Applied Soil Ecology 214 (2025) 106340

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Driving soils to change: Tyre wear particles modulate microbial-mediated soil functions and performance of vegetable crops

D. Kundel [a,*], M. Bigalke [b], B. Stehle [a], M. Hammer [a], K.N. Nitzsche [b], A. Fliessbach [a]

[a] Soil Sciences Department, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland
[b] Department of Soil Mineralogy and Soil Chemistry, Institute of Applied Geosciences, Technical University of Darmstadt, 64287 Darmstadt, Germany

ARTICLE INFO

Keywords:
Tyre wear particles
Microbial activity
Vegetable production
Agriculture
Soil pollution

ABSTRACT

Tyre wear particles (TP) enter soils through various pathways, accumulate over time and can affect plant-soil systems. However, the effects of TP, particularly on the microbiota, are not well understood and the potential risks to soil ecosystems remain uncertain. We added TP at concentrations ranging from 0 % to 3 % (*w*/w) to a sandy loam soil and conducted an experiment with lettuce and leek. We assessed how TP affect plants, the colonisation of their roots with arbuscular mycorrhizal fungi (AMF), soil enzymatic activities and microbial catabolic profiles. Exposure to 3 % TP reduced root and shoot biomass by 35 % and 23 %, respectively, relative to the control, while lower TP levels showed some stimulating effects. Also, a TP addition of 3 % increased plant copper and zinc concentrations but decreased plant nitrogen. We did not find effects on AMF colonisation, yet, these findings are based on the staining method only and should be studied in more depth using additional methods. Some soil enzymes showed a high sensitivity to even the lowest TP concentrations. Furthermore, in soils containing 3 % TP, we measured a reduction in the microbial biomass carbon (up to −20 %), an increase in the microbial metabolic quotient (up to +62 %), and significant changes in the catabolic capacity of soil microbes. Our data show that TP can affect the microbial basis of the soil food web and specific processes related to soil nutrient and energy flow, with ultimate effects on plants. Although the mechanisms cannot be fully displayed with the data at hand, our study stresses the urgent need to implement measures that reduce TP pollution to safeguard soil quality and allow the production of healthy food crops.

1. Introduction

Plastic and its decay products, microplastics (MP), enter soil e.g. as plastic residues in urban composts, digestate application, leftovers from plastic mulch film or through littering (Riveros et al., 2021). Yet, tyre particles (TP), created through the abrasion of rubber material while driving, are nowadays considered to be one of the most significant contributors to terrestrial MP pollution (Baensch-Baltruschat et al., 2020). The annual TP emission rates into the environment were summarised across 13 countries and predicted to exceed 3.3 Mt, translating into a global per capita TP emission of 0.23–4.7 kg year^{-1} with an average of 0.81 kg year^{-1} (Kole et al., 2017). Airborne transport, subsequent deposition, and runoff from roads with no connection to a wastewater system result in the contamination of water bodies, air and soils (Sieber et al., 2020). Tyre particle concentrations in roadside soils are difficult to quantify, yet likely in the range between 0.1 and 117 g kg^{-1} soil (Baensch-Baltruschat et al., 2020; Müller et al., 2022; Sieber et al., 2020; Wik and Dave, 2009). Published data on TP directly extracted from soils are, however, still extremely scarce (Mennekes and Nowack, 2022) leaving us with a significant level of uncertainty when predicting the effects of this global change driver on soil ecosystems.

Tyres are composed of a complex mixture of natural and synthetic carbon polymers. Although not completely made of artificial polymers, they are usually classified as MP (Ding et al., 2023; Hartmann et al., 2019; Lassen et al., 2015). Tyres also contain fillers such as soot and carbon black, silica and chalk, softeners including oil and resin, vulcanisation agents, (e.g. sulphur and zinc oxides), and various additives (e. g. *N*-phenyl-N′-(1,3-dimethylbutyl)-*p*-phenylenediamine (6PPD), benzothiazole or 2-Mercaptobenzothiazole) to enhance tyre durability (Wagner et al., 2018). Moreover, TP produced through abrasion, frequently accumulate environmental pollutants on their surfaces, including antibiotics (Fan et al., 2023) and heavy metals (Glaubitz et al., 2023), which transfers each particle into a unique combination of inherent chemicals and external contaminants and results in a complex

---

[*] Corresponding author.
E-mail address: dominika.kundel@fibl.org (D. Kundel).

https://doi.org/10.1016/j.apsoil.2025.106340
Received 5 March 2025; Received in revised form 2 June 2025; Accepted 21 July 2025

cocktail of chemicals introduced into the environment.

Despite the assumed massive emissions of TP into soils and the presence of substances in tyres that raise concerns about their environmental impacts, we lack investigations in soil systems to assess the risk of TP contamination for the manifold ecosystem services. The few available studies exploring the impact of TP on the plant-soil system, showed, e.g. plant available Zn concentrations in TP-contaminated soils to increase to levels that may be detrimental to plant health (Leifheit et al., 2022). Zinc toxicity was earlier suggested as one of the main reasons for morphological alterations and reduced plant growth (Bowman et al., 1994; Schulz, 1987). Zeb et al. (2024a) identified sixteen different polycyclic aromatic hydrocarbons (PAHs) leaching from TP and Castan et al. (2022) showed that certain tyre wear-derived compounds transformed into stable metabolites in lettuce leaves. Given the largely unknown toxicity of these substances, the findings raise great concern regarding the impacts of TP on soil and plant quality and human health. Indeed, Leifheit et al. (2022) reported alterations in various biogeochemical soil parameters upon the addition of TP. These changes included an increase in soil pH and soil microbial respiration and reduced plant biomass upon exposure of plants to 10 mg TP g^{-1}. Smolders and Degryse (2002) observed elevated levels of soil nitrification in response to TP, an effect they attribute to the release of Zn and an increase in pH; yet also the discharge of additives is considered important in this context (Rogge et al., 1993). Just recently, Wei et al. (2025) showed the impacts of TP on carbon and nitrogen cycling, the denitrifying community, greenhouse gas emissions and fertilizer N loss. While these studies have provided novel and important insights regarding the effects of TP, investigations focussing on microorganisms remain limited (Ding et al., 2023), there are still too few studies assessing soil microbial responses in soils (Leifheit et al., 2022; Wei et al., 2025; Xu et al., 2024; Zeb et al., 2024a, 2024b) to comprehensively outline the risks for soil quality.

Even less research is available on how TP affect specific groups of microorganisms such as arbuscular mycorrhizal fungi (AMF), which are root-associated symbionts with an essential role in nutrient uptake and enhancing plant stress resistance. Wang et al. (2020) found dose-dependent negative effects of biodegradable polylactic acid particles on AMF diversity and community composition, but limited effects of non-biodegradable MPs such as polyethylene. De Souza Machado et al. (2019) found an eight-fold increase in root colonisation upon exposure to polyester fibres at 0.2 % (*w*/w), while 2 % (w/w) polyester terephthalate fragments reduced colonisation by about 50 %. These findings highlight that the impact on AMF is shaped by the chemical composition and structure of MP, suggesting that results from these studies may not necessarily apply to tyre-derived particles. To date, however, no data is available on how TP act on AMF. Changes in AMF communities, however, may affect the functioning of an ecosystem at different levels (Powell and Rillig, 2018).

To explore the impacts of TP on the plant-soil system, we conducted an experiment under controlled conditions exposing leek over 12 and lettuce over 7 weeks to TP concentrations ranging from 0 % to 3 % w/w. We used a sandy loam soil and examined how TP affect root and shoot biomass, and shoot nutrient and trace metal concentrations as crucial endpoint measurements with insights into yield quantity and quality. Moreover, we quantified the impact of TP addition on AMF root colonisation, which, to our knowledge, has not been explored in this context before. We measured the activity of seven extracellular soil enzymes, given their significance for nutrient cycling and potential as early indicators of soil fertility changes. Finally, using the MicroResp™ method, we assessed the size and stress indices of microbial communities and investigated impacts on microbial catabolic profiles to reveal potential shifts in soil microbial community functions in response to TP exposure. The assay assumes that microbial communities characterised by a high diversity can also utilise a wider range of structurally different carbon substrates when compared to less diverse communities (Creamer et al., 2009).

We hypothesised that soil contamination with TP would (i) negatively affect plant performance, reflected by a decrease in overall biomass and a significant rise in trace metal concentrations, particularly Zn, and (ii) exert an influence on the soil biota by negatively affecting AMF, causing changes in enzymatic activity, and triggering shifts in microbial catabolic profiles.

2. Material and methods

2.1. Experimental design and setup

Our experiment contained two crops (lettuce, leek) and five TP concentration levels (0 %, 0.1 %, 0.5 %, 1 % and 3 % TP weight/weight calculated based on dry soil) with eight replicates, resulting in 80 pots. The TP concentrations were chosen based on TP concentrations reported in the literature (Baensch-Baltruschat et al., 2020; Müller et al., 2022; Sieber et al., 2020; Wagner et al., 2018; Wik and Dave, 2009). We produced TP from a mixture of randomly chosen, old passenger car tyres (Table S1): Using an electric planer, fine tyre flakes were created, transferred into a mortar, crushed in liquid nitrogen and then pulverised in an ultra-centrifugal mill (ZM 200 Retsch, Haan, Germany) over a 350 μm sieve (Fig. S1). The particle size distribution was analysed based on 1009 particles, yielding a mean Feret diameter of 201 μm with a standard deviation of 180 μm. The particle size of the artificially produced TP thus falls within the range of 40 nm to 400 μm, which is the size spectrum reported for TP (Gustafsson et al., 2008; Kreider et al., 2010). Organic carbon content of the TP material was 66 %. High resolution surface images of the produced TP material assessed by Scanning Electron Microscopy are presented in Fig. S2. To assess the trace element content of the TP material, triplicates of 200 mg TP were digested in 10 ml of nitric acid at 200 °C using a MARS 6 Microwave Digestion System (CEM Corporation, Matthews, NC). The data is provided as Table S2.

The test soil for the experiment was a sandy loam collected from the top 20 cm of an organically managed grassland at Full-Reuenthal (Aargau, Switzerland). The soil was sieved to 2 mm and stored at 4 °C in the dark until further use. Basic soil characteristics and element concentrations are provided in Table S3. At the set-up of the experiment, the required soil dry weight (DW) equivalent was thoroughly mixed with the respective amount of freshly produced TP and brought to 50 % of the maximum water holding capacity (mWHC) by adding tap water. The soil was filled into 1 l plastic pots (Hortima Baumschulbedarf AG, Hausen, Switzerland) and slightly compacted to reach the same bulk density in all pots. Seeds of leek (*Allium porrum* var. Fantic) and lettuce (*Lactuca sativa* var. Till) were pre-grown in commercial potting soil. We selected 1-month-old seedlings of comparable sizes from the seedling trays and cleaned roots off the potting substrate. One lettuce seedling and three leek seedlings were planted into each pot. All pots received 30 ml of tap water and the soil surface was covered with a thin layer of quartz sand (KK 0,6/1.2, grain size 0.6–1.2 mm, Trafor AG, Basel, Switzerland) to reduce water loss and to create a physical barrier preventing soil-dwelling pests from entering the soil. All pots were placed in a greenhouse on plastic saucers and distributed randomly on two tables.

2.2. Maintenance of the experiment

Pots were randomised within and across the greenhouse tables twice a week. Water loss from the pots was assessed gravimetrically twice a week and pots were brought back to 50 % mWHC by adding tap water. We utilised an ammonium nitrate (NH_4NO_3) fertilizer solution, supplying 10 mg N per pot weekly for leek and 5 mg nitrogen (N) per pot for lettuce, respectively, during the initial four weeks. Later, we used a commercial synthetic fertilizer with additional micronutrients (7 % N, 5 % P_2O_5, 6 % K_2O, trace elements: 0.01 % B, 0.002 % Cu, 0.02 % Fe, 0.010 % Mn, 0.001 % Mo, 0.002 % Zn) providing 14 mg N per pot and week to leek over 6 weeks and 6 mg N per pot and week to lettuce over 2 weeks. We treated the plants twice against fungus gnat larvae (*Bradysia* *impatiens*) using Substral® Celaflor TrauermückenEx (Evergreen Garden Care GmbH, Mainz, Germany) with the active ingredient Azadirachtin A (registration number 024436-86) dissolved in water. The plants were grown at a minimum temperature of 20 °C with a 16-hour photoperiod. Natural light was complemented by artificial light if needed.

2.3. Harvest of the experiment

After seven weeks for lettuce and 12 weeks for leek, the plants reached a growth stage where the pot size became limiting and the experiment was ended. At harvest, we measured the plant height and stem diameter of leek and assessed lettuce head diameter and SPAD values using a chlorophyll meter (SPAD-502Plus, Konica Minolta). The aboveground plant parts were cut at the soil surface. The sand layer was carefully removed from the soil surface and discarded. After washing, we determined the fresh weight (FW) and the DW of the shoots, the latter after drying at 60 °C to constant weight. The roots were collected from the soil and washed over a 1 mm sieve. A subsample of roots was stored in 50 % ethanol to assess root colonisation rates with AMF, the rest was dried at 60 °C to constant weight to assess the root biomass. The root-free soil was thoroughly mixed by hand and sieved to 2 mm. Samples were stored at 4 °C for the MicroResp™ test assay, at −20 °C for extracellular enzyme activity assays, and air-dried to determine the soil pH in a soil: water ratio of 1:2.5.

2.4. Plant analyses

The dried shoot biomass was ground in a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany). The total carbon (C), sulphur (S) and N contents in the ground biomass samples were determined using a CNS elemental analyser (HEKAtech, Wegberg, Germany) at the Institute of Geography and Geoecology at the Karlsruhe Institute of Technology (KIT). The standard deviation based on the replicate analysis ($n = 9$) of Hay powder (CRM code BCR-129 HAY POWDER) was 0.2 % for C, 0.05 % for N, and 0.01 % for S, respectively. Approximately 100 mg of powdered sample was weighed into perfluoroalkoxy polymer (PFA) vials (Savillex, Eden Prairie, United States) and digested in HNO_3-H_2O_2-HF (4:1:0.1) by stepwise heating to 140 °C. Samples were evaporated and re-dissolved in 0.5 M HNO_3 for further analysis. Aliquots of the digested plant samples were diluted and analysed for the major cations calcium (Ca), potassium (K), and sodium (Na) as well as for total phosphorus (P) using inductively coupled plasma atomic emission spectroscopy (ICP-AES 5100 VDV, Agilent, Santa Clara, United States) at the KIT. Trace elements (copper (Cu), manganese (Mn) and zinc (Zn)) were analysed using a PlasmaQuant MS Elite ICP-MS (Analytik Jena, Jena, Germany) at the Institute of Applied Geosciences at the Technical University of Darmstadt. The recovery rates of Mn, Cu and Zn in the European Reference Material (ERM) CD281 rye grass standard (European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium), digested together with the biomass samples, ranged from 90 % to 99 %. The standard deviation based on the replicate analysis of ERM CD281 ($n = 6$) was <3.5 %.

2.5. Soil biota

2.5.1. Root colonisation rates with arbuscular mycorrhizal fungi

The percentage of root colonisation with AMF was assessed after staining roots in a vinegar-ink solution (Vierheilig et al., 1998). Briefly, roots were rinsed with water, cut into pieces of approximately 1.5 cm, bleached in 10 % potassium hydroxide at 80 °C for 25 min, acidified in 1 % hydrochloric acid at room temperature for 2 h, stained in a 5 % ink-vinegar solution (Black Parker Quink ink) at 80 °C during 20 min and stored in 50 % glycerine for at least four weeks before the presence of AMF structures (vesicles, hyphae or arbuscules) were evaluated by microscopy. For each sample, we aligned approximately 25 root fragments in parallel on microscope slides in a few drops of a 50 % glycerine solution and sealed the mounted samples with coverslips. We then determined the length of roots colonised by fungal structures using the magnified intersections method (McGonigle et al., 1990) at 25× magnification.

2.5.2. Fluorometric enzyme assay

The extracellular enzyme activity (EEA) was measured in triplicates and by following the principles of Marx et al. (2001) and Bell et al. (2013). In brief, EEA was assessed upon the addition of seven substrates involved in N-, C- and P-cycling (Table S4), which were either coupled to 4-methylumbelliferone (MUB) or 7-amino-4-methyl-coumarin (AMC), both being synthetic fluorescent dyes (Sigma-Aldrich/Merck, Darmstadt, Germany). For the EEA test assay, 100 ml sterile water was added to 1 g of sieved (<2 mm) and frozen soil, shaken on a universal shaker (100 rpm, 30 min), ultrasonicated for 30 s at 60 % amplitude (Sonopuls HD 2200.2, Bandelin, Berlin, Germany) and stirred. Then, 50 μl of the soil slurry was added to a 96-well microplate containing either 50 μl of 0.1 M MES buffer for MUB-linked substrates or 0.05 M TRIZMA buffer for the AMC-linked substrate. Finally, 100 μl substrate solution was added, resulting in a substrate concentration of 50–150 μM depending on the substrate. Immediately after substrate addition, samples were shaken for 120 s in a microplate reader (Infinite M Nano$^+$, Tecan Group Ltd., Männedorf, Switzerland) and the background fluorescence was measured (355 nm excitation, 460 nm emission). The actual enzymatic activity was assessed after incubating the plates in the dark for 3 h at room temperature. In addition, standard plates were prepared for each soil sample and the MUB or AMC standard in their respective buffers and the fluorescence measured as described above.

2.5.3. Community-level physiological profiling (MicroResp™)

The catabolic profiles of the microbial communities were assessed using the MicroResp™ test assay (Bongiorno et al., 2020; Campbell et al., 2003; Creamer et al., 2016). In brief, 96-well detection plates were prepared as described in the MicroResp™ protocol (Cameron, 2007). Soils were added to 96-deep-well plates and incubated for six days at 20 °C. We included a standard soil in each deep-well plate along with each soil sample to quantify the variation between plates. After the incubation, we measured the initial colourimetric values (Tecan plate reader Infinite M Nano+, monochromator absorbance/fluorescence; Tecan Group, Männedorf, Switzerland) of the detection plates at 570 nm (T0 reading). For the test, we used seven structurally different carbon substrates (Table S5). A volume of 25 μl of each substrate solution was added to the deep-well plates containing the test soil in eight technical replicates per substrate. The deep-well plates were left to stand at room temperature for 30 min to release CO_2 derived from calcite dissolution, closed with the detection plates from the MicroResp™ system and incubated at 20 °C for 5 h before the colourimetric values of the detection plates were assessed again at 570 nm (T1 reading). Due to our focus on comparing treatment effects rather than determining maximum respiratory potential through optimised glucose amendment, the reported microbial biomass carbon (MBC) data from MicroResp™ test should be interpreted as relative proxies.

2.6. Data handling, statistical analyses and data visualisation

In the EEA test assay, we used the MUB and MUC standard curves to transfer raw fluorescence data of samples and substrates into potential EEA expressed as nmol*(g soil DW*h)$^{-1}$ (Bell et al., 2013). For each sample, we removed obvious outliers and calculated averages from the technical triplicates. To observe more general trends in the potential soil C-, N-, and P-cycling, C acquisition was represented by the summed activities of β-1,4-glucosidase (BG), β-D-cellobiosidase (CB), β-xylosidase (XYL) and α-glucosidase (AG), N acquisition was represented by the sum of β-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP), and P acquisition was represented by alkaline phosphatase (PHOS) activity (Bell et al., 2013). Since the potential EEA was on different scales, we standardized the data using z-scores.

We normalised the T1 readings of the MicroResp™ test using the T0 absorbance before calculating the CO_2 concentration in the headspace (Brolsma et al., 2015), and then converting them into absolute respiration rates (μg CO_2–C g^{-1} dry potting substrate h^{-1}) as indicated in the MicroResp™ manual (Cameron, 2007). For each sample, we removed obvious outliers before calculating averages from the technical replicates. For leek, the standard soil indicated the first run to deviate strongly, so we removed this data, leaving us with 6 instead of 8 biological replicates per TP concentration. We then calculated (i) the soil basal respiration from the soil samples supplemented with deionised water, (ii) the microbial biomass carbon (MBC) according to Anderson and Domsch (1978) as MBC = 40.04 × Glucose-induced respiration+0.37, (iii) the microbial metabolic quotient qCO_2 (expressed as mg CO_2-C g^{-1} MBC^{-1}) (Anderson, 2003; Anderson and Domsch, 1993) as qCO_2 = basal respiration × 1000 × MBC^{-1}, (iv) the multiple substrate-induced respiration as the total CO_2 flux from all substrates, and (v) the Shannon functional diversity index (H′) as described earlier (Bongiorno et al., 2020).

All data were analysed using R (R Core Team, 2023) through RStudio version 2023.3.0.386 (Posit team, 2023). Graphs were produced using the ggplot2 package (Wickham, 2016). The effects of TP addition on the response variables were analysed separately for the two crops using Bootstrap-Coupled ESTimation statistics and graphs implemented in the dabestr package (Ho et al., 2019). Five thousand bootstrap samples were taken, and the confidence intervals were bias-corrected and accelerated (BCa bootstrap) to accurately handle skewed distributions. Any reported *p*-value represents the probability of observing the effect size or greater under the null hypothesis of zero difference between treatment and control. The raw data are presented alongside Cumming estimation plots, which depict mean differences plotted as bootstrap sampling distributions for each of the TP groups against the control. AMF root colonisation rates, plant nutrients and trace metal concentrations were measured in the control treatments and the 3 % TP group only.

Effects of TP addition on the joint microbial catabolic profiles from the MicroResp™ were assessed with a permutational analysis of variance (PERMANOVA) using the vegan::adonis2 function (Anderson, 2008) with 999 permutations and Hellinger distances (Euclidean distance on Hellinger transformed data). Missing values in the respiration matrix were replaced with the group mean to avoid whole rows being lost in the downstream data analysis. We confirmed that the multivariate dispersion in the five TP levels was comparable using the vegan::betadisp function, followed by pairwise comparisons of group mean dispersions to avoid confounding dispersion and location effects. In case of significant effects, we performed multilevel pairwise comparisons using the pairwise.adonis function (Martinez Arbizu, 2020) with FDR correction of *p*-values for multiple testing. For a graphical representation of our data, we performed a redundancy analysis (RDA) using the vegan::rda function and assessed the significance of the constrained ordination by applying Monte Carlo permutation tests. The data of this study is available under https://doi.org/10.5281/zenodo.14893022.

3. Results

3.1. Effects of TP on plants

After 7 weeks of growth, we harvested the lettuce plants and measured SPAD values as a quick, non-destructive estimate of chlorophyll content that can provide insights into photosynthetic activity and assessed the diameter of lettuce heads as an important market parameter. Exposure of plants to 3 % TP significantly reduced the SPAD values of lettuce (−17 %) relative to the control, while no differences were recorded at lower TP concentrations (Fig. S3 A, Table S6). Lettuce head diameter tended to decrease at 0.1 % and 0.5 % TP exposure compared to the control; however, a statistically significant reduction was only observed at 1 % TP exposure (−3.4 %), with no significant differences to the control at 3 % TP exposure (Fig. S3 B, Table S6). Leek plants were harvested after 12 weeks, and we measured the height of the plants and the stem diameter as general indicators of plant growth performance. The height of leek plants did not change upon TP exposure (Fig. S3 C, Table S6). The effects of TP on the stem diameter of leek were biphasic: relative to the control, stems showed a trend of thickening at 0.1 % TP exposure, which intensified to significant differences (+19 %) at 0.5 % TP exposure. However, this trend reversed at higher TP levels, with no significant differences at 1 % TP and even a tendency for thinner stems at 3 % TP exposure (Fig. S3 D, Table S6).

Lettuce root DW did not change when exposed to concentrations up to 1 % TP; however, a significant reduction (−10 %) relative to the control was observed when exposed to 3 % TP (Fig. 1, Table S7). Shoot DW of lettuce was not affected by 0.5 % TP exposure, but both lower and higher TP concentrations decreased shoot DW (Fig. 1, Table S7), with the largest decrease (−23 %) relative to the control found upon exposure to 3 % TP. Leek root DW increased significantly at 0.1 % TP exposure (+47 %) when compared to the control. At 0.5 % TP exposure, although there was still a trend towards higher root DW, the effects were smaller (+23 %) and did not reach statistical significance (Table S7). Exposure to 1 % TP tended to negatively impact root DW relative to the control, with a further significant decrease (−35 %) observed at 3 % TP (Fig. 1, Table S7). The shoot DW of leek significantly increased when plants were grown at 0.1 % TP (+9 %), 0.5 % TP (+13 %), and 1 % TP (+12 %). However, values decreased at 3 % TP exposure to levels comparable to the control (Fig. 1, Table S7).

We assessed the effect of TP addition on macro- and micronutrients in plant material, focusing on the comparison between 3 % TP and the control. The exposure to TP changed the macro- and micronutrient concentrations in the shoots (Table S8). From the ten elemental concentrations we assessed, N, Cu, and Zn showed statistically significant and consistent changes across the two crops: In both crops, the concentrations of N declined in the 3 % TP group relative to the control (lettuce: −4 %, leek: −6 %). The exposure to 3 % TP further significantly increased Cu concentrations (lettuce: +5 %, leek: +21 %), as well as Zn concentrations (lettuce: +58 %, leek: +27 %). The Mg concentrations did not change in either crop upon TP exposure. Furthermore, the S, Ca, and P concentrations did not change upon TP exposure in leek but increased relative to the control in lettuce. The Na concentrations decreased in the 3 % TP group relative to the control in lettuce but increased in leek. Tissue C and K concentrations did not change upon TP exposure in lettuce, while in leek the C concentrations increased and the K concentrations decreased.

3.2. Effects of TP addition on soil and soil biota

We measured the soil pH because it influences the availability of soil nutrients, focusing on the control and the 3 % TP group. There was one particularly influential observation in the data of lettuce, which we identified based on Cook's Distance as the outlier detection method. When replacing this observation with the group-specific mean, the impact of 3 % TP on soil pH changed from +0.093 [95 % CI 0.028, 0.189] to +0.063 [95 % CI: 0.02, 0.139] relative to the control. No effects of TP on pH were measured in soil cultivated with leek (+0.008 [95 % CI −0.039, 0.055]).

We stained intraradical AMF structures in the control treatments and the 3 % TP treatment group. The overall root colonisation rate was moderate (median 40 %) and we could not measure changes in root colonisation rates upon 3 % TP exposure, neither in lettuce (+4 % points [95 % CI −2.7, 12.5]) nor leek (+3 % points [95 % CI −3.7, 13.3]).

We conducted fluorometric enzyme assays to assess extracellular soil enzymatic activity because it is a sensitive indicator for changes in soil fertility. We show the effects of TP on the summed activities of enzymes involved in C-, N- and P-cycling, but give details on the activity of each enzyme in the supplementary material (Table S9 and Figs. S4 and S5). The activity patterns of C-, N-, and P-cycling enzymes upon TP exposure

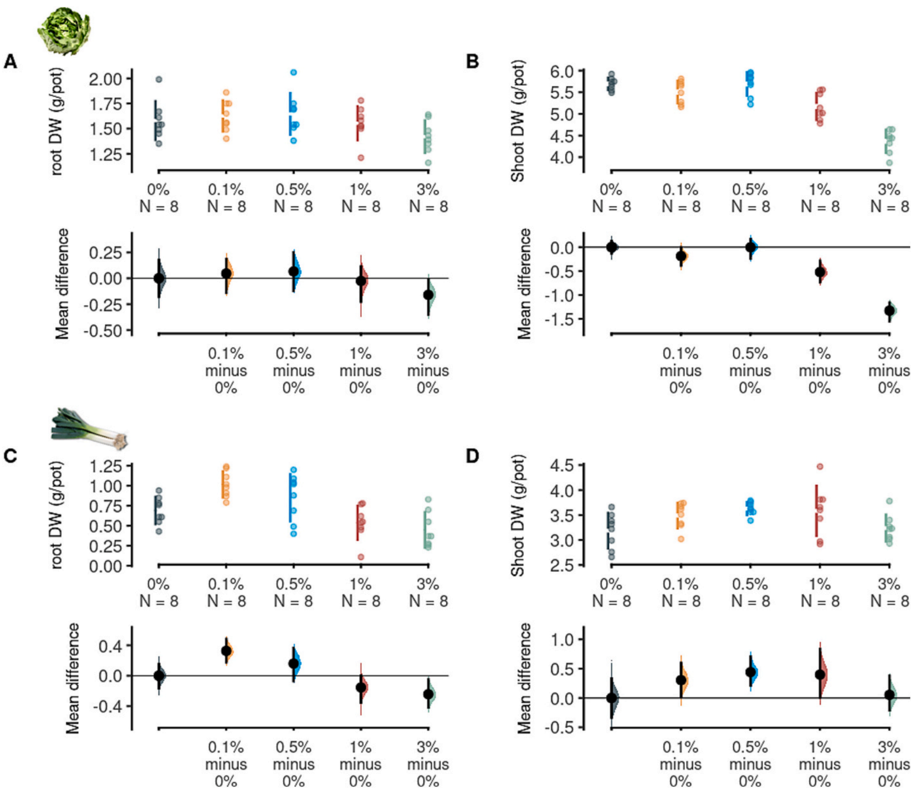

Fig. 1. Effects of tyre particles (TP) on dry weight (DW) of lettuce shoots (A), lettuce roots (B), leek shoots (C), and leek roots (D). For each response, the raw data is displayed on upper panels, lower panels are Cumming estimation plots depicting effect sizes as mean differences plotted as bootstrap sampling distributions for each of the 4 TP groups (0.1 %, 0.5 %, 1 %, 3 %) against the control (0 %). Vertical error bars are 95 % confidence intervals. Effect sizes excluding zero are associated with significant differences in the classical frequentist sense ($p \leq 0.05$).

reacted similarly under the cultivation of both crops but showed crop-specific differences in effect magnitude (Table S11). Under the cultivation of lettuce, C-cycling activities did not change upon exposure to 0.1 % or 0.5 % TP, however, C-cycling activity decreased in the 1 % TP (−11 %) and 3 % TP (−12 %) groups relative to the control (Fig. 2A). Under leek, C-cycling activity showed a slight increase at TP levels of 0.1 %, 0.5 %, and 1 % compared to the control (+12 %, +5.5 %, and +9.8 %), yet activity in the 3 % TP group was lower (−11 %) than in the control (Fig. 2D).

The activity of the N-cycling enzymes increased under the cultivation of both crops when exposed to TP. In lettuce, differences were measurable only upon exposure to 3 % TP with about 12.5 % higher values in the 3 % TP relative to the control group (Fig. 2B). The potential N-cycling activity under leek increased by 10 %, 13 %, 15 %, and 17 % relative to the control and upon exposure to 0.1 %, 0.5 %, 1 % and 3 % TP (Fig. 2E). In both crops, the activity of the P-cycling enzyme remained comparable to the control upon TP exposure of 0.1 % and 0.5 %. However, the potential P-cycling activities dropped relative to the control at 1 % (lettuce: −16 %, leek: −10 %) and 3 % TP exposure (lettuce: −10 %, leek: −12 %) (Fig. 2C and F).

The soil microbial catabolic profiles were analysed using the MicroResp™ test. From the respiration profiles, we derived a range of parameters to describe microbial communities as detailed in the method section. Soil basal respiration increased relative to the control under lettuce and upon exposure to 3 % TP (+37 %), whereas for leek, the only statistically significant change was a decrease upon 0.5 % exposure (−19 %) (Table S10). The microbial biomass carbon decreased under the cultivation of both crops when exposed to TP. Under lettuce, values decreased relative to the control when soils were exposed to concentrations of 1 % TP (−10 %) and 3 % TP (−14 %). Under cultivation of leek, microbial biomass carbon dropped in all TP groups, with significant reductions of −11 %, −18 %, −17 %, and −20 % in response to 0.1 %, 0.5 %, 1 % and 3 % TP relative to the control. The microbial metabolic quotient increased in soils from both crops: under lettuce values increased upon exposure to 1 % TP (+23 %) and 3 % TP (+62 %) relative to the control, while for leek it changed only upon 3 % TP exposure (+23 %) (Fig. 3C and G; Table S10). The MSIR under lettuce remained stable across the TP concentration range (Fig. 3D, Table S10). In contrast, it decreased under cultivation of leek and upon exposure to all TP concentrations with reductions of −16 %, −17 %, −15 %, and −7 % for 0.1 %, 0.5 %, 1 %, and 3 % TP exposure relative to the control (Fig. 3H, Table S10). The Shannon function diversity index did not change upon TP exposure under either crop (Table S10).

The response of the assimilation of each carbon substrate upon TP exposure is provided (Table S10, Figs. S6 and S7). When analysing the catabolic profiles jointly using the PERMANOVA test, we found stronger effects of TP addition on the microbial catabolic profiles under cultivation of lettuce ($R^2 = 0.40$, F = 5.87, Pr(>F) = 0.001) than leek ($R^2 = 0.228$, F = 1.844, Pr(>F) = 0.056). The Redundancy Analysis (RDA) confirmed a significant relationship between TP addition and the catabolic profiles with 39 % and 22 % of the variance being accounted for by TP addition under lettuce and leek (Fig. 4A and B). For lettuce, multi-level pairwise comparison indicated significant differences in the joint catabolic profiles between the 3 % TP group and all other TP concentrations (0 %, 0.1 %, 0.5 %, and 1 %), while for leek, catabolic profiles differed only between the control and 3 % TP exposure group (Table S12). The main substrates accounting for at least 10 % of the variability in the data profiles on axis 1 were identified as D-glucose, alpha-ketoglutaric acid, and oxalic acid and in the case of lettuce, also plain water (Fig. 4A and B).

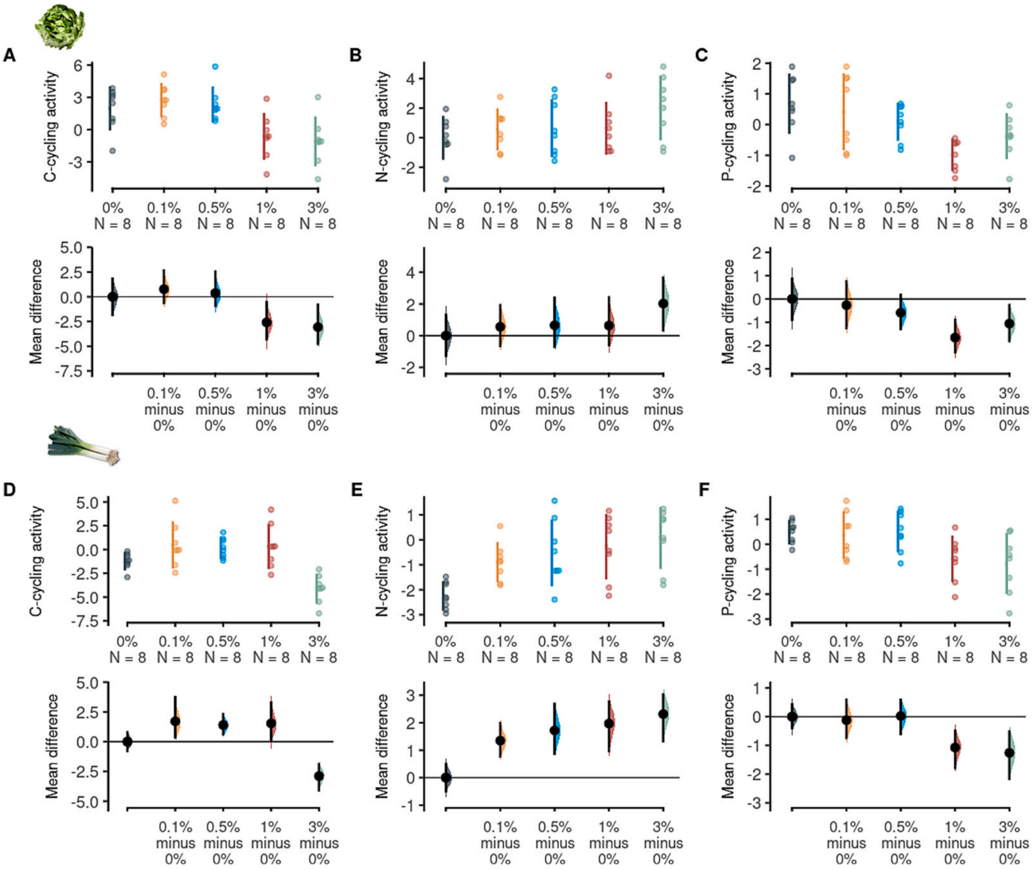

Fig. 2. Effect of tyre particle (TP) addition on the standardized (z-scaled) extracellular enzymatic C-cycling (A, D), N-cycling (B, E) and P-cycling (C, F) in soils under cultivation of lettuce (A–C) and leek (D–F). The raw data is displayed on upper panels, lower panels are Cumming estimation plots depicting effect sizes as mean differences plotted as bootstrap sampling distributions for each of the 4 TP groups (0.1 %, 0.5 %, 1 %, 3 %) against the control (0 %). Vertical error bars are 95 % confidence intervals. Effect sizes excluding zero are associated with significant differences in the classical frequentist sense ($p \leq 0.05$). C-cycling: scaled and summed activities of β-1,4-glucosidase, β-D-cellobiosidase, β-xylosidase and α-glucosidase, N-cycling: scaled and summed activities of β-acetylglucosaminidase and leucine aminopeptidase, P-cycling: scaled activity of alkaline phosphatase activity.

4. Discussion

4.1. TP affect plant biomass biphasically

Our data partly support hypothesis 1, showing that plants grown in the presence of TP can exhibit declines in shoot and root biomass (Table S7, Fig. 1). TP concentrations of 3 % reduced lettuce shoot biomass by almost a quarter and root biomass by around 10 %. Leek root biomass was drastically boosted at low TP exposure levels (up to +47 %), but stimulating effects diminished at 1 % TP. At 3 % TP exposure, shoot biomass was reduced by as much as −35 % relative to the control.

Earlier studies reported contrasting effects of TP exposure on plant biomass: Zeb et al. (2024a) found no biomass decline in mung bean (*Vigna radiata*) exposed to up to 1.5 % TP for 32 days, but Kim et al. (2022a) observed enhanced shoot and leaf growth in *V. radiata* after a 28-day exposure to 0.1 % and 1 % TP. In line with our results, Leifheit et al. (2022) found decreased shoot biomass in leek at 1 % TP after a six-week growth period; yet, there were no stimulating effects at lower TP exposure in contrast to our results. However, Lv et al. (2024) showed that low TP concentrations (0.6–3 mg L^{-1}) stimulated the growth of a marine microalgae, while high TP concentrations (15 and 75 mg L^{-1}) reduced it. The phenomenon of biphasic dose-response curves is referred to as hormesis. It describes a situation when low doses of an effector enhance the response variable, whereas higher doses diminish the effect or even reduce it below control levels (Lushchak, 2014). Mechanistically, TP are known to leach trace metals including Zn and Cu (Smolders and Degryse, 2002; Sommer et al., 2018), which play a role as crucial micronutrients for plants and microbes (Broadley et al., 2012) and may thus have stimulating effects on plants at low TP concentrations. Leifheit et al. (2022) showed that TP addition increased soil Zn levels. However, only TP concentrations exceeding 3 % (combined with the soil background Zn levels) increased soil Zn concentrations beyond typical soil levels, which, according to a recent analysis of the LUCAS topsoil database, are usually below 167 mg kg^{-1} (Van Eynde et al., 2023). Kim et al. (2022b) assessed the effects of TP originating from passenger cars and found a positive correlation between soil Zn (and sulphur) concentrations and root growth. Similarly, earlier studies reported higher Zn concentrations in plant tissue upon contact with tyre material (Handreck, 1996), with Zn tissue concentrations generally reflecting concentrations in the soil solution (Poschenrieder et al., 1995). Likewise, the plants in our study had enhanced Zn (up to +58 %) and Cu concentrations (up to +21 %) in shoot tissue upon exposure to 3 % TP (Table S8), suggesting uptake of these elements from TP. With foliar concentrations below 30 mg Zn kg^{-1} and 8.5 mg Cu kg^{-1}, the measured values did not reach phytotoxic levels, which typically commence at 100 mg Zn kg^{-1} for sensitive crops (Marschner, 2011) and 20–30 mg Cu kg^{-1} (Hodenberg and Finck, 1975; Marschner, 2011; Reuter et al., 1981), suggesting that the plants in our experiment did not cross the thresholds for these trace elements. The sandy loam used in our study has a relatively low clay content, providing few binding sites for Cu and Zn. This could increase their concentration in the soil solution compared to soils with a higher proportion of clay. In contrast, the neutral soil pH could have contributed to Zn and Cu adsorption and the formation of less soluble compounds, thus limiting further plant uptake and

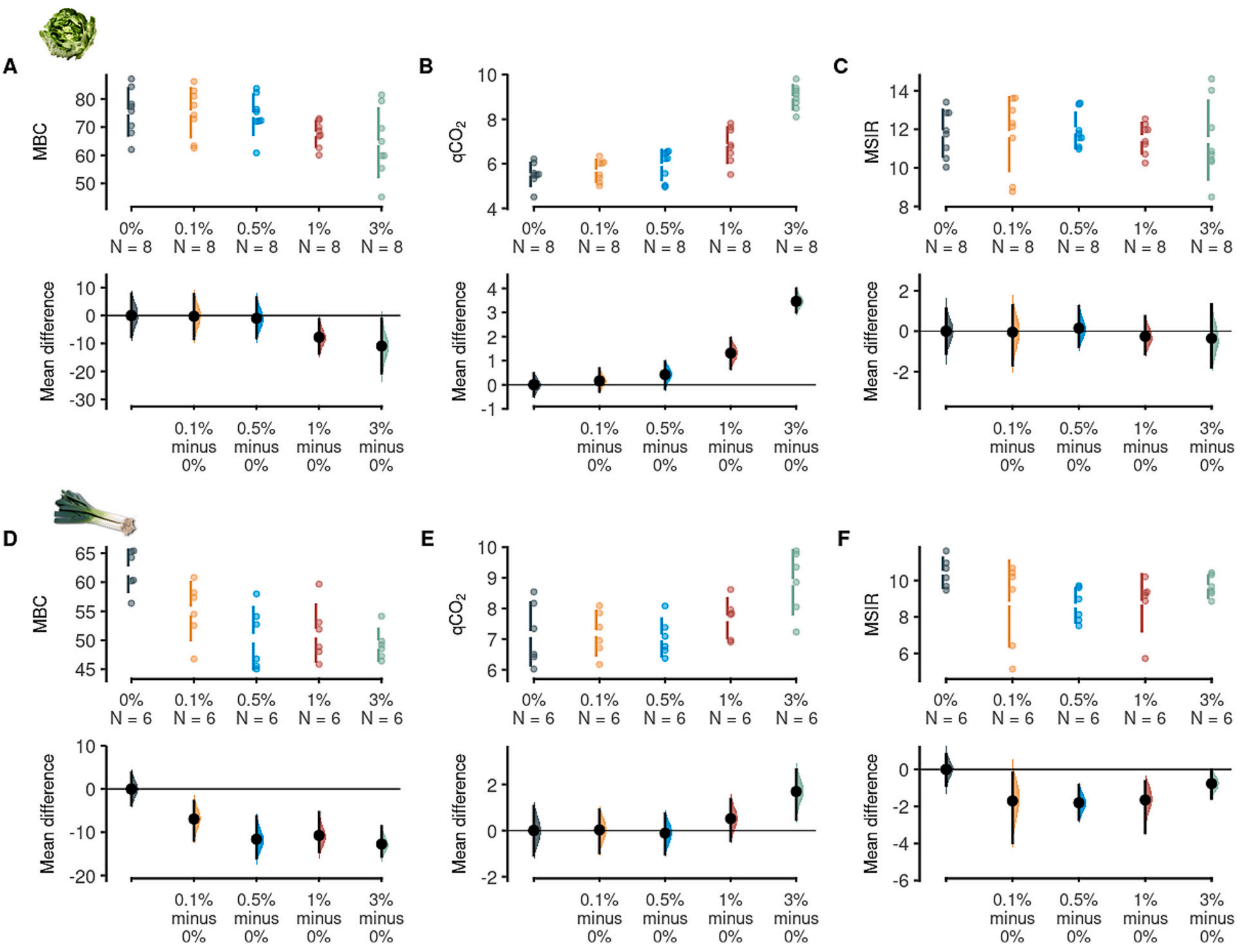

Fig. 3. Effect of tyre particle (TP) concentration in soils under cultivation of lettuce (A–C) and leek (D–F) on microbial biomass carbon (MBC; 3A and 3D), microbial metabolic quotient (qCO2) expressed as mg CO2-C g-1 MBC-1 (3B and 3E), and the multiple substrate-induced respiration (MSIR; 3C and 3F). For each response, the raw data is displayed on upper panels, lower panels are Cumming estimation plots depicting effect sizes as mean differences plotted as bootstrap sampling distributions for each of the 4 TP groups (0.1 %, 0.5 %, 1 %, 3 %) against the control (0 %). Vertical error bars are 95 % confidence intervals. Effect sizes excluding zero are associated with significant differences in the classical frequentist sense ($p \leq 0.05$).

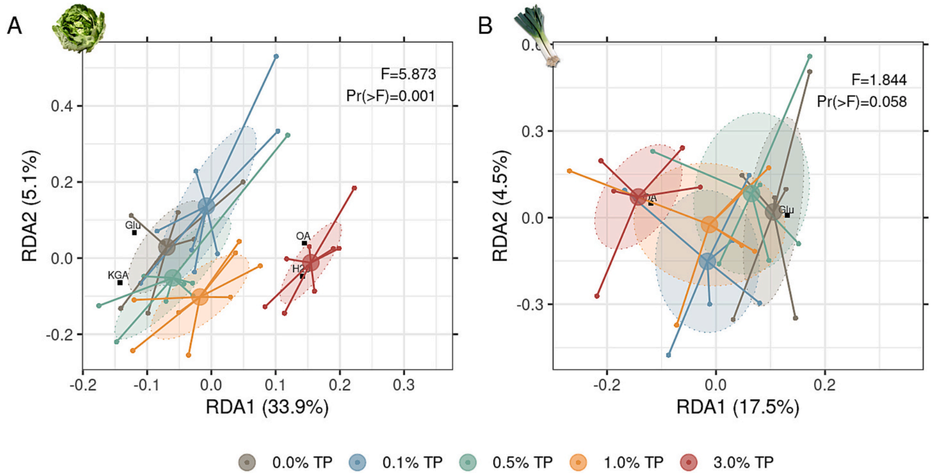

Fig. 4. Redundancy analysis (RDA) of catabolic expression profiles (MicroResp-Test) in soil with different concentrations of tyre particle (TP) under cultivation of lettuce (A) and leek (B) and upon the addition of 8 substrates (water (H_{2O}); D-glucose (GLU); L-alanine (ALA); gamma-aminobutyric acid (ABA); *n*-acetyl-glucosamine (NAG); oxalic acid (OA); alpha-ketoglutaric acid (KGA); xylan (XYL)). Ordination plots are based on Hellinger distances and constrained using TP concentration as the explanatory variable. Small black dots: location of the individual community profiles (samples); larger dots: group centroids; lines connect centroids to their respective data points, illustrating dispersion. Black squares: contributions of substrates accounting for >10 % of the variability in respiration profiles along axis 1; ellipses indicate uncertainty (95 % confidence intervals).

preventing phytotoxic effects. An inverse stable isotope spiking approach (Yan et al., 2021) could further elucidate the fate and distribution of Zn in the soil by analysing its isotopes and conducting source tracing calculations (Künzli et al., 2024).

Importantly, other trace metals, such as chromium, cadmium or lead, could have leached from TP. These trace metals have no nutritional value and impair, e.g. the root and shoot development at low concentrations (DalCorso, 2012). Once they accumulate, they can further disturb various physiological functions, including photosynthesis, and cause shifts in plant nutritional composition (DalCorso, 2012), yet we did not measure these and thus, can only speculate about their impact. Interestingly, however, we measured reduced SPAD values (−17 %) in lettuce (Fig. S3), a proxy for chlorophyll content, next to significant changes in plant nutritional composition (Table S8). Notably, we observed but did not quantitatively assess alterations in root structure following TP exposure, particularly in leek, where the prevalence of finer roots at harvest could have affected plant nutrient acquisition. In line with this, Liu et al. (2023a, 2023b) showed that elevated MP concentrations had adverse effects on the growth of peanut plants and plant N uptake. More specifically, MP damaged the plasma membranes of root cells and resulted in oxidative plant stress. Similar findings have been reported by others, showing that MPs can physically block root surfaces when particles attach, ultimately reducing plant performance (Gao et al., 2021; Huo et al., 2022).

Apart from trace metals, TP contain various organic compounds: Zeb et al. (2024a) identified sixteen different PAHs from TP and Castan et al. (2022) showed that different TP-derived compounds and a wide range of subsequent transformation products with largely unidentified toxicities are taken up by plants and can accumulate in plant tissue. While we used worn-out tyres for the current experiment, Castan et al. (2022) further demonstrated that TP derived from used tyres continue to leach a substantial number of compounds. Although we did not analyse the chemical composition of the pore water in our experiment and therefore cannot identify the specific chemicals responsible for promoting or inhibiting plant growth, we assume that TP-derived compounds—such as organic contaminants, potentially toxic trace elements, or so far unidentified substances—ultimately impaired plant performance at higher exposure levels while at lower exposure levels, trace metals with nutritional values may have had a fertilisation effect.

Negative effects on plants may also come from TP-induced alterations in microbial community composition, which can affect the functional attributes of microbial communities, thereby ultimately influencing plant growth and health. This was shown by O'Brien et al. (2022) who found TP leachate to disrupt plant-microbe interactions and change plant properties. Effects of TP on plants can also be mediated by changes in physical soil properties: the addition of MP can induce changes in soil structure, increase soil bulk density, and decrease water-stable aggregates, and these changes ultimately also influence living organisms, including plants (De Souza Machado et al., 2019). However, Kim et al. (2022b) found that the origin of TP — whether from cars, bicycles, or e-scooters — was important for the effects on soil physical and chemical properties: TP from bicycles and e-scooters influenced some parameters but car-derived TP at concentrations of 0.1 % and 1 % caused no changes in bulk density, electrical conductivity, pH, soil organic matter, or water-holding capacity. Since our analyses focused on the living soil, we lack the data to assess the role of TP in explaining the observed patterns through changes in soil physical properties.

4.2. TP has effects on the microbial basis of the soil food web

Our findings partly support hypothesis 2, indicating that TP exposure impacts soil microorganisms: in both plants, TP did not significantly affect AMF root colonisation. However, it increased extracellular soil N-cycling, reduced C- and P-cycling sensitively, influenced microbial community metrics and shaped distinct microbial catabolic profiles.

AMF can be negatively affected by soil pollutants, thereby influencing soil ecosystem functioning at many levels (Powell and Rillig, 2018). As postulated by Leifheit et al. (2021), AMF may be susceptible to MP: while the fungus can dwell under certain levels of trace metals or hydrocarbons and may even help plants deal with such soil contaminants, higher concentrations of soil pollutants can negatively affect the establishment of the symbiosis (Cabello, 1997; Joner and Leyval, 2003; Wang et al., 2020). This can be observed and measured as reduced root colonisation, reduced spore formation, a decline in intraradical fungal structures and a smaller soil hyphal network. We did not find measurable effects on root colonisation rates with native AMF when plants were grown in the presence of 3 % TP. This may indicate AMF tolerance to TP pollution, however, the staining method may have failed to detect small changes in root colonisation over the short TP exposure and growth period. We suggest using a variety of additional methods to detect AMF such as qPCR (Bodenhausen et al., 2021; Thonar et al., 2012) or amplicon sequencing (Delavaux et al., 2021; Schlaeppi et al., 2016; Tedersoo et al., 2018) to explore the potential impacts of TP on this important symbiotic interaction in more depth. Studying AMF in the context of soil pollution with TP is also crucial to understand their role in transporting chemicals from TP or MP into the bulk soil, similar to how they expand the rhizosphere by transporting substances through their hyphal network (Rillig et al., 2024).

Soil enzymes mediate key steps in the carbon, nitrogen, and phosphorus cycles, enabling nutrient uptake by soil organisms. However, enzyme synthesis requires resources that could otherwise support microbial growth. According to the optimality hypothesis, microbes balance this trade-off by adjusting enzyme production: investing less when substrates are abundant and more when they are limited to maintain growth (Calabrese et al., 2022). Our observation of rising N-cycling activities could thus suggest an increased microbial demand for N to maintain microbial growth rates. Despite key differences in experimental design, Zhu et al. (2024) reported that exposure to 0.9 % TP significantly increased β-1,4-*N*-acetylglucosaminidase activity five weeks after addition in an incubation experiment at 100 % soil mWHC. In contrast, Zeb et al. (2024a) reported a reduction in soil urease activity in a controlled pot experiment with mung bean plants, even at the lowest TP exposure level of 0.1 % after 32 days of treatment. Similarly, urease activity in soils planted with *Ipomoea aquatica* also declined relative to the control following exposure to 0.1 % and 1 % TP (Zeb et al., 2024b). Moreover, our findings contrast with previous studies on phosphorus cycling. In our study, phosphorus cycling declined in both crops at TP exposures of 1 % and 3 % (Fig. 2c, f). In contrast, Zeb et al. (2024a) reported increased soil alkaline phosphatase activity at 1.5 % TP, and Zeb et al. (2024b) observed enhanced phytase activity at 0.1 % and 1 % TP. When investigating carbon cycling, we observed decreased cellulase activity at high TP exposure in both crops (Fig. 2a, d), consistent with the decline in dehydrogenase activity reported by Zeb et al. (2024a). However, in contrast, Zeb et al. (2024b) found that dehydrogenase activity in soils planted with *Ipomoea aquatica* remained comparable to the control following exposure to TP. Recent studies have shown that TP can leach dissolved organic carbon into the soil, increasing the labile carbon content by releasing lipids, proteins, amino sugars, and carbohydrate-like compounds (Wei et al., 2025). Such changes may eventually trigger a “priming” effect, stimulating microbial metabolic activity (Rillig et al., 2021). However, in our study it remains uncertain whether TP degradation significantly influenced substrate levels in the soil solution within such a short time frame: TPs are notoriously hard to degrade and there is no evidence of positive priming in the data collected on microbes; instead, the data suggest a general decline in microbial biomass and activity, accompanied by increased microbial stress (qCO_2), even at low TP concentrations. In general, it is known that EEA reacts sensitively to soil pollutants including trace metals and PAHs (Gianfreda et al., 2005; Smreczak et al., 1999), and as shown in a global meta-analysis, pollutants have enzyme-specific effects (Aponte et al., 2020). Soil pollutants can directly act on the enzyme, e.g. by covalent bindings to active sites of an enzyme (Gianfreda et al., 2005).

Additionally, MP or TP-induced alterations in physical soil properties—such as water-holding capacity, soil density (de Souza Machado et al., 2018), and soil structure (Wan et al., 2019) —can influence enzymatic activity, e.g. by disrupting water film-connected soil pores and consequently shifting enzyme-substrate interactions. Additionally, adsorption and desorption processes to TP have been described (Hüffer et al., 2019) and will likely influence substrate availability. Shifts in soil enzymatic activity may also be associated with changes in soil microbial community composition (Gianfreda et al., 2005; Kandeler et al., 1996; Xu et al., 2024) as microbial species possess distinct genetic and metabolic capabilities, which determine the types and quantities of enzymes produced. The ultimate effects of MP and TP on extracellular enzymatic activity remain highly context-dependent: A recent meta-analysis incorporating 1812 observations across 9 MP types, highlights that factors such as soil pH, identity of the plant presence, exposure duration, MP type and size collectively shape the response of soil enzymes to MP exposure (Liu et al., 2023a, 2023b). Furthermore, as with other types of MP, numerous factors add complexity to understanding the impact of TP on soil enzymatic activity. These include, e.g. soil storage conditions before experiments (Kim et al., 2022b) and whether TP is introduced abruptly or gradually (Zhu et al., 2024).

In both crops, the microbial biomass carbon as assessed from the MicroResp™ test declined upon TP exposure, with measurable effects (−11 %) in leek appearing already at TP concentrations as low as 0.1 % (Table S10). Alongside, the microbial metabolic quotient, which measured the efficiency of microbial respiration relative to the size of its biomass, increased by up to 62 % when exposed to 3 % TP (Fig. 3). This effect can be attributed to a greater allocation of carbon towards cell defence and repair mechanisms, at the expense of cell growth, indicating a microbial stress response. Finally, the decrease in total CO_2 flux from all substrates (MSIR rate; Fig. 3) signifies a decrease in the overall microbial activity. These effects likely stem from the multiple pathways through which TP influences soil systems, such as TP-derived leachates and indirect effects mediated by physical and chemical shifts in soil properties.

Microbial community functioning in the soils changed upon TP exposure as reflected in the distinct microbial catabolic profiles that separated the different TP concentrations (Fig. 4). Under lettuce cultivation, shifts in functional community composition were observed between the 3 % TP group and all other TP exposure groups and for leek between the 3 % TP group and the control (Table S12). Notable shifts in microbial communities upon TP-exposures were noted in earlier studies (Ding et al., 2022; Wei et al., 2025; Xu et al., 2024; Zeb et al., 2024a). As a potential driver of these shifts, Zn has been mentioned as the main toxicity-causing agent (Liu et al., 2022). Zn levels in our study are unlikely to have crossed toxic thresholds for plants, however, soil microorganisms are significantly more susceptible to trace metal exposure (Giller et al., 1998, 2009). Although trace metals can induce shifts in microbial community assemblies, there are no distinct thresholds for microbial metal tolerance (Giller et al., 2009, 1998) or depend on site characteristics (Bünemann et al., 2006). A recent study further highlights the role of leachable additives in explaining TP toxicity: Ding et al. (2022) identified five trace metals in TP and TP leachate, along with six organic compounds (acetophenone, 4-methylaniline, benzothiazole, octadecylacetate, N-cyclohexylfomamide, and dicyclohexylamine), that are toxic to microbes, thereby providing the first causal link between chemical additives and tyre toxicity in soil or sediment.

Shifts in microbial community composition may also be influenced by the particle itself, which functions as a novel ecological niche. Similar to the “plastisphere” (Rillig et al., 2024; Wang et al., 2022; Zettler et al., 2013), the terms “TWPsphere” (Ding et al., 2023) and “Tyre plastisphere” (Zhang et al., 2025) have been coined: the tyre plastisphere describes the distinct physical and chemical properties of the particle, with conditions that contrast with the surrounding habitat. These conditions select a unique microbial community, likely including microbes that can degrade TP. Shifts in soil microbial communities have previously been reported in the context of TP pollution. For example, Zeb et al. (2024b) observed reduced Shannon diversity and increased Simpson evenness in the rhizosphere following the addition of 1 % TP, suggesting that TP may suppress bacterial diversity by promoting dominant taxa while inhibiting others. They also reported compositional changes in microbial community structure. Similarly, Zeb et al. (2024a) found that TP exposure significantly altered soil microbiota, affecting the relative abundance of several bacterial and fungal genera in both bulk and rhizospheric soils.

4.3. Limitations and significance of results

Our TP were artificially produced from worn-out tyres. In the environment, pure rubber particles like the ones utilised in our current study are rarely encountered. Normally, particles formed during vehicular travel become coated with inorganic materials derived from road surfaces and brake wear, which constitute 10–50 % of the particle volume (Sommer et al., 2018). Thus, artificially produced and real-world tyre particles have widely different shapes and chemical properties (Baensch-Baltruschat et al., 2020). Due to the distinct physical and chemical properties of particles produced by different methods, their impact on the plant-soil system can vary significantly, thus, experimental results obtained from artificially produced tyres are not directly applicable to environmental scenarios. Furthermore, in real-world scenarios, TP accumulate gradually in soil, eventually reaching the concentrations reported in the literature. This implies that a part of the TP has been present in the soil for years, potentially having already lost most of its leachable compounds and undergone surface ageing. The importance of considering the delivery rate—specifically, whether particles are introduced gradually or all at once—was also emphasised by Zhu et al. (2024). Our experimental design, constrained by time limitations, differed from this natural process by introducing the entire TP amount at once. To partially mitigate this discrepancy and simulate long-term leaching effects, we utilised particles from old, worn tyres. However, it is important to acknowledge that despite this measure, the experimental conditions still deviate from those found in nature. The one-time addition of all particles likely amplified the soil system's response in terms of microbial adaptation, transport, and the availability of TP-derived leachates. Ideally, this study should be repeated using soils that have been stored for a longer period after TP contamination to reflect more realistic scenarios and better mirror longer-term effects.

Our data also showed that for some response variables, the effects of TP addition differed for soils under lettuce and leek cultivation, indicating that the type of crop grown may influence how TP affects the plant-soil system. This is supported by earlier studies showing species-specific effects of MP exposure on germination, oxidative stress, and root growth in lettuce, maize, radish, and wheat (Gong et al., 2021; Huo et al., 2022). However, a direct comparison of the susceptibility of lettuce and leek in our study is limited by the different experimental durations of 7 and 12 weeks: Zeb et al. (2024b) observed that polycyclic aromatic hydrocarbons (PAHs) in soil and TP decreased over time, suggesting that the toxicity of TP change with their incubation time. Moreover, when marine microalgae were exposed to TP, the particulate- and leachate-toxicity decreased with increasing age of the TP (Lv et al., 2024). In this light, it is not possible to discern species-specific susceptibility from the different exposure times and age-dependent effects in the current experimental set-up.

4.4. Summary and conclusions

We assessed the effects of artificially produced TP on the plant-soil system under controlled conditions using lettuce and leek as model plants. Our data indicate clear negative effects on plants when exposed to high TP levels, regardless of the plant species. The impacts of lower TP levels appeared more variable, likely influenced by differences in exposure time (7 vs 12 weeks) and species identity. While we assume the negative effects primarily to be linked to toxic compounds leaching from TP, alterations in soil physical properties or effects mediated via microorganisms may have been equally important. The observed effects of TP on extracellular enzymatic activities and functional attributes of soil microbial communities may suggest that TP could trigger a cascade of effects in the soil food web. Given the pivotal role of soil microbes in nutrient cycling and energy flow, these alterations could potentially impact higher trophic levels, influencing overall ecosystem functioning. Our study clearly emphasises the pressing importance of implementing measures to mitigate TP pollution of soils to preserve soil quality, plant development and guarantee safe production of food crops.

CRediT authorship contribution statement

D. Kundel: Project administration, Writing – original draft, Investigation, Formal analysis, Writing – review & editing, Funding acquisition, Conceptualization. **M. Bigalke:** Project administration, Conceptualization, Writing – review & editing, Funding acquisition. **B. Stehle:** Writing – review & editing, Investigation, Resources. **M. Hammer:** Writing – review & editing, Investigation. **K.N. Nitzsche:** Investigation, Writing – review & editing. **A. Fliessbach:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve the readability and language in parts of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Funding

This project is funded by the Federal Office for Agriculture (FOAG) and the MINAGRIS Project which is funded by the European Union's Horizon 2020 Programme for Research & Innovation under Grant Agreement number 101000407.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106340.

Data availability

The data of this study is available under https://doi.org/10.5281/zenodo.14893022.

References

Anderson, T.H., 2003. Microbial eco-physiological indicators to asses soil quality. Agric. Ecosyst. Environ. 98, 285–293. https://doi.org/10.1016/S0167-8809(03)00088-4.

Anderson, M.J., 2008. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215–221. https://doi.org/10.1016/0038-0717(78)90099-8.

Anderson, T.H., Domsch, K.H., 1993. The metabolic quotient for CO2 (qCO2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol. Biochem. 25, 393–395. https://doi.org/10.1016/0038-0717(93)90140-7.

Aponte, H., Meli, P., Butler, B., Paolini, J., Matus, F., Merino, C., Cornejo, P., Kuzyakov, Y., 2020. Meta-analysis of heavy metal effects on soil enzyme activities. Sci. Total Environ. 737, 139744. https://doi.org/10.1016/J.SCITOTENV.2020.139744.

Baensch-Baltruschat, B., Kocher, B., Stock, F., Reifferscheid, G., 2020. Tyre and road wear particles (TRWP) - a review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. Sci. Total Environ. 733, 137823. https://doi.org/10.1016/J.SCITOTENV.2020.137823.

Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. J. Vis. Exp., e50961 https://doi.org/10.3791/50961.

Bodenhausen, N., Deslandes-Hérold, G., Waelchli, J., Held, A., van der Heijden, M.G.A., Schlaeppi, K., 2021. Relative qPCR to quantify colonization of plant roots by arbuscular mycorrhizal fungi. Mycorrhiza 31, 137–148. https://doi.org/10.1007/S00572-020-01014-1.

Bongiorno, G., Bünemann, E.K., Brussaard, L., Mäder, P., Oguejiofor, C.U., de Goede, R.G.M., 2020. Soil management intensity shifts microbial catabolic profiles across a range of European long-term field experiments. Appl. Soil Ecol. 154, 103596. https://doi.org/10.1016/j.apsoil.2020.103596.

Bowman, D.C., Evans, R.Y., Dodge, L.L., 1994. Growth of chrysanthemum with ground automobile tires used as a container soil amendment. HortScience 29, 774–776. https://doi.org/10.21273/HORTSCI.29.7.774.

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F., 2012. Function of nutrients: micronutrients. In: Marschner's Mineral Nutrition of Higher Plants: Third Edition, pp. 191–248. https://doi.org/10.1016/B978-0-12-384905-2.00007-8.

Brolsma, K.M., Vonk, J.A., Hoffland, E., Mulder, C., de Goede, R.G.M., 2015. Effects of GM potato Modena on soil microbial activity and litter decomposition fall within the range of effects found for two conventional cultivars. Biol. Fertil. Soils 51, 913–922. https://doi.org/10.1007/s00374-015-1031-2.

Bünemann, E.K., Schwenke, G.D., Van Zwieten, L., 2006. Impact of agricultural inputs on soil organisms—a review. Soil Research 44, 379–406. https://doi.org/10.1071/SR05125.

Cabello, M.N., 1997. Hydrocarbon pollution: its effect on native arbuscular mycorrhizal fungi (AMF). FEMS Microbiol. Ecol. 22, 233–236. https://doi.org/10.1111/J.1574-6941.1997.TB00375.X.

Calabrese, S., Mohanty, B.P., Malik, A.A., 2022. Soil microorganisms regulate extracellular enzyme production to maximize their growth rate. Biogeochemistry 158, 303–312. https://doi.org/10.1007/S10533-022-00899-8. /FIGURES/5.

Cameron, C., 2007. MicroResp™ Technical Manual—A Versatile Soil Respiration System. Macaulay Institute, Craigiebuckler, Aberdeen, Scotland, UK.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl. Environ. Microbiol. 69, 3593–3599. https://doi.org/10.1128/AEM.69.6.3593-3599.2003.

Castan, S., