0.24 0.49 0.91 0.56
TCy-RD 0.13 0.22 0.33 0.48 -0.30 -0.58 -0.21 0.13 -0.23 -0.11 -0.20 -0.16 0.36 0.72 0.97 0.58 0.50 0.64
W-RD
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GRE-RD
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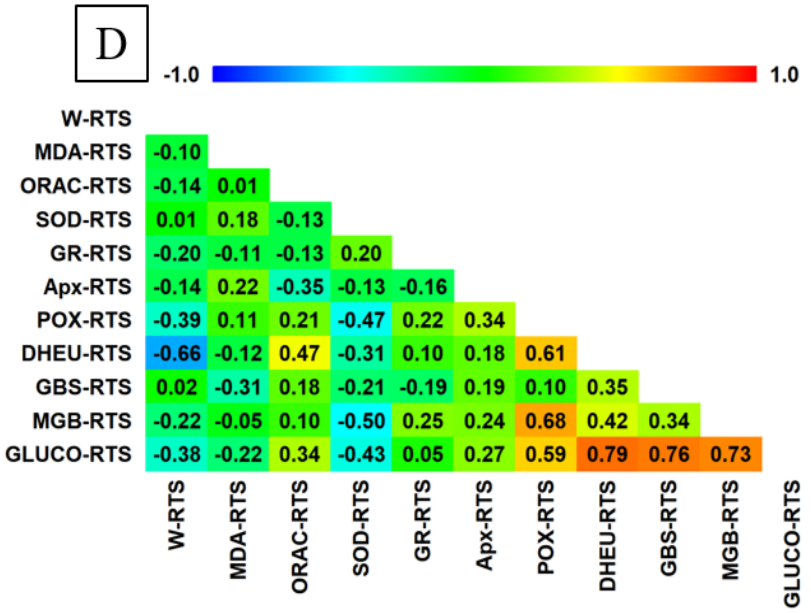
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Apx-RTS -0.14 0.22 -0.35 -0.13 -0.16
POX-RTS -0.39 0.11 0.21 -0.47 0.22 0.34
DHEU-RTS -0.66 -0.12 0.47 -0.31 0.10 0.18 0.61
GBS-RTS 0.02 -0.31 0.18 -0.21 -0.19 0.19 0.10 0.35
MGB-RTS -0.22 -0.05 0.10 -0.50 0.25 0.24 0.68 0.42 0.34
GLUCO-RTS -0.38 -0.22 0.34 -0.43 0.05 0.27 0.59 0.79 0.76 0.73
W-RTS
MDA-RTS
ORAC-RTS
SOD-RTS
GR-RTS
Apx-RTS
POX-RTS
DHEU-RTS
GBS-RTS
MGB-RTS
GLUCO-RTS

Figure S4. Significant Pearson correlations ($p < 0.05$) were observed between the soil and plant parts parameters. A: Soil; B: leaves; C: radish; D: rootlets. Abbreviations: LVS, Leaves; RD, Radish; RTS, Rootlets; ACP, Acid phosphatase; AN, Available nitrogen; AP, available phosphorus; UR, Urease; BG, β-glucosidase; CH, carbohydrates; DHA, Dehydrogenase; DOC, Dissolved organic carbon; DTC, Dissolved total carbon; NH4, Ammonium; NO3, Nitrate; pH, pH; PO, polyphenols; BR, Soil basal respiration; MBC, Microbial biomass-C; 16S, Gen 16S RNA; nifH, Gen *nifH*; amoA, gen *amoA*; nirS, Gen *nirS*; nosZ, Gen *nosZ.* GLUCO, Total glucosinolates; GRE, Glucoraphanin; GBS, Glucobrassicin; MGB, Methoxyglucobrassicin; DH-EU, Dehydroeuricin; TCy, Total anthocyanin; 1-Cy, CY-3-O-(SI)soph-5-O-glu; 2-Cy, CY-3-O-(FE)soph-5-O-(MA)shop; 3-Cy, CY-3-O-(FE-SI)soph-5-O-soph + CY-3-O-(pCoA)soph-5-O-(MA)glu; 4-Cy, CY-3-O-(pCoA)soph-5-O-(MA)glu + CY-3-O-(FE-SI)diglu-5-O-glu; 5-Cy, CY-3-O-(SI)soph-5-O-(MA)glu + CY-3-O-(FE)soph-5-O-(MA)glu; 6-Cy, CY-3-O-(pCoA-SI)soph-5-O-(MA)soph or CY-3-O-(diFE)soph-5-O-(MA)soph; 7-Cy, CY-3-O-(pCoA-SI)soph-5-O-(MA)glu + CY-3-O-(pCoA-SI)soph-5-O-(MA)soph or Cy-3-O-(pCoA-SI)soph-5-O-(MA)soph + Cy-3-O-(FE-SI)soph-5-O-(MA)glu; 8-Cy, Cy-3-0-(pCoA-SI)soph-5-O-(MA)glu; SOD, Superoxide dismutase; APX, Ascorbate peroxidase; GR, Glutathione reductase; POX, Peroxidase; ORAC, ORAC; MDA, MDA; W, fresh weight.

