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Interactive impacts of microplastics and arsenic on agricultural soil and plant traits

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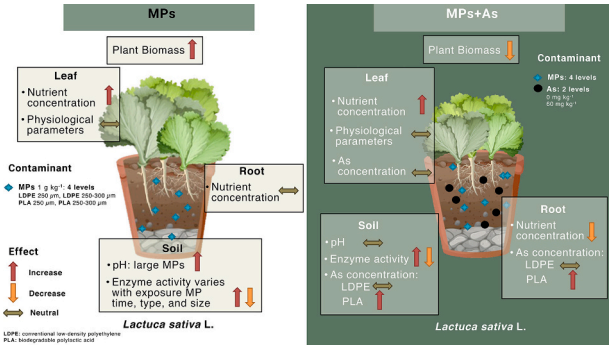
HIGHLIGHTS

- Microplastics (MPs) increase biomass of lettuce plants.
- The effect of MPs varies with exposure time, polymer type, and size.
- Soil pH is increased by larger size of MPs (250–300 μm).
- Arsenic (As) concentration in soil and roots is increased by PLA, but not by LDPE.
- As-MPs co-contamination negatively affects plant growth and root nutrients.

GRAPHICAL ABSTRACT



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ABSTRACT

The ability of microplastics (MPs) to interact with environmental pollutants is currently of great concern due to the increasing use of plastic. Agricultural soils are sinks for multipollutants and the safety of biodegradable MPs in field conditions is questioned. However, still few studies have investigated the interactive effects between MPs and metals on the soil-plant system with agricultural soil and testing crops for human consumption. In this work, we tested the effect on soil and plant parameters of two common MPs, non-degradable plastic low-density polyethylene and biodegradable polymer polylactic acid at two different sizes (<250 μm and 250–300 μm) in association with arsenic (As). Lettuce (*Lactuca sativa* L.) was used as a model plant in a small-scale experiment lasting 60 days. Microplastics and As explained 12 % and 47 % of total variance, respectively, while their interaction explained 21 %, suggesting a higher toxic impact of As than MPs. Plant growth was promoted by MPs alone, especially when biodegradable MPs were added (+22 %). However, MPs did not affect nutrient concentrations in roots and leaves. The effect of MPs on enzyme activities was variable depending on the time of exposure (with larger effects immediately after exposure), the type and size of the MPs. On the contrary, the co-application of MP and As, although it did not change the amount of bioavailable As in soil in the short and medium term, it resulted in a significant decrease in lettuce biomass (−19 %) and root nutrient concentrations, especially when polylactic acid was applied. Generally, MPs in association with As determined the plant-soil

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toxicity. This work provides insights into the risk of copollution of MPs and As in agricultural soil and its phytotoxic effect for agricultural crops. However, the mechanisms of the joint effect of MP and As on plant toxicity need further investigation, especially under field conditions and in long-term experiments.

1. Introduction

Plastic-contaminated soils by plastics are an emergency that needs to be tackled. The widespreadness and persistence of plastics and their residues in agricultural soils have become a threat to animals and humans (Tian et al., 2022; Shaoliang Zhang et al., 2023c). Within plastic contaminants, microplastics (MPs) are defined plastic particles ranging in size from 0.1 to 5000 μm (EFSA, 2016) and can be produced directly in their original state (primary MP) or result from deterioration, alteration, or degradation of larger plastic products (secondary MP). Microplastic contamination in agroecosystems can greatly vary according to cropping system: the concentration of MP in soils ranged from approximately 540 particles kg^{-1} with crops under the usual management techniques (Corradini et al., 2021) to 8885 particles kg^{-1} in fields cultivated with long-term plastic mulch (Li et al., 2022). The massive use of plastics in agriculture, either direct (*e.g.*, use of mulch covers, greenhouse crop production, use of string, nets, and pipes for irrigation) and indirect (*e.g.*, inadequate waste disposal, irrigation with wastewater, use of potentially contaminated soil amendments, such as compost and biosolids), is the main determinant of diffuse MP pollution in agricultural soil with potential transfer to food (FAO, 2021; Javed et al., 2023). Although there is still no standardized protocol for the detection of MPs in agricultural fields, the most common methods are based on visual observation (counting under stereomicroscopy), spectroscopy (Fourier transform infrared spectroscopy, FT-IR; Raman spectroscopy), and chromatography (gas chromatography; mass spectrometry), after extraction based on sieving, filtration, or density separation/flotation techniques (Q. Liu et al., 2022a; Pérez-Reverón et al., 2022).

Microplastics can have direct and indirect effects on the soil-plant system. They can directly alter the physical-chemical and biological parameters of soil, for example pH, soil aggregation, porosity, soil nutrients, microbial activity, and community assemblages (Huang et al., 2022; F. Wang et al., 2022b). Modifications to these parameters influence soil quality and fertility, which in turn may affect plant growth and nutrient uptake. The persistence of MPs in soil can create accumulation concerns (bioaccumulation/biomagnification) for both plants and soil microbes (Shaoliang Zhang et al., 2023c; F. Wang et al., 2022a; Q. Wang et al., 2022). The smaller MPs could enter the roots through the 'crack-entry mode' (Li et al., 2020) or by exploiting discontinuous areas at the apex of immature root and the growth area of the lateral root (Hua et al., 2024), and then translocate to edible parts of the plant (Dong et al., 2021a). Furthermore, the superficial adhesion of MPs to roots or seeds can inhibit water and nutrient uptake, with consequent negative effects on seed germination, plant growth and development (Hua et al., 2024; Y. Zhang et al., 2023b). Considering the potential threat of MPs, biodegradable polymers have been developed to replace conventional nondegradable plastics (Ainali et al., 2022; Chu et al., 2023). Biodegradable plastics can be degraded through biological activities, and this process under realistic environmental conditions can take a few years compared to conventional plastics (Zhou et al., 2023). This leads to an increase in MP production over short periods with a greater impact on soil quality (Fan et al., 2022; Liao and Chen, 2021).

The negative impact of MPs on plants may also be enhanced by the ability of the MP to act as carriers of other contaminants. In the case of coexistence in soil of MPs and heavy metals, significant interactions can occur (Li and Wang, 2023; Ivy et al., 2023). On the one hand, structural and chemical characteristics of MPs (*e.g.*, polarity, functional groups, or crystallinity) may alter the bioavailability and toxicity of metals; for example, biodegradable plastics may adsorb more heavy metals on their surface than conventional plastics, due to their rapid degradation rate and changes in surface functional groups (Fan et al., 2022). On the other hand, metal toxicity could determine structural damage to plant cells, facilitating the entry of MPs into plants (Dong et al., 2021a). In particular, arsenic (As) is a metalloid that is highly toxic to all organisms (Ahmed et al., 2022; Fatoki and Badmus, 2022) and widely distributed in soils due to both natural processes and anthropogenic activities. Exposure to As induces significant physiological and metabolic stresses in plants, reducing their growth and productivity (Martínez-Castillo et al., 2022). The toxicity of As to plants is closely related to the bioavailability of As in the soil, which in turn is regulated by site-specific physical and chemical soil parameters. Infact, plants are able to absorb only bioavailable soil As (Pedron et al., 2017). Some studies have reported that the bioavailability of metals in the presence of MPs is reduced and that the increased porosity and roughness of MPs, after a long period of weathering and oxidation in the soil, can favor the adsorption of As onto the surface of MP *via* hydrogen bonding (Dong et al., 2021b; Li and Wang, 2023; Mora et al., 2023). Other mechanisms involved in the interaction between MP surfaces and As include electrostatic and non-covalent interactions, carbon-π-bonding, complexation, and precipitation (Ivy et al., 2023; Mora et al., 2023; Premarathna et al., 2023). However, the bioavailability of As and the adsorption processes are also regulated by other factors, such as CEC and pH, which in turn are influenced by MP (Chen et al., 2023).

Although soil protection is an essential goal of the new environmental policies and strategies (EC, 2019), knowledge about MP pollution in soil and associated ecological risks is still very limited. Indeed, the complexity of the processes involved, including the different polymeric types, sizes, shapes, and concentrations of MPs, coupled with and the current limitations in the techniques of MP analysis in complex matrices, have led to contradictory results without reaching definitive conclusions on the real impact of the MP in agricultural systems (F. Wang et al., 2022b; Zhao et al., 2023, 2022). Thus, in this study, we tested the following hypotheses: (i) MP contamination negatively affects plant growth by reducing nutrient uptake and microbial activity in soil, and the effect varies according to type and size of MPs; (ii) the cocontamination of MPs and As reduces soil As bioavailability with positive effects on plants and soil microbial activities. We investigated the effects of MPs and As pollution, at environmentally relevant concentrations and at MP sizes commonly found in agricultural soil, on chemical and biological soil parameters, growth response, and nutrient uptake of plants. Two MPs at two sizes (*i.e.*, <250 μm and 250–300 μm) were tested: conventional low-density polyethylene (LDPE) and biodegradable polylactic acid (PLA). Lettuce (*Lactuca sativa* L.) was selected as the model plant and used in a small-scale experiment lasting 60 days.

2. Materials and methods

2.1. Soil characterization

The soil used in the experiment as substrate was an agricultural soil collected at 0–20 cm soil depth from a field at the 'Enrico Avanzi' Center for Agri-Environmental Research of the University of Pisa, San Piero a Grado, Pisa, Italy (43° 40′ N lat; 10° 19′ E long, 1 m above sea level and 0 % slope). Physicochemical analyses of the soil used in the experiment were carried out on <2 mm air-dried soil. The analytical procedures are given in Supplementary Methods 1. The soil was a sandy loam (79.7 % sand, 12.0 % silt and 37.9 % clay) with 2.14 % total organic carbon, 8.15 pH, 1289 μS cm^{-1} electrical conductivity, cation exchange capacity 15.3 $Cmol_{(+)}$ kg^{-1}, 0.15 % total N, 16.3 mg kg^{-1} available P, 2.98 g kg^{-1} Ca,

0.19 g kg^{-1} K, 0.18 g kg^{-1} Na, 0.13 g kg^{-1} Mg, 0.14 mg kg^{-1} NH_4^+, 10 mg kg^{-1} NO_3^- (Table S1). The soil was a poorly drained alluvial loam, classified as Typic Xerofluvent by the USDA system (USDA, 1975) and as Fluvisol by FAO (IUSS, 2006). Although there is no history of metal and plastic pollution at this site, As background values and some suspected plastic particles (>1 mm) were detected in the soil. The As background was 6.29 ± 0.19 mg kg^{-1}, in the range of values found at sites close to the research area that confirms the natural origin of the metal (Costagliola et al., 2010; Giannaccini et al., 2012). To check the presence in the soil of baseline MP pollution (<1 mm), a sequential wet extraction based on plastic particle flotation was performed following Corradini et al. (2019) with slight modifications. Details of the applied method are given in Supplementary Methods 1. This allowed us to analyze the shape and size of MPs. Digital images were captured using LAS software (Leica Application Suite v.4.12, Leica Microsystems GmbH, Wetzlar, Germany). The plastic particles were also chemically examined by Fourier transform infrared spectrometry (FTIR) in Attenuated Total Reflectance (ATR) using the Jasco FT/IR6200 spectrophotometer equipped with the PIKE MIRacle ATR accessory. The recovered plastics (~0.5 g) were subjected to 128 scans, in the spectral range 4000–650 cm^{-1}, with a collection time of 2 min. To determine the polymer type, we compared the collected spectra with the Spectra Analysis software (Spectra Manager™, Jasco Corporation, Tokyo, Japan).

2.2. Preparation of MPs

Conventional low-density polyethylene and biodegradable polylactic acid were selected as target plastics for the experiment. Low-density polyethylene was chosen as a model for conventional plastics because it is one of the most commonly used polymers in the manufacture of mulch for agricultural purposes (H. Liu et al., 2022b; Wang et al., 2020). Its fragmentation over time can cause widespread MP contamination in soil (Rong et al., 2021). Polylactic acid was chosen as a biodegradable plastic model because it is currently one of the most common alternatives to conventional plastic mulches (Ainali et al., 2022).

Virgin pellets of conventional LDPE (20 17070M, density 0.917 g cm^{-3}) and biodegradable PLA (Ingeo 2002D®, density 1.25 g cm^{-3}) were purchased from Repsol (Madrid, Spain) and NatureWorks LLC (Plymouth, USA), respectively. The pellets of each polymer (with a diameter of approximately 5 mm) were washed and then ground with a mechanical grinder (IKA MF 10B Grinder, Staufen, Germany) equipped with a 1 mm mesh sieve. The residence time for the grinding cycle was approximately 5 min for LDPE and 3 min for PLA.

Before grinding, the pellets were frozen overnight at −20 °C to prevent overheating of the polymer during grinding and thus limit polymer thermodegradation (Kefer et al., 2021). To obtain MP sizes representative of those present in agricultural soil (Chen et al., 2020; Liu et al., 2018), the LDPE and PLA particles recovered from the grinding process were further sieved using two steel sieves with mesh sizes of 300 μm and 250 μm, respectively, stacked on a calibrated Retsch AS200 vibratory sieve shaker and with a shaking cycle of approximately 30 min. The two class sizes of LDPE and PLA tested in this study were: <250 μm and 250–300 μm. The MPs obtained were stored in sealed glass beakers and kept refrigerated for characterization and subsequent setup of the experiment. Chemical and thermal analyses of plastic particles were performed on the original pellets and on the fractions produced by grinding, before their use in the pot experiment. Details of the applied methods are given in Supplementary Methods 2.

2.3. Contamination of the soil

Approximately 20 kg of soil sample was artificially spiked with an As solution to reach a concentration of 60 mg kg^{-1} to simulate a realistic environmental scenario (Chirenje et al., 2003; Dong et al., 2020a). Following the geochemical characterization of agricultural land soil in Europe (Cicchella et al., 2015; Reimann et al., 2009), As reaches values up to 62.2 mg kg^{-1} in Italian agricultural topsoil, well above the contamination threshold value in Italian soils (20 mg kg^{-1} for residential/recreational use) given by the current Environmental Framework Decree (Legislative Decree 152/2006, 2006). The highest As values are recorded in north Italy between Milano and Aosta, in the west of Padova, and in the south-west of Firenze. Other high values also occur in the Roman Neapolitan Volcanic Province, along the Apulia region, in Sardinia near Cagliari and in central Calabria.

The As solution, prepared by dissolving the $As_2O_5 \cdot xH_2O$ salt (Sigma-Aldrich, USA) in distilled water, was added and mixed with the soil until homogeneity. The contaminated soil was equilibrated for ~30 days prior to the experiment. The total concentration of As in the spiked soil was 45.9 ± 1.1 mg kg^{-1}.

Microplastics were applied at a dose of 0.1 % (w/w), equivalent to 1 g kg^{-1} soil (dry weight). This content was chosen according to the most recent studies on the effect of MPs on the growth and production of various crops, such as *Lactuca sativa* L. (Wang et al., 2021), *Cucurbita pepo* L. (Colzi et al., 2022), *Brassica napus* L. (Jia et al., 2022), and *Daucus carota* L. (Lozano et al., 2021b). Before addition, the MPs were cleaned with 0.1 M HCl and distilled water to remove any elements from the surface. The application of MPs was carried out separately for each pot to ensure the correct MP concentration. Soil and MPs were manually mixed with a spoon for about 5 min in aluminum containers prior to placing them in the pots. Plastic pots (height 9 cm, diameter 13 cm) were filled with 500 g of dry soil. The pots were further stabilized for two weeks prior to the beginning of the experiment. During the stabilization period, all pots were watered using a glass straw placed inside the pots to supply water from the bottom to the top to avoid MP leaching.

2.4. Set-up of the experiment

A full factorial experiment with five levels of MP (no MPs, LDPE250, LDPE250-300, PLA250, PLA250-300) and two levels of As contamination (0 and 60 mg As kg^{-1}) with four replicates was arranged in a completely randomized design. Treatments are described in Table S3. Lettuce (*Lactuca sativa* L., var. crispa) was selected as it is proposed as a model species to study the ecological effects of contaminants (EPA, 2012). The lettuce seeds were germinated on moist filter paper in a Petri dish for three days at 25 °C in the dark. After lettuce seedling emergence, four uniform pregerminated seeds were transplanted into each pot (replicate). The pots were placed in a controlled environment phytotron (Monti & C., Pistoia, Italy) at a photosynthetic photon flux density of 400–500 μmol m^{-2} s^{-1} with a day/night photoperiod of 16/8 h, temperature of 26/20 °C and relative humidity of 55/75 %. Inside the phytotron, the position of the pots was changed twice a month. All pots were watered three times a week by gently sprinkling approximately 30 ml of deionized water (Lozano et al., 2021b). From the third week, pots were fertilized once a week with a half-strength Hoagland solution. The experiment lasted 60 days, from seedling transplanting to harvest.

2.5. Soil analyses

At the beginning of the experiment (T0, before seeding) and at the end of the experiment (TF, 60 days), the soil of each pot was air-dried, sieved at 2 m and analyzed for As concentration. Arsenic concentration was determined by acid digestion with nitric acid (HNO_3, 65 %, v/v) and hydrogen peroxide (H_2O_2, 30 %, v/v) (EPA, 1995a) in a microwave oven FKV-ETHOS 900 (MILESTONE Srl, Bergamo, Italy) with pulsed emission. Bioavailable As concentration (the exchangeable fraction most readily absorbed by plants; Grifoni et al., 2017) was assessed by extraction (1:2.5 ratio of soil mass to solution volume) with sodium nitrate ($NaNO_3$, 0.05 M). The concentration of As in the digested soil and extracts was determined by inductively coupled plasma optical emission spectrometry (ICP-OES 5900 Agilent, Santa Clara, CA, USA). Soil enzyme potential activities were measured using the fluorogenic methylumbelliferyl (MUF) substrates method (Marx et al., 2001; Vepsäläinen

et al., 2001), before seeding and at the end of the experiment. The following hydrolytic enzymes, known to be involved in the biogeochemical cycles of carbon (C), nitrogen (N), and phosphorus (P) (Nannipieri et al., 2002) were analyzed: β-glucosidase (β-gluc, EC 3.2.1.21; cellulose degradation), *N*-acetyl-β-glucosaminidase = chitinase (NAG, EC 3.2.1.30; degradation of chitin), and acid phosphatase = phosphatase (Phosph, EC 3.1.3.2: mineralization of organic phosphorus). Furthermore, the butyrate esterase (Buty Est, EC 3.1.1; indicator of total microbial activity), a nonspecific esterase involved in C cycle and ester bond hydrolysis, was investigated. Before starting the analysis, all soil samples were adjusted at 60 % water holding capacity and kept at 25 °C for 3 days in the dark. A suspension of soil (2 g) and deionized water (50 ml) was sonicated (40 s) and pipetted into 96-well black microplates. Artificial substrates based on MUF (β-gluc: 4-MUF β-D-glucoside; NAG: 4-MUF-*N*-acetyl-β-glucosaminide; Phosph: 4-MUF-phosphate; Buty Est: 4-MUF-butyrate) and buffer (Na-acetate buffer, pH 5.5) were added to the wells. Subsequently, the microplates were fluorometrically measured (excitation wavelength 360 nm; emission 450 nm) with an automated fluorometric plate reader (Infinite® F200PRO Tecan) after 0, 30, 60, 120, and 180 min of incubation at 30 °C. Arsenic and enzymatic analyses were performed in triplicate for each replicate pot.

2.6. Phytotoxicity test

The phytotoxicity of MP and As in the soil was assessed using the Germination Index test (GI%) on *Lepidium sativum* L. (ISO, 2016). This species, which has a rapid germination and root growth, was selected for its sensitivity to contaminants, including plastics (Liwarska-Bizukojc, 2022). The test was carried out in Petri dishes filled with 10 g of soil samples from all treatments and moistened with deionized water to saturation. A Whatman N°1 filter with 10 seeds of *Lepidium sativum* was placed on the soil surface. Hydrated quartz sand was used as a negative control. Four replicates were carried out for each treatment and negative control. The Petri dishes were closed and placed in a germination chamber in the dark for 72 h at 25 ± 1 °C. The GI% was calculated by combining the number of germinated seeds and the length of the root compared to the negative control sampled at 72 h, according to the following equation.

$$GI\% = \frac{G_s * L_s}{G_n * L_n} * 100$$

where G_s and L_s are the mean germinated seeds and mean root length (cm), respectively, in the tested soil samples, and G_n and L_n are the mean germinated seeds and mean root length (cm), respectively, in the negative control.

2.7. Plant analyses

After 60 days of growth (TF), plants were harvested and biomass was partitioned into leaves and roots. The fresh weight (FW) of leaves and roots per pot was determined and the dry weight (DW) was assessed after drying in the oven at 40 °C until constant weight. The concentration of As and nutrient elements, such as potassium (K), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), sodium (Na) and zinc (Zn), in dry samples (0.3 g) was determined after acid digestion with HNO_3 (65 %, v/v) and H_2O_2 (30 %, v/v) (EPA, 1995b). The digestion solution was diluted with Milli-Q water and the elements were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES 5900 Agilent, Santa Clara, CA, USA). The As content in the leaves and roots was calculated as the product between the element concentration in the leaves or roots and the DW of the leaves and roots. Furthermore, the As content in the whole plant was calculated by the sum of the As content in the leaves and roots, and the As concentration in the whole plant was obtained by dividing the total dry weight of the plant by the As content in the whole plant.

The effectiveness of the plant to translocate As from roots to shoots was determined by the translocation factor (TrF), calculated as the ratio between the As concentration in leaves and the concentration in roots. The ability of the plant to concentrate As into plant parts was assessed by calculating the bioconcentration factor (BCF), the ratio between the concentration of As in leaves or roots and the concentration of As concentration in soil.

At TF, immediately before the destructive sampling of plants, chlorophyll (Chl), flavonols (Flv) and anthocyanin (Anth) contents and Nitrogen-Flavonol Index (NFI; Chl/Flv ratio) were measured using the MPM-100 Multipigment Meter (ADC BioScientific Ltd., UK). Measurement was carried out on the upper side of the leaves (four leaves for each pot), avoiding the main veins (Dainelli et al., 2023).

2.8. Experimental quality assurance and control

Precautions were taken to minimize the possible MP contamination of the samples (Scopetani et al., 2020). The pots used were made of hard plastic, nondegradable within the established experimental duration. The presence of MPs in deionized water and reagents used was checked by filtration and visual inspection under a stereomicroscope. For the quality control of chemical analyzes, two standard solutions (0.5 and 2 mg L^{-1}) were used every 10 samples during the analysis procedure (CRM ERM - CD281 for plants and CRM ERM - CC141 for soil as certified reference materials). Blank samples were also run for the correction of the background and to identify possible sources of error. The detection limit for As was 5 μg L^{-1} and the recovery of the spiked samples ranged between 93 and 101 % with 1.91 standard deviation.

2.9. Statistical analysis

Soil and plant parameters at TF (60 days) were analyzed by two-way analysis of variance (ANOVA) with MP and As as fixed factors. Arsenic concentration in soil, As concentration and content in leaves, roots, and whole plant, As TrF and BCF in leaves and roots at TF were analyzed by one-way ANOVA with MPs as fixed factor. Additionally, As concentration in soil at T0 and TF was analyzed by a two-way ANOVA with MPs and time as fixed factors. Data were transformed to ln and arcsine when needed to fulfill the assumptions of the ANOVA, which was carried out according to the completely randomized design. The Tukey-B post-hoc significant difference test was used for comparison among treatments. Means and standard errors (SE) given in tables and figures are for untransformed data. Statistical analyzes were performed using the SPSS software package (version 25.0, SPSS Inc., Chicago, IL, USA).

All soil and plant parameters were analyzed by the Cluster/Similarity Profile test (SIMPROF), which allowed to segregate the samples into clusters based on their similarity/homogeneity. The data were initially square-root transformed and normalized (Clarke and Warwick, 2001), visualized by a Draftsman plot [based on Pearson correlations (*r*), −1 to +1] and then Euclidean distance was calculated. The significance of the correlations was calculated using the SPSS software package (version 25.0, SPSS Inc., Chicago, IL, USA). A correlation map was drawn and edited by Adobe Illustrator 2021. Cluster analysis was based on hierarchical agglomerative clustering with group average linkage. SIMPROF was performed using 999 permutations and a significant level of 5 % to objectively define the groups within the dendrogram and infer the effect of MPs and As. In the dendrogram, slices were drawn at statistically supported resemblance levels. Furthermore, the permutational analysis of variance (PERMANOVA) was used to test the effect of MP and As as fixed factors. The *P* value in PERMANOVA [*P*(MC)] was calculated using the Monte Carlo test (999 permutations) (Anderson and Braak, 2003). The explained variance was calculated and portioned among the sources of variation (mean effect of MPs and As, MPs x As interaction, residual). Since PERMANOVA is sensitive to differences in multivariate location (average community composition of a group) and dispersion (within-group variability), the analysis of homogeneity of multivariate

dispersion (PERMDISP) (Anderson, 2006) was performed to check the homogeneity of dispersion among groups (beta-diversity) (Anderson et al., 2006). When PERMANOVA was statistically significant, principal coordinate analysis (PCO) was performed to visualize the most relevant patterns in the data. In the PCO plot, the clusters were visualized based on the resemblance levels of the cluster/SIMPROF analysis. The circle in the PCO plot, whose diameter is 1.0, allows the reader to understand the scale of the vectors in the vector plot. All multivariate analyzes were performed using PRIMER 7 and PERMANOVA + software (Anderson et al., 2008; Clarke and Gorley, 2006).

3. Results and discussion

3.1. Microplastic background in soil and manufactured MPs

A quantitative analysis of the initial MPs in the soil could not be performed and only a visual analysis using a stereomicroscope was performed. In fact, standard methods for the quantification of MPs in a soil sample are not yet available, mainly due to problems associated with the considerably heterogeneous composition of soil (L. Yang et al., 2021b). However, the visual analysis of MP indicated that the level of MP (<1 mm) pollution in the soil was not relevant for this study. The ATR-FTIR spectroscopic analysis allowed to identify the MPs collected from the soil (background) as polyethylene (PE) and polypropylene (PP)

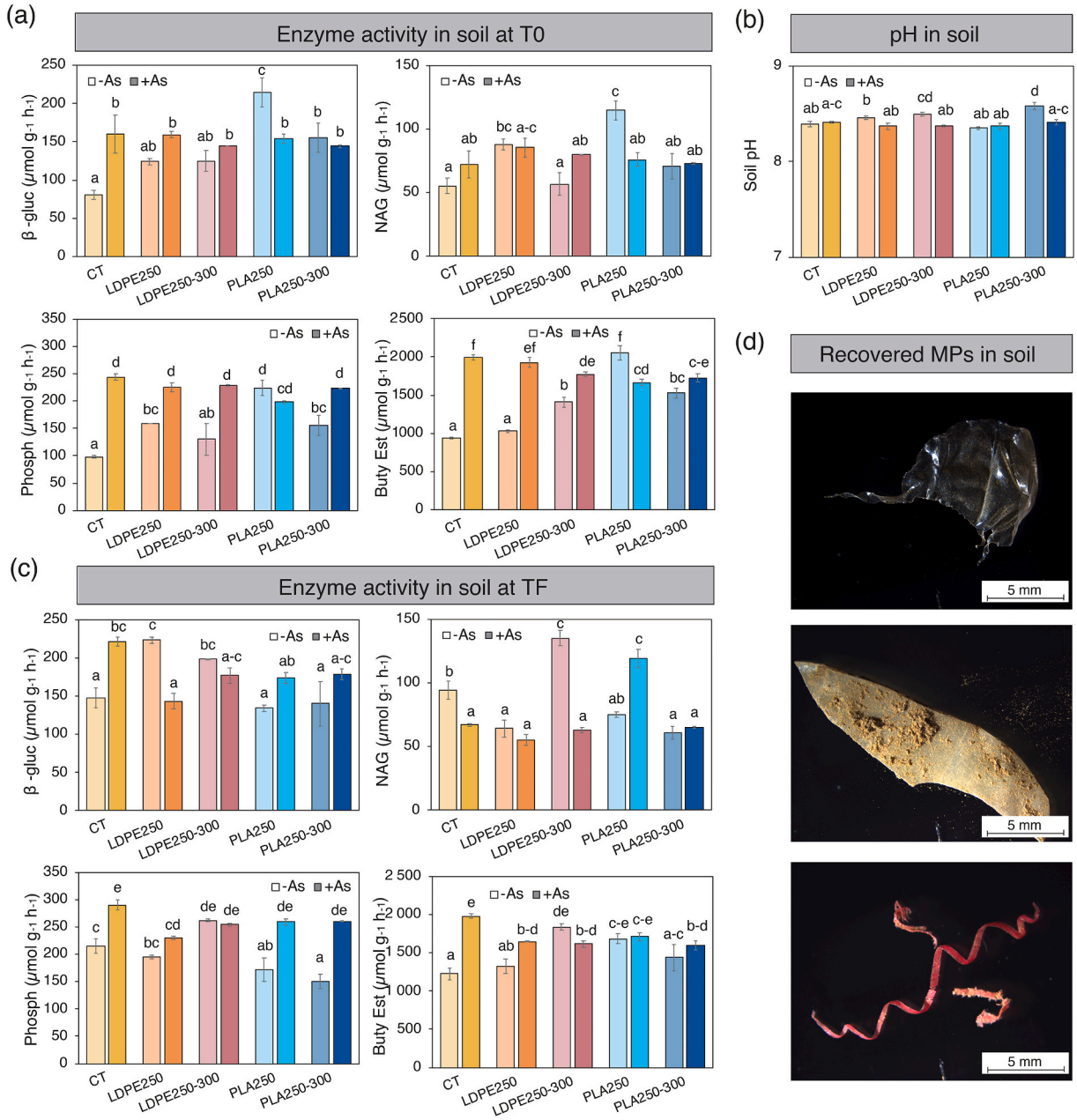

Fig. 1. Effect of the interaction of microplastics (MPs) and arsenic (As) application on enzyme activities in soil at T0 (a) and TF (c) (T0, 0 days; TF, 60 days) and on soil pH at TF (b). Images of the plastic particles manually collected from soil at T0 and visualized under a Leica M205 C stereomicroscope (d). The analyzed enzymes in soil are: β-glucosidase (β-gluc), *N*-acetylglutamate synthase (NAG), acid phosphatase (Phosph), and butyrate esterase (Buty Est). Codes of MP treatments are described in Table S3. Data from figures in (a), (b), and (c) are mean ± SE ($n = 4$). Different letters over the bars indicate significant differences according to the ANOVA and Tukey-B test ($P < 0.05$). Light colours of the bars indicate no As application (-As), while dark colours indicate the application of 60 mg As kg^{-1} soil (+As).

(Fig. S1). Detailed descriptions of ATR-FTIR spectra and the main structural difference between PE and PP are reported in Supplementary Results 1. Differential scanning calorimetry (DSC) thermograms (Fig. S2) of virgin pellets and manufactured MPs of low-density polyethylene (LDPE) and biodegradable polylactic acid (PLA) (<250 μm and 250–300 μm), utilized in this study, suggested that the grinding process did not affect the general thermal behavior of the materials and crystallinity, as shown in Table S3. The values of the parameters obtained from the DSC curves of LDPE and PLA are given in Table S4 and illustrated in Supplementary Results 2. After visual observation under stereomicroscope (Fig. 1d), manufactured MPs were classified based on their morphology as fragments, *i.e.* irregular shaped hard particles that appear to be broken down from a larger piece of litter, according to the GESAMP (GESAMP, 2019).

3.2. Effects of MPs and As on phytotoxicity

To evaluate the phytotoxicity of the soil, treated and untreated, *Lepidium sativum* germination was measured after 72 h of exposure to contaminants. The results of the test showed that the soil used was not phytotoxic to the plants, since all seedlings showed GI% values between 96.1 % and 105 % (Table S5). In fact, high values of GI% (>90 %) indicate reduced phytotoxicity and good quality of the contaminated soil. Furthermore, the addition of MPs and As to the soil did not influence the germination of *Lepidium sativum*. In fact, the two-way ANOVA did not indicate statistically significant differences in GI% between control soil and soil treated with MP and/or As (Table S6). Similarly to our results, Liwarska-Bizukojc (2022) found no differences between germination of seeds not exposed to plastic materials and seeds exposed to PLA, PP, and polyhydroxybutyrate of a size range of 3–5 mm. The author assumed that in the early stage of growth, the seedlings take advantage of the seed nutrient and energy reserves. On the contrary, Pflugmacher et al. (2020) recorded from the first day of the test an inhibitory effect of new and aged polycarbonate (PC) granules (size: 3 ± 1 mm) on *Lepidium sativum* germination and attributed the toxic effects to the release of chemicals present on the surface rather than to the physical effect of particles. Similarly, Bosker et al. (2019) found a significant reduction in the germination rate of *Lepidium sativum* after 8 h of exposure to nano- and micro-plastics (<100 nm and <5 mm) with increased adverse effects with increasing plastic sizes. In the present study, the presence of the paper filter might have overshadowed the direct physical interaction between MPs and the seeds. Indeed, the inhibition of germination found by Bosker et al. (2019) and Pflugmacher et al. (2020) in seeds of *Lepidium sativum* exposed to plastic particles was attributed to a physical blockage of the pores on the surface of the seed capsule. In contrast, in a soil-free system, seeds can recover and germination can increase to 100 %, whereas the soil can wrap seeds together with MPs and MPs can continue to block the pores.

3.3. Effects of MPs and As on soil

3.3.1. Soil pH

After 60 days of exposure (TF), the interaction between As application and MP type significantly affected soil pH (Fig. 1b, Table S7). Without As application, LDPE250–300 and PLA250–300 induced a significant increase in soil pH by 1.7 % compared to the control soil and the other MP treatments (Fig. 1b). On the contrary, with As application, soil pH was not modified by any MP types, supporting the lack of interaction As-MPs found by Dong et al. (2021b). Thus, under no As application, soil pH was more affected by MPs of large size. Several experiments performed with different types of MPs, in terms of shape, size and polymer, confirmed an increase in soil pH (Lozano et al., 2021a; Wang et al., 2021; W. Yang et al., 2021a; Zhao et al., 2021). However, the mechanisms by which MPs influence soil pH, a key soil parameter that affects a variety of microbial processes (Zhao et al., 2021), have not been fully clarified. The pH of the soil depends on multiple factors related to physicochemical and biological properties the soil, the intrinsic properties of contaminants, the presence of plants and the interactions between these factors (F. Wang et al., 2022b). Zhao et al. (2021) observed increases in soil pH according to the shape of polymers with higher values with foams and fragments compared to films. In fact, microplastic foams and fragments increased soil pH possibly due to high soil aeration and porosity (Lozano et al., 2021b) or due to the release and leaching of plastic additives (Kim et al., 2020; Rillig et al., 2019). In disagreement with our results, the influence of the size of MPs was highlighted by Dong et al. (2021b), who reported a decrease in soil pH and a greater impact of small particle MPs (0.1–1 μm) (polystyrene - PS and polytetrafluoroethylene - PTFE) compared to large particles (10–100 μm). Accordingly, other studies reported negative or neutral influences of MPs on pH (Feng et al., 2022; Yu et al., 2023; Shuwu Zhang et al., 2023a). However, the magnitude of the effects of MPs on soil pH observed in our study and other studies is small and could not influence the chemical and agronomic properties of the soil.

3.3.2. Soil As bioavailability

The bioavailable fraction of As in the soil at the beginning of the experiment (T0) did not vary according to the MP treatments and the As concentration differed between the untreated and As-treated conditions (1.11 ± 0.13 *vs* 4.78 ± 0.17 mg As kg^{-1}; $P < 0.001$). At the end of the experiment (TF), bioavailable As was found only in As-treated soils and the highest concentration was detected in the LDPE250–300, PLA250 and PLA250–300 treatments, which showed on average about 6 % higher values than the other treatments (Table S8). On the other hand, the bioavailable As over time in the As-treated soil did not vary with MP application, while it decreased significantly when MPs were not applied (−6.4 % from T0 to TF) (Fig. 2b). Therefore, our hypothesis that MPs application reduces soil As bioavailability was not confirmed by the results.

The effect of MPs on the bioavailability of As in the soil was previously investigated by Dong et al. (2021b) who found that MPs (PS 0.1–1 μm and PTFE 10–100 μm) induced a reduction in As availability (AsIII and AsV) and the effect was greater at the higher dose of MPs. The reduction might be due to the MP's higher surface absorbing capacity that decreases the bioavailability of As in the soil solution. The disagreement between these findings and our results may depend on the duration of the study (throughout the rice production cycle), as ageing could change the physical and chemical characteristics of MP, or may depend on MP type, dose and size, and initial soil pH (Dong et al., 2021b). On the other hand, similar to our results, MPs (PE and PLA, 100–250 μm) increased the bioavailability of As in riverine sediments after 65 days of exposure (Qin et al., 2022), probably due to microbial alterations. The disagreement found between studies could be attributable to the different species of As in the soil due to the experimental conditions, such as pH, or the As extraction method, but also to the type, dose, and size of MP used (An et al., 2023; Chen et al., 2023).

3.3.3. Soil enzymatic activity

A statistically significant interaction between MP and As was observed in soil enzyme activities at the beginning (T0) and the end of the experiment (TF) (Table S7). At T0, under no-As addition, β-gluc (involved in cellulose degradation) increased only with PLA in both sizes (+129 %) (Fig. 1a). Moreover, As addition significantly increased β-gluc in control soil (+98 %), while decreased the enzyme activity by 28 % with PLA250. No changes due to As were found in the soil treated with all other MPs. At TF, with no-As addition, β-gluc was increased only with LDPE at both sizes (+43 %) (Fig. 1c). Following As addition, β-gluc increased in the control soil (+50 %) and decreased with LDPE250 (−36 %), while it did not change with the other MPs. Recently, β-gluc was stimulated by PE foams (1.28 mm × 1.26 mm) as well as by PE and degradable Mater-Bi® with diameters of 20 μm-5 mm (Santini et al., 2023; Zhao et al., 2021). Stimulation of β-gluc could be related to the release of dissolved organic carbon (DOC) from plastics into the soil that

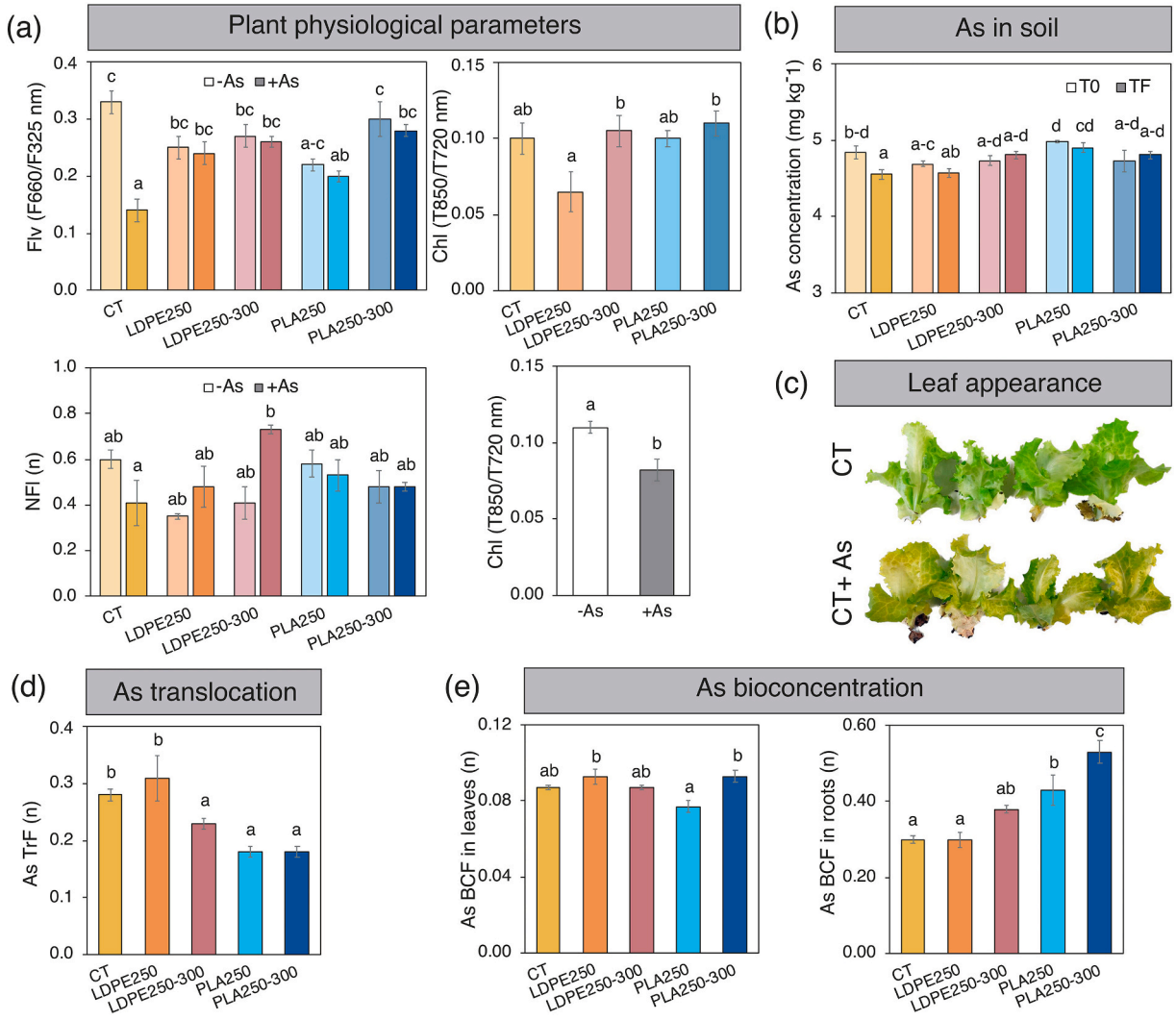

Fig. 2. Physiological parameters of lettuce (*Lactuca sativa* L.) (a): effect of the interaction of microplastics (MPs) and arsenic (As) application on Flavonol (Flv) and Nitrogen-Flavonol Index (NFI); effect of MPs on Chlorophyll content (Chl), and effect of As on Chl. Effect of the interaction of MPs and time of exposure (T0, 0 days; TF, 60 days) on As concentration in soil under As application (b). Images of untreated and As-treated leaves at TF (c). Effect of MPs on translocation factor of As (As TrF) of As-treated plants (d). Effect of MPs on As bioconcentration (As BCF) in leaves and roots of As-treated plants (e). Data from figures in (a), (b), and (e) are recorded at TF. Codes of MP treatments are described in Table S3. Data are mean ± SE (n = 4). Different letters over the bars indicate significant differences according to ANOVA and Tukey-B test ($P < 0.05$). In the figures reporting the effect of the interaction MPs and As, light colours of the bars indicate no As application (-As), while dark colours indicate the application of 60 mg As kg^{-1} soil (+As).

is rapidly used by microorganisms, potentially accelerating the decomposition of organic carbon from the soil and increasing CO_2 emissions (Cao et al., 2023). In fact, in our experiment, LDPE stimulated β-gluc at TF, when the release of C due to the ageing phenomena should be the greatest, while biodegradable MP appears to act only in the short term (T0). The stimulation effect of PE on β-gluc can also be related to the long-term effect of this plastic on the pore space of the soil, thus affecting the water and air flows and promoting soil microbial activity (Zhao et al., 2021). Other authors indicated an increase in β-gluc with PE application (<2 mm) regardless of the concentrations tested (0.1 % and 1 % w/w) (Diao et al., 2023), while PLA (20 to 50 μm, 2 % w/w) and other polymers (PP; PS; PC; polyamide, PA; polyester, PES; polyurethane, PU) in different shapes did not cause any effect (Zhao et al., 2021). These contradictory results may also be related to the differential effect of the incubation time on polymers that determines variable release of additives and fluctuations in the soil (micro)biota (Kim et al., 2020; Yi et al., 2021).

Similarly to β-gluc, at T0 and in the absence of As, NAG increased with LDPE250 and PLA250 compared to the control (+83 %) (Fig. 1a). However, when As was added, NAG was unchanged with LDPE250 and reduced by 34 % with PLA250, restoring enzyme activity to values similar to those of the other treatments. At TF and in the absence of As, NAG increased by 44 % with LDPE250–300 and decreased by 34 % with LDPE250 and PLA250–300 compared to the control (Fig. 1c). Furthermore, under As application, NAG decreased in control soil and with LDPE 250–300 (−29 % and −53 %, respectively), while it increased with PLA250 (+59 %). In general, the highest NAG activity was recorded with LDPE250–300 without As and PLA250 with As. In contrast to our results, no changes or decreases in NAG activity were found with PE (125 μm) at rates of 1 % and 5 %, respectively (Y. Liu et al., 2022c). These authors suggested that the high-rate addition of MP produced an unfavorable environment for N cycle by soil microorganisms. Furthermore, it was observed that the application of PE at a rate of 5 % (*w*/w) did not affect the activity of NAG when the particle size was small (300 μm), whereas it stimulated the activity of NAG when the size was higher (600 μm) (Ma et al., 2023). They indicated that larger MPs modify the physical environment within soil pores, leading to the promotion of the activity of N-related enzymes and the hydrolyzation of N-containing

organic matter. Finally, R. Liu et al. (2023b) found no significant variation in NAG activity between PLA (40 μm) at five rates (0, 0.1 %, 1 %, 5 % and 10 %, w/w). However, it should be considered that in our study the MP rate was 0.1 % and the particle sizes were higher than those tested by R. Liu et al. (2023b).

At T0, the activity of Phosph, linked to organic P mineralization, increased significantly in soil without As and treated with MP, except for LDPE250-300, compared to the control (on average by 83 %) (Fig. 1a). The addition of As promoted Phosph activity in all treatments, with significant increases in control soil (149 %) and with LDPE250, LDPE250–300 and PLA250–300 (42 %, 75 %, 44 %, respectively). On the contrary, at TF and without As, the presence of biodegradable MPs resulted in a reduction in Phosph activity compared to the control (−25 %), while with LDPE250–300 it was higher than in the control (+22 %) and LDPE250 did not change (Fig. 1c). Furthermore, the application of As stimulated Phosph activity in the control soil (+35 %) and the PLA-treated soils (on average 61 %). Fei et al. (2020), in a 50-day microcosm experiment, observed a significant increase in Phosph with LDPE (678 μm) at a concentration ranging from 1 % to 5 %. A similar positive influence was found in the present study with LDPE, supporting the stimulating effect of MP on Phosph activity due to the reduction of bulk density and the increase in soil porosity and aeration (de Souza Machado et al., 2018; Zhang et al., 2019). On the contrary, other authors (Liang et al., 2021; Lozano et al., 2021a) reported negative effects of MPs on Phosph or no change with the application of PE (<2 mm) and other polymers at 0.1 %, 0.4 % and 1 % (w/w) rates up to one month of incubation (Diao et al., 2023; Zhao et al., 2021).

Significant differences in Buty Est activity were found at T0 without As addition, and similar to the other enzymes, all MP, except LDPE250, increased enzyme activity (from 50 % with LDPE250–300 to 119 % with PLA250) (Fig. 1a). The addition of As significantly increased Buty Est in the control and LDPE-treated soils, while it was not effective in the PLA-treated soils. At TF and without As, Buty Est was higher with LDPE250–300 and PLA250 compared to the control (+43 %), while As application increased enzyme activity only in the control (+61 %) (Fig. 1c). To the best of our knowledge, this is the first study to investigate the activity of Buty Est in soils contaminated with MP. However, since Buty Est is a proxy of the microbial biomass, the data do not support our hypothesis that MPs reduce soil microbial activities, but suggest that the co-contamination of As with MPs promote microbial activities. Our results could depend on the resistant microorganisms growing on the debris of dead sensitive microbes, changing the microbial synthesis of the enzymes and possibly stimulating the microbial process that inactivates pollutants (Nannipieri et al., 2012). These indirect effects could have masked the direct inhibitory effects on soil enzymes. However, the results on the contamination of MPs and As should be interpreted with caution due to several reasons: (i) the activity of enzymes can be or not equal to microbial activity because many enzymes can exist and accumulate as protected enzymes in the soil matrix, remaining catalytic; (ii) changes in the speciation of the metal used as cocontaminant with MPs can determine changes in the responses of the enzymes during the assays; (iii) results may be subjected to environmental factors, management, or perturbations (Nannipieri et al., 2018, 2012). Therefore, there is still a large research gap in the impact of MPs in association or not with heavy metals on soil microbial activity and abundance, and there is the need to combine the conventional biochemical methods of evaluation with molecular tools allowing one to disentangle microbial communities in soil.

3.4. Effects of MPs and As on plant

3.4.1. Plant growth

A significant interaction between MP and As was observed in the fresh weight of lettuce leaf, root and plant, as demonstrated by the results of the two-way ANOVA (Table S7). Without As, leaf FW was higher in soil treated with MPs than in the control (on average + 20 %), with the exception of LDPE250–300 (Fig. 3a). On the contrary, root FW was similar to the control with all MPs, except for PLA250–300, which promoted root biomass (+41 %). The modifications of leaf development with MPs are illustrated in Fig. 4b. No significant differences between MPs were detected in the leaf and root FW (Table S7). Furthermore, the application of As did not significantly modify the leaf and root FW in control soil and in the root treated with LDPE250, while in the soil treated with all other MPs it significantly reduced the leaf and root FW (Fig. 3a). The effect of the interaction of As with PLA250–300 is visualized on leaves and roots in Figs. 4b and 5b. The DW of the leaves and root showed a response pattern similar to that of FW, while the water content of the leaves and roots was not significantly affected either by the treatments or by their interactions (data not shown). Not in line with our hypothesis, when As was not applied, the total FW of the plant increased with MP, with the exception of LDPE250–300, which showed whole plant biomass similar to the control (Fig. 3a). However, no significant differences were detected between MPs without As. The application of As did not modify the FW of the whole plant in the control, while, contrary to our hypothesis, it reduced the FW of the whole plant in soil treated with MP (Fig. 3a). The highest reduction was recorded with PLA250 and PLA250–300 (−37 % and −45 %, respectively), due to the reduction of root FW (−69 % and −75 %, respectively; Fig. 5b). Thus, MPs appear to have amplified the phytotoxic effect of As, suggesting a higher toxicity of MPs with As than with As exposure alone.

Few research studies have investigated the effects of MPs on plants and even less have analyzed the combined effect of MPs and As (Y. Dong et al., 2022a; Ivy et al., 2023). In our study, the increases in plant growth with all types of MP are in line with the general promotion of soil enzyme activities and the uptake of plant nutrients. Similarly, W. Yang et al. (2021a) found a promotion of maize growth with HDPE at a rate of 10 % and with PLA at lower rates (0.1 % and 1 %) (size 100–154 μm for both MPs). Accordingly, in the study of Shah et al. (2023), the biomass of *Glycine* max L. plants increased significantly with polyvinylchloride - PVC, PE and PS (10 μm, 10 % w/w). Microplastics decrease soil bulk density, leading to increased soil macroporosity and aeration, facilitating the penetration of roots into the soil and thus their growth (de Souza Machado et al., 2019). Such an increase in root biomass promotes water and nutrient uptake and is likely to stimulate higher rhizodeposition and mycorrhizal colonization with consequent increase in shoot biomass (Pellegrino et al., 2020, 2022). Furthermore, de Souza Machado et al. (2019), studying the effect of various fossil-based plastics on onion biomass, reported no effect or increases of MPs on total biomass in relation to MP type and size, and explained this pattern by the release of elements from the polymers. In contrast, the inhibition of lettuce growth by smaller MPs (45–75 μm; polyethylene terephthalate - PET, and bio-based plastic polyethylene 2,5-furan-dicarboxylate - PEF, at doses of 0.5 %, 1.0 % and 2.0 %) was attributed to MP adsorption at the root surface, resulting in a barrier to water and nutrient uptake (Zhang et al., 2022).

Wang et al. (2021) found that PE (<500 μm) at a dose of 10 % significantly reduced lettuce biomass after 45 days of exposure to three concentrations of soil cadmium (Cd) (0.49, 1.75, and 4.38 mg kg^{-1}), while no significant changes were found at MP doses of 0.1 % and 1 %. The authors attributed this inhibition to increased Cd availability in the soil, resulting in increased bioaccumulation of Cd in plants. On the contrary, in our study, detrimental effects on plant growth were detected under As application at a low MP rate (0.1 %), although no significant effects on As bioavailability in soil were found in the presence of MPs. Furthermore, W. Yang et al. (2021a) found that the combination of MPs (HDPE and PLA) with ZnO nanoparticles generally leads to a growth reduction of maize growth. In contrast, the addition of MPs (PS and PTFE; sizes of 0.1–1 μm and 10–100 μm and doses of 0.25 % and 0.5 %) to As-contaminated soil promoted increased biomasses in rice roots, stems, leaves and grains, thus alleviating the phytotoxic effect of As (Y. Dong et al., 2022a).

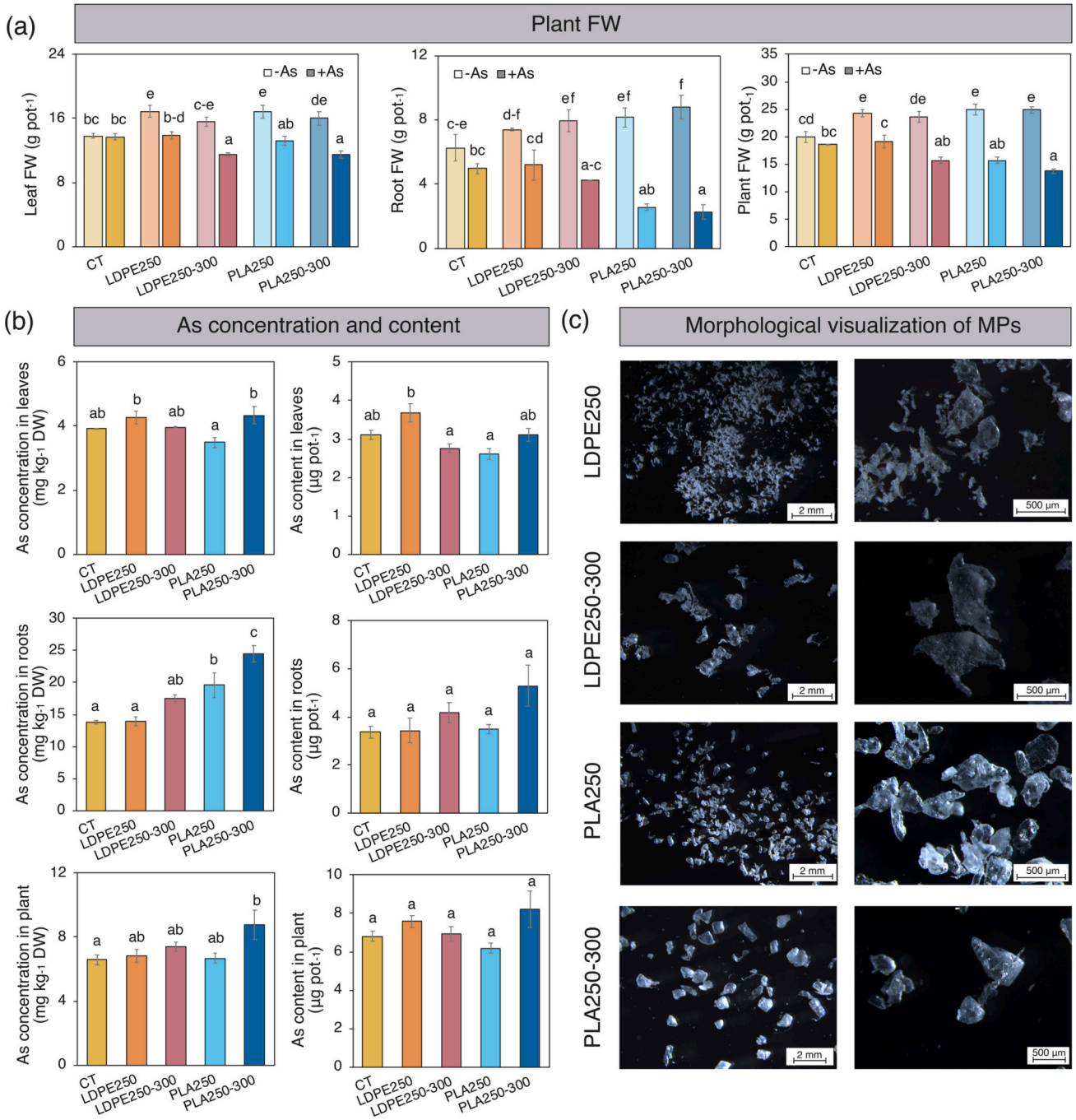

Fig. 3. Effect of the interaction of microplastics (MPs) and arsenic (As) application on lettuce (*Lactuca sativa* L.) fresh weight (FW) of leaves, roots and whole plant at TF (60 days) (a). Effect of MPs on As concentration and content in leaves, roots and whole plant in As-treated plants at TF (b). Images of the manufactured MPs used in the experiment and visualized under a Leica M205 C stereomicroscope (c). Codes of MP treatments are described in Table S3. Data are mean ± SE (n = 4). Different letters over the bars indicate significant differences according to ANOVA and Tukey-B test ($P < 0.05$). In the figures reporting the effect of the interaction MPs and As, light colours of the bars indicate no As application (-As), while dark colours indicate the application of 60 mg As kg^{-1} soil (+As).

3.4.2. Plant As concentration and content

The effect of the application of MPs on the concentration and content of As in lettuce plants treated with As is shown in Fig. 3b and Table S9. In general, As concentration in roots was approximately 4.5 times higher than in leaves and progressively increased from control and LDPE250 to LDPE250–300 and PLA250 and to PLA250–300. The roots of plants treated with PLA250–300 exhibited the highest As concentration, that is 78 % higher than the control. On the contrary, the highest As concentrations in leaves were reported with LDPE250 and PLA250–300, intermediate with the control and LDPE250–300 and the lowest with PLA250. However, variations in As concentration in leaves were small among treatments. No significant differences in the As content were observed in the roots (Table S9). On the contrary, the As content in the leaves varied according to MPs, and leaves under LDPE250 accumulated the highest amount of As, while leaves under LDPE250–300 and PLA250 accumulated the lowest amount of As (Fig. 3b; Table S9). The As content in the leaves under LDPE250–300 was 37 % higher than under LDPE250–300 and PLA250. The concentration of As in the whole plant was highest with PLA250–300, the lowest without MP and intermediate with the other MPs, while the As content did not vary between

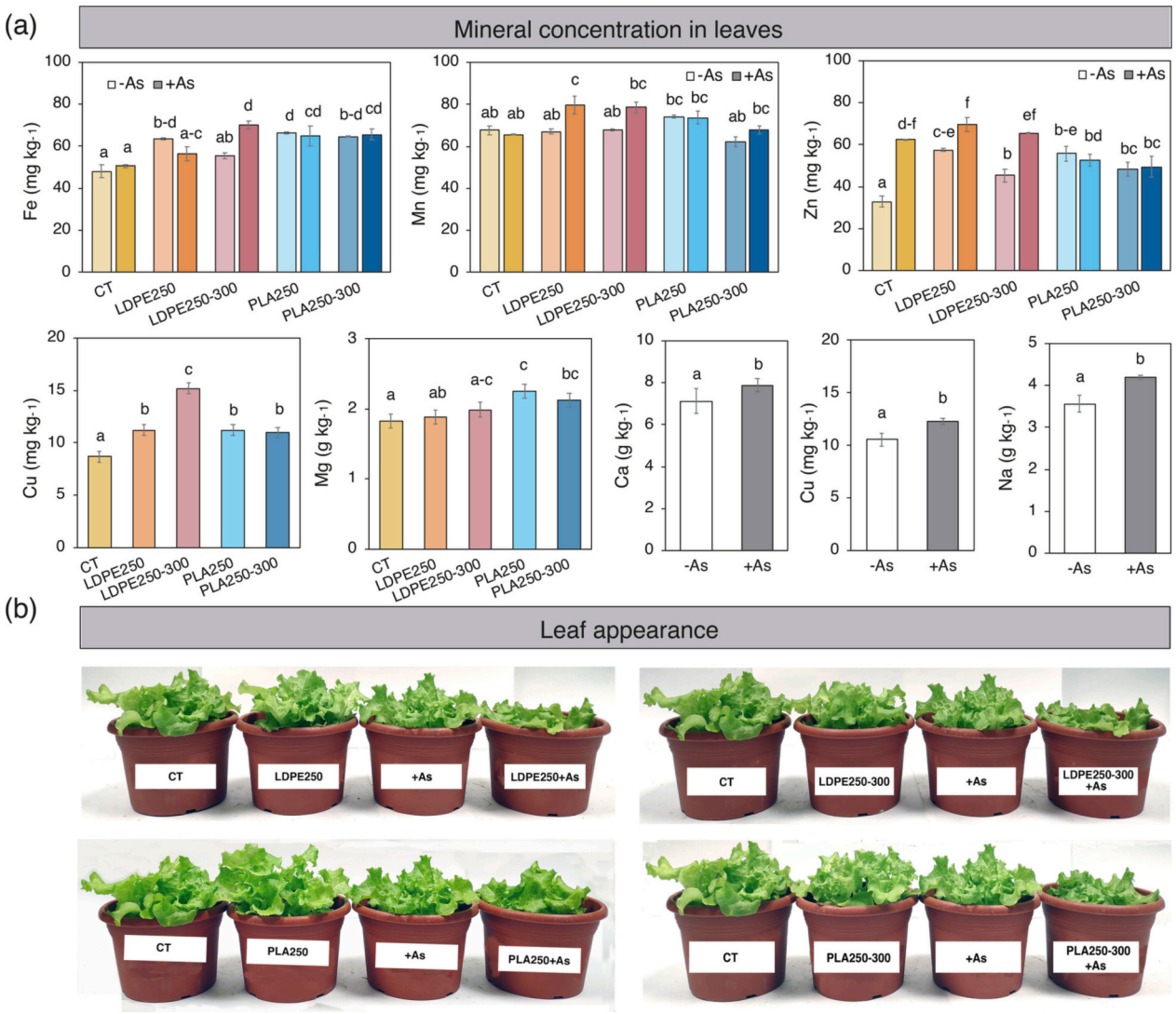

Fig. 4. Mineral concentration in leaves of lettuce (*Lactuca sativa* L.) (a): effect of the interaction of microplastics (MPs) and arsenic (As) application on Fe, Mn and Zn; effect of MPs on Cu and Mg; effect of As on Ca, Cu and Na. (b) Images of plants treated with MPs. Data and images are recorded at TF (60 days). Codes of MP treatments are described in Table S3. Data are mean ± SE (n = 4 for interaction MPs x As; $n = 8$ for MPs mean effect; $n = 20$ for As mean effect). Different letters over the bars indicate significant differences according to ANOVA and Tukey-B test ($P < 0.05$). In the figures reporting the effect of the interaction MPs and As, light colours of the bars indicate no As application (-As), while dark colours indicate the application of 60 mg As kg^{-1} soil (+As).

treatments (Fig. 3b; Table S9).

The accumulation pattern of As in lettuce was also investigated by calculating the translocation and bioconcentration factors. In general, TrF was below 1 in all treatments (values ranging from 0.18 to 0.31) (Fig. 2d), suggesting that lettuce was not able to transfer the metal to the aerial part of the plants. However, MPs significantly reduced As TrF, except for LDPE250, which showed values similar to the control (Table S9). The TrF reduction was on average about 30 % with LDPE250–300 and PLA in both sizes. In general, the ability to concentrate As by roots was greater than that of leaves, since BCF in roots ranged from 0.30 to 0.53 and BCF in leaves from 0.001 to 0.004 (Fig. 2e). In roots, BFC progressively and significantly increased from control and LDPE250 to LDPE250–300, PLA250, PLA250–300, while, in leaves, BCF showed small variations and a significant increase only in plants treated with LPDE250 and PLA250–300 *versus* plants treated with PLA250 (+21 %).

Similarly to other studies on lettuce (Yañez et al., 2019) and on nonhyperaccumulating As species, such as *Cannabis sativa* L. (Grifoni et al., 2021), our study showed that lettuce plants preferentially stored the toxic metal in the root system. Therefore, lettuce can be considered as an excluder plant species, implying a limited translocation rate in the edible aerial part, even when As concentrations in soil are high, exceeding the Italian legislative limits (20 mg As kg^{-1}; Legislative Decree 152/2006, 2006). Contrary to the results of Yu et al. (2020), who found that MPs reduce the bioavailability of As, Cu, chromium (Cr) and nickel (Ni), by converting the mobile form into stable organic forms, in the present study, the enhanced concentration of As in roots was presumably caused by a weak adsorption of As on the MP surface with consequent potential reductions of leached As.

3.4.3. Plant physiological parameters and mineral element concentrations

In the absence of As, no change in Flv was reported in leaves, while when As was applied, a significant reduction was reported only in the control (−58 %) (Fig. 2a; Table S7). In Fig. 2c the effect of As on the leaf appearance is visualized. The flavonol content in plants treated with MP plus As was similar to the values observed when As was not applied, supporting an alleviation of the phytotoxic effect of As. On the contrary, Chl was significantly affected by the mean effect of MP and As (Table S7). No significant variation in Chl was observed applying MP, while application of LDPE250 significantly reduced Chl compared to

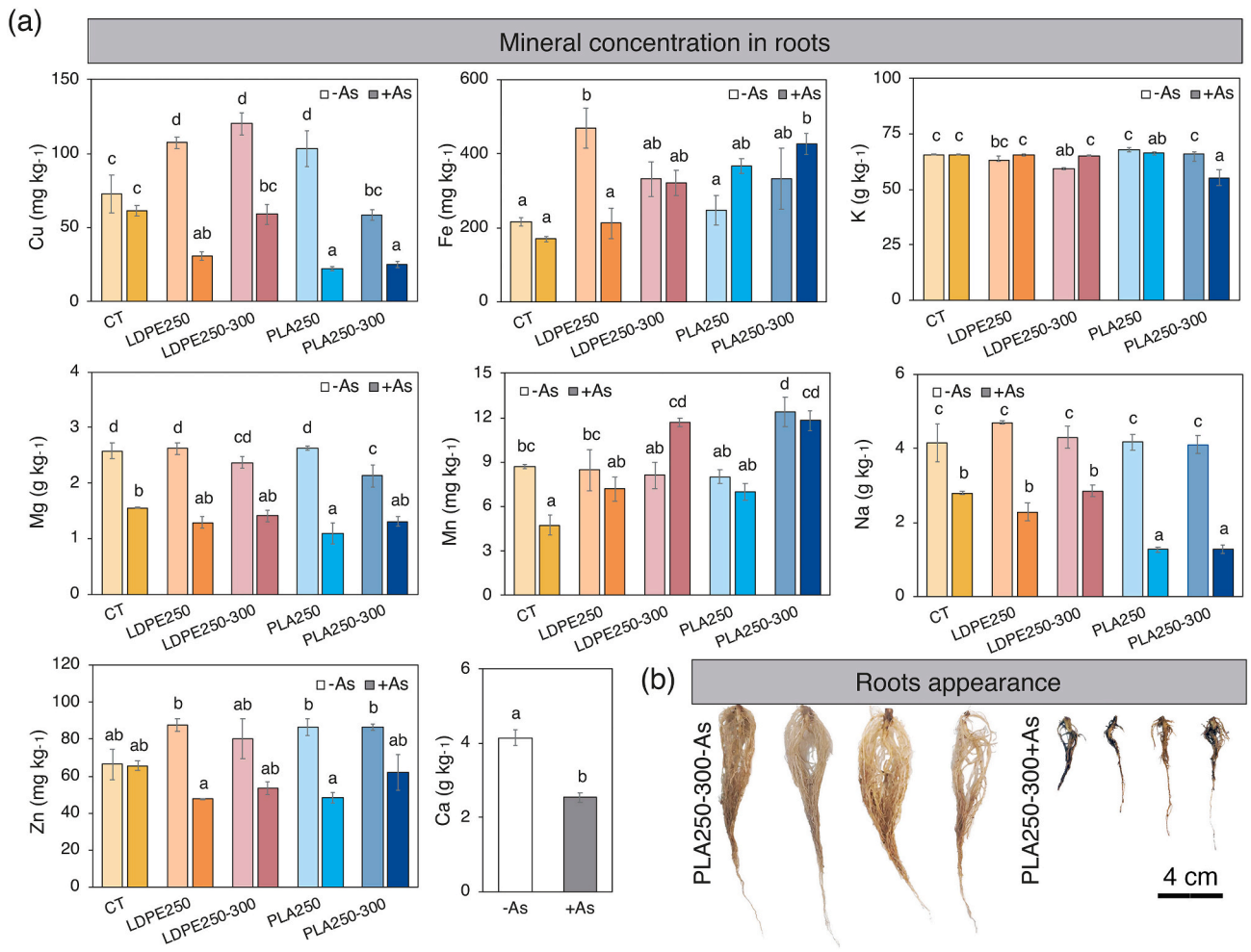

Fig. 5. Mineral concentration in roots of lettuce (*Lactuca sativa* L.) (a): effect of the interaction of microplastics (MPs) and arsenic (As) application on Cu, Fe, K, Mg, Mn, Na and Zn; effect of As on *Ca.* (b) Images of roots with PLA250–300 without As (PLA250–300-As) and with PLA250–300 with As (PLA250–300 + As). Data and images are recorded at TF (60 days). Codes of MP treatments are described in Table S3. Data are mean ± SE (n = 4 for interaction MPs x As; n = 20 for As mean effect). Different letters over the bars indicate significant differences according to ANOVA and Tukey-B test ($P < 0.05$). In the figures reporting the effect of the interaction MPs and As, light colours of the bars indicate no As application (-As), while dark colours indicate the application of 60 mg As kg^{-1} soil (+As).

LDPE250–300 and PLA250–300 (−43 %) (Fig. 2a). As expected, application of As reduced Chl in leaves (−46 %). Additionally, the Nitrogen-Flavonol Index (Chl/Flv ratio) when As was not applied did not vary between treatments, while with As a significant difference was only recorded between the control and LDPE250–300, supporting the beneficial effect of LDPE250–300 in alleviating the phytotoxic effect of As. Finally, Anth did not change between treatments (Table S7; on average 0.16 ± 0.03 multiplex ANTH units; Cerovic et al., 2008). Previously, the addition of PS (10 μm), PTFE (10 μm), and As(III) was reported to reduce the photosynthetic rate in hydroponic experiments (Dong et al., 2020b). Accordingly, exposure to PE nanoplastic significantly reduced the Chl content in rapeseed leaves (R. Dong et al., 2022b). Additionally, Zong et al. (2021) reported that contamination with Cu, Cd and MP (584 nm) increased Chl content and photosynthetic activities and reduced the accumulation of reactive oxygen species, supporting our result on the mitigation effect of LDPE250–300 on As phytotoxicity.

The concentration of Cu, Fe, K, Mg, Mn, Na, and Zn in the roots was significantly affected by the interaction of MP and As, while the concentration of Ca in the root was affected only by the mean effect of As (Table S10; Fig. 4a). The application of MPs without As generally did not modify the concentration of Cu, Mg, Na, and Zn in roots, whereas the co-contamination had a general negative impact on the root concentration. However, the detrimental effect was recorded at variable intensity among MPs, with the highest reduction with PLA250. Similarly, MPs without As generally did not modify the concentration of Fe, K, and Mn in roots, whereas with MPs and As only slight reductions were observed. Altered root activity in absorbing nutrients from the soil is a general indicator of the response of the plant to contaminants, affecting metabolism, growth, and development. On the one hand, As can reduce nutrient uptake and translocation directly through structural damage to root cell membranes (Gusman et al., 2013) or indirectly by promoting oxidative stress (Zulfiqar and Ashraf, 2022). On the other hand, MPs can physically hinder water and nutrient absorption by obstructing root cell pores (Ivy et al., 2023). This absorption inhibition was also found by Xu et al. (2022), who reported a significant influence on ionomic metabolism in lettuce roots in response to PS microplastic beads (200 nm diameter), with significant reductions in K, Ca, Mg and Fe in the roots. However, in our study, exposure to MPs did not affect the concentrations of nutrients in the roots, although root growth, soil microbial biomass, and cellulose degradation activity were promoted. The neutral response observed to MPs could be due to the limited exposure time of plants to contaminants. Probably, compared to As, the effect of MPs can be observed over longer periods, when weathering produced significant physical and/or chemical alterations in soil and contaminants, thus affecting plants (Duan et al., 2021).

The concentration of Fe, Mn and Zn in leaves was significantly affected by the interaction of MPs and As, while Ca, Cu and Na were affected by the mean effect of As and Cu and Mg by the mean effect of

MP (Fig. 4a; Table S10). The application of MPs without As generally increased the concentration of Fe and Zn in leaves, whereas no changes were observed for Mn. Moreover, the concentration of Cu and Mg in the leaves were generally promoted by the application of MPs. These increases were associated with a general promotion of leaf biomass, suggesting an increase in total nutrient uptake due to the stimulation of the activity of some soil enzymes. Since soil enzyme activities, produced by microbes, regulate soil organic matter decomposition and nutrient cycling, variation in the enzyme pattern has major impacts on the uptake of nutrients by plants (Fei et al., 2020; Oladele et al., 2023; Shah et al., 2023). Similarly, increases in Fe and Zn concentration were reported in cowpea shoots under low concentration of PE contamination (0.5 % w/w) (Oladele et al., 2023). Additionally, Y. Liu et al. (2023a) also found that contamination with PET and PLA (~51 μm size) increased the Fe and Mn content in rice roots and shoots at a dose of 2 % (w/w). However, most studies reported a negative effect of MPs on plant nutrient uptake. As an example, Colzi et al. (2022) reported negative effects on nutrient concentration (K, Ca, Mg, Fe, Zn, Cu, Mn) in zucchini leaves after the application of MPs (PP, PE, PET and PVC) at increasing concentrations, attributing this effect to the polymer's ability to induce

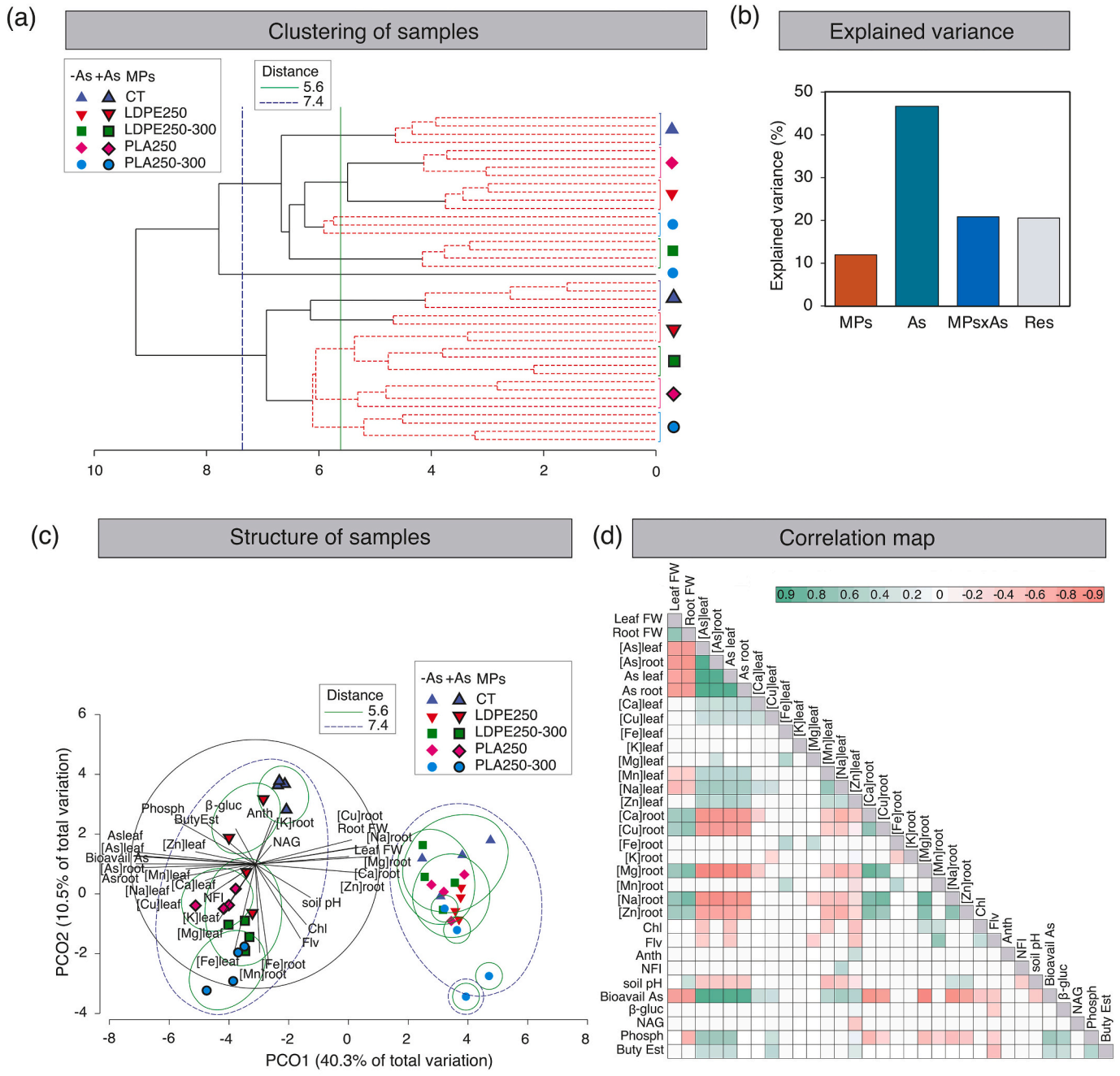

Fig. 6. Dendrogram of samples following Cluster/Similarity Profile test (SIMPROF) analysis of the effect of microplastics (MPs) [low-density polyethylene (LDPE, at 0.1 % w/w) and polylactic acid (PLA, at 0.1 % w/w) at two sizes] and arsenic (As) (0 and 60 mg As kg^{-1}) on soil and plant parameters (a). Explained variance partitioning into mean effects of MPs and As, interaction of MPs and As (MPsxAs) and residuals (Res) (b). The SIMPROF was performed using 999 permutations and groups in the dendrogram are defined at significant level of 5 % (red-dashed lines). In the dendrogram, slices are drawn at statistically supported resemblance levels (*i.e.*, green slice; 5.5; violet slice: 7.4). The variance partitioning is calculated by permutational analysis of variance (PERMANOVA). *P* value in the PERMANOVA [P (MC)] is calculated using the Monte-Carlo test (999 permutations). Data are square root transformed and normalized and Euclidean distance are calculated. Codes of treatments are described in Table S3. Treatments were replicated four times.

oxidative stress. Similarly, the application of PS (100 nm, 5 % w/v) reduced or did not modify the concentration of nutrients (Mg, Fe, Mn, Cu, Zn) in wheat shoots (Lian et al., 2020), while the application of PS and PVC (~10 μm) at increasing doses reduced the concentration of Ca and K in rice shoots (Ma et al., 2022). This was explained by the blocking of MP of cell connections or pores of the cell wall for the transport of nutrients (Hua et al., 2024; Y. Zhang et al., 2023b). Another explanation was the indirect effect of microbial growth due to the release of C by MPs and the immobilization of nutrients by microbes leaving little or no nutrients to plants. Our contrasting results might be explained by the promotion of nutrient availability in soil with MPs that can be determined by the improvement of soil physical structure and the consequent increase of microbial biomass and enzyme activities, proxy of organic matter degradation.

Unexpectedly, the concentration of Ca, Cu and Na in leaves was higher with the application of As (Fig. 4a; Table S10), suggesting that the applied contamination of As enhanced the transport from the roots to the shoots. When As was applied with MPs, the co-contamination did not modify or slightly increase the concentration of Fe, Mn and Zn in leaves (Fig. 4a; Table S10). The response of these nutrients could suggest a mitigation effect of MPs on As phytotoxicity and that most of As is likely present as As/MP complexes rather than as free ions, as reported in several studies for other metals (Zong et al., 2021). Thus, As immobilization can further improve nutrient cycling by microbes and plant nutrient uptake. This pattern may also be explained by the fact that the addition of MPs to the soil may increase the release of additives, such as phthalates, which are reported to promote nutrient cycles (Wang et al., 2018). However, the effect of MPs on metal phytotoxicity needs further investigation.

3.5. Overall response to MPs and As

Cluster analysis allowed identifying three SIMPROF clusters that were consistent with As treatment (Fig. 6a). In detail, at a similarity distance of 7.4, two clusters without As-treated were identified, one composed of the control soil, LDPE250, LDPE250–300, PLA250 and PLA250–300, and the other composed of one sample of PLA250–300, and one cluster composed of all As treated samples. Furthermore, at a distance of similarity of 5.6, 12 clusters were identified. Under no application of As, the control soil clustered far from all samples treated with MPs. The LDPE250 and PLA250 treatments grouped together and had a behavior more similar to PLA250–300 that clustered separately from them and formed four distinct groups. LDPE250–300 formed a homogenous cluster far from the others. On the contrary, in As application, LDPE250 showed a behavior similar to the control and LDPE250–300, since two samples grouped with the control, while the other two samples grouped with LDPE250–300. Finally, PLA250 and PLA250–300 formed two distinct groups, well separated from the other treatments. Accordingly, the PERMANOVA showed that the MP and As mean effect and their interaction significantly affected the parameters of the soil and the plant (Table S11). Microplastics and As explained 12 % and 47 % of the variance, respectively, while their interaction explained 21 %, suggesting a higher toxic impact of As than MPs (Fig. 6b). In the PCO biplots, the first axis explained 40.3 % of the total variation, while the second axis explained 10.5 % (Fig. 6c). Along the first axis, the samples were distributed in two groups, -As and +As, proving that the major driver of variation is As. This is also depicted by the clouds that represent the similarity distance of 7.4. The -As treated samples were mainly associated with a higher concentration of Cu, Na, Mg, Ca, and Zn in roots and higher leaves and root FW, whereas the +As treated samples were associated with a higher concentration of Zn, Mn, Ca, Na, and Cu in leaves, and, as expected, to a higher concentration and content of As in leaves and roots and bioavailable As in soil. Along the second axis, the samples were separated according to MP treatments. This is also clear when one observes the clouds representing the distance of similarity of 5.6. Indeed, similar to the cluster analysis (Fig. 6a), in the -As cloud, the LDPE250 and PLA250 grouped together, while the control and LDPE250–300 were more similar to each other, and the PLA250–300 samples clustered in four separate groups. In the +As cloud, the MP and control groups were greatly discriminated along the second axis, and the pattern of similarities changed with respect to the -As samples. In detail, while LDPE250 and PLA250 remained close to each other and PLA250–300 was far from the others, the control and LDPE250–300 were plotted in two well-separated groups, and PLA250–300 formed a homogeneous group. Therefore, to summarize, the application of MPs determined overall similar effects on plant and soil parameters, whereas the co-contamination produced a clear differentiation. In fact, within the +As cloud, the control soil was characterized by a high concentration of K in the roots and anthocyanin content in the leaves, while LDPE250 was characterized by high enzyme activities (β-gluc, Phosph, Buty Est) and PLA250, LDPE250–300 and PLA250–300 with a high NFI, and a high concentration of K, Mg, and Fe in the leaves, and Fe and Mn concentrations in the roots.

The correlation map between soil and plant parameters highlighted positive correlations between the concentration and content of As in leaves and roots on the one side, and Ca, Cu, Mn, Na and Zn in leaves on the other side, and negative correlations between As concentrations and contents in leaves and roots on the one side, and Ca, Cu, Mg, Na, and Zn in roots on the other side (Fig. 6d). These correlations indicate a higher toxic effect of As on the root than on the leaves. Indeed, we observed greater reductions in biomass in roots than in leaves. The concentration and content of As in leaves and roots were also negatively correlated with soil pH and positively correlated with the enzyme activities in soil, such as Phosph and Buty Est, supporting our hypothesis that co-contamination of As with MP promotes microbial activity. Furthermore, the concentration in leaves of some nutrients were positively correlated between each other (that is, Ca *vs* Cu/Na; Cu *vs* Na; Fe *vs* Mg; Mn *vs* Na/Zn, Na *vs* Zn). Similarly, positive correlations were detected between some nutrient concentrations in leaves and roots (Fe_{leaf} *vs* Fe_{root}, Fe_{leaf} *vs* Mn_{root}, Mg_{leaf} *vs* Fe_{root}) and in roots (Ca_{root} *vs* Cu_{root}; $Ca/Mg/Na/Zn_{root}$; Cu_{root} *vs* $Mg/Na/Zn_{root}$; Fe_{root} *vs* Mn_{root}; Mg *vs* Na/Zn_{root}; Na_{root} *vs* Zn_{root}). On the contrary, some nutrients in leaves and roots were negatively correlated (that is, Ca_{leaf} *vs* Ca/Mg_{root}; Cu_{leaf} *vs* K_{root}; $Mg/Mn/Na/Zn_{leaf}$ *vs* Na_{root}; Mn/Na_{leaf} *vs* Ca/Cu_{root}; $Mn/Na/Zn_{leaf}$ *vs* Mg/Zn_{root}). This pattern suggests that the balance of chemical elements of plants may be regulated by other factors, such as contaminants, in addition to the need to maintain fixed ratios of nutrients to maintain physiological processes and for the size scaling of plants (Elser et al., 2010). As expected, leaf and root biomass was highly positively related, as well as As concentration in roots and leaves, positively correlated with As content\ in roots and leaves and bioavailable As in soil. Finally, an expected positive correlation was detected between β-gluc and Phosph/Buty Est and between Phosph and Buty Est, while a negative correlation was detected between leaf and root As concentration and content and leaf and root fresh weight.

4. Conclusions

In our study, soil contamination with conventional and biodegradable MPs of larger size (250–300 μm) buffered the effects of As on soil pH. Indeed, the pH was slightly increased by larger MPs, but when MPs were applied with As, this effect disappeared. Our hypothesis that MP contamination reduces plant growth by reducing nutrient uptake and soil microbial activity was not supported by our results. MPs generally promoted plant growth, did not induce changes in root nutrient concentrations, and did not affect or increased the leaf nutrient content and soil enzyme activities. The increased plant growth may be due to the release of DOC in the soil and the resulting promotion of soil microbial biomass and functionality. Furthermore, the microbial growth might be driven by improving the physical structure of the soil. The effect of MPs on enzyme activities varied depending on the time of exposure, *i.e.*, with greater effects immediately after MP contamination and on the type and

size of MPs. On the contrary, the effect on plant growth and nutrient concentration was similar between MP types and sizes, probably due to the limited exposure time of plants to contaminants.

The application of As did not modify the growth of the plant and reduced the concentration of some nutrients in the roots. However, As promoted most of the soil enzymes studied, suggesting the growth of degrading microbes both immediately after application and in the medium term.

Our hypothesis that co-contamination of MPs and As reduces the bioavailability of As in soil with positive effects on plants and soil microbial activities was not supported by our results, since the co-application of MPs and As did not modify the amount of bioavailable As in soil in the short and medium term, generally it did not modify soil enzymes and reduced plant growth and nutrient concentration in roots. Therefore, MPs in association with As determined plant and soil toxicity. However, the mechanisms of the joint effect of MPs and As on plant/soil toxicity need further investigation, especially in field conditions and long-term experiments.

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CRediT authorship contribution statement

Martina Grifoni: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Elisa Pellegrino:** Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. **Leonardo Arrighetti:** Formal analysis, Writing – review & editing. **Simona Bronco:** Writing – review & editing. **Beatrice Pezzarossa:** Writing – review & editing. **Laura Ercoli:** Conceptualization, Data curation, Funding acquisition, Methodology, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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