Table S2. DistLM analysis showing soil independent variables that significantly influenced *R. sativus* characteristics in soil with MPs.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	*P*	Prop.
UR	111.28	0.34474	0.844	1.88E-02
BG	168.12	0.52598	0.68	2.84E-02
ACP	236.79	0.74973	0.507	4.00E-02
DHA	97.774	0.30219	0.844	1.65E-02
CH	168.58	0.52743	0.667	2.85E-02
PO	1228.9	4.7138	0.016	0.20753
BR	272.91	0.86963	0.43	4.61E-02
pH	607.42	2.0574	0.114	0.10258
DTC	131.05	0.40737	0.749	2.21E-02
DOC	231.38	0.73192	0.514	3.91E-02
NO3	89.042	0.27479	0.874	1.50E-02
NH4	145.67	0.45396	0.716	2.46E-02
AN	198.64	0.62476	0.584	3.35E-02
AP	318.47	1.0231	0.355	5.38E-02
MBC	945.27	3.4191	0.04	0.15963
16S	641.27	2.186	0.095	0.10829
nifH	1061.3	3.9306	0.024	0.17923
amoA	869	3.0958	0.054	0.14675
nirS	1182.8	4.4925	0.013	0.19973
nosZ	355.82	1.1507	0.304	6.01E-02

SEQUENTIAL TESTS

Variable	AICc	SS (trace)	Pseudo-F	*P*	Prop.	Cumul.
PO	113.87	1228.9	4.7138	0.015	0.20753	0.20753
MBC	112.09	957.81	4.3596	0.008	0.16175	0.36928

Best solution

AICc	R^2	RSS	No.Vars	Selections
112.09	0.36928	3734.9	2	6;15

Percentage of variation explained by individual axes

Axis	% explained variation out of fitted model		% explained variation out of total variation	
	Individual	Cumulative	Individual	Cumulative
1	89.51	89.51	33.05	33.05
2	10.49	100	3.88	36.93

Abbreviations: ACP, acid phosphatase; AN, available nitrogen; AP, available phosphorus; UR, urease; BG, β-glucosidase; CH, carbohydrates; DHA, dehydrogenase; DOC, dissolved organic carbon; DTC, dissolved total carbon;

NH4, Ammonium; NO3, Nitrate; pH, pH; PO, polyphenols; BR, soil basal respiration; MBC, microbial biomass-C; 16S, gen 16S rRNA; *nifH*, gen *nifH*; *amoA*, gen *amoA*; *nirS*, gen *nirS*; *nosZ*, gen *nosZ.*

SUPPLEMENTARY REFERENCES

Keshri J, Mishra A, Jha B (2013) Microbial population index and community structure in saline–alkaline soil using gene targeted metagenomics. Microbiological research, 168(3), 165-173. https://doi.org/10.1016/j.micres.2012.09.005

Kim DD, Park D, Yoon H, Yun T, Song MJ, Yoon S (2020) Quantification of nosZ genes and transcripts in activated sludge microbiomes with novel group-specific qPCR methods validated with metagenomic analyses. Water Research, 185, 116261. https://doi.org/10.1016/j.watres.2020.116261

Rong L, Zhao L, Zhao L, Cheng Z, et al. (2021) LDPE microplastics affect soil microbial communities and nitrogen cycling. Science of the Total Environment 773:. https://doi.org/10.1016/j.scitotenv.2021.145640

Wang Y, Chen Y, Xue Q, Xiang Q, Zhao K, Yu X, Chen Q, Ma M, Jiang H, Zhang X, Penttinen P, Gu Y (2021) The abundance of the nifH gene became higher and the nifH-containing diazotrophic bacterial communities changed during primary succession in the Hailuogou Glacier chronosequence, China. Frontiers in Microbiology, 12, 672656. https://doi.org/10.3389/fmicb.2021.672656

Yang YD, Hu YG, Wang ZM, Zeng ZH (2018) Variations of the *nirS-, nirK*-, and *nosZ*-denitrifying bacterial communities in a northern Chinese soil as affected by different long-term irrigation regimes. Environmental Science and Pollution Research, 25, 14057-14067. https://doi.org/10.1007/s11356-018-1548-7

III. CONCLUSIONES GENERALES

El primer artículo original realizado en esta tesis evidencia que la presencia de MPs en el suelo y en ausencia de plantas puede aumentar el carbono orgánico total y el amonio, pero disminuir tanto el nitrato y la actividad de enzimas esenciales como la fosfatasa ácida y la β-glucosidasa. Estas alteraciones, observadas en un Andisol, sugieren que los MPs afectan los ciclos de nutrientes y la actividad microbiológica, siendo más pronunciadas en tratamientos con PA y en dosis elevadas de MPs. Además, las variaciones en el pH y la conductividad eléctrica destacan la influencia de los MPs en parámetros fundamentales del suelo, impactando su fertilidad y calidad. Además, se pudo determinar que el fósforo total y el carbono orgánico total pueden ser utilizados como predictores químicos de alteraciones en propiedades microbiológicas de Andisoles contaminados con MPs. La importancia de estudiar este orden de suelo radica en su alta capacidad de almacenamiento de carbono y debido a su rol importante en la agricultura de Chile.

En el segundo artículo original de esta tesis se cultivó *R. sativus* en un Andisol contaminado con MPs, evidenciando que los MPs no solo alteran propiedades de suelo, sino que también el crecimiento y la fisiología de *R. sativus* dependiendo tanto del MPs adicionado como de la parte de la planta estudiada (hoja, rábano o raicillas). Los MPs incrementaron el contenido relativo de clorofila y la biomasa, aunque se observó un aumento del estrés oxidativo en los tejidos radiculares, especialmente bajo tratamientos con PP y LDPE. Además, los MPs modificaron la composición y por ende los perfiles de compuestos bioactivos tales como las antocianinas y glucosinolatos en *R. sativus*, lo cual tiene implicancias para defensa, la calidad nutricional y por ende beneficios para la salud humana. Además, se pudo determinar que los polifenoles en el suelo y el C de la biomasa microbiana surgen como predictores clave de las respuestas de *R. sativus* a la contaminación por MPs,

con posibles efectos a largo plazo sobre las interacciones planta-microbio y la fertilidad del suelo.

Los resultados obtenidos en esta investigación confirman parcialmente la hipótesis planteada, evidenciando que la presencia de MPs en un Andisol impacta tanto sus propiedades químicas como biológicas, aunque con efectos complejos y dependientes del tipo de MP y su concentración. Se observó que los MPs pueden alterar los ciclos de nutrientes, afectando la disponibilidad de nitrógeno y la actividad enzimática del suelo, lo que indica una perturbación en los procesos microbiológicos esenciales. Sin embargo, a diferencia de lo esperado, el crecimiento de *R. sativus* no se vio afectado, al contrario, se evidenció un incremento en la biomasa y el contenido relativo de clorofila, a pesar del aumento del estrés oxidativo en las raíces. El aumento del crecimiento vegetal en presencia de MPs no debe considerarse un resultado positivo desde la perspectiva de la salud humana, ya que los MPs pueden ingresar a la cadena alimentaria y producir efectos adversos como reacciones alérgicas y daños en el tracto gastrointestinal, debido a una posible relación con la enfermedad inflamatoria intestinal. Además, los MPs modificaron la composición de compuestos bioactivos en la planta, lo que puede tener implicancias tanto para la defensa de la planta contra patógenos como en su valor nutricional.

Estos hallazgos resaltan la necesidad de investigaciones a largo plazo y en estudios en campo para evaluar los efectos de los MPs en los ciclos biogeoquímicos, la seguridad alimentaria y la productividad agrícola, además de su impacto en la interacción suelo-planta-microorganismos. En conjunto, el estudio enfatiza la urgencia de desarrollar prácticas agronómicas y políticas públicas para reducir la contaminación por MPs en suelos agrícolas, garantizando la sostenibilidad y la seguridad de los cultivos ante la creciente acumulación de estos contaminantes emergentes en el ambiente terrestre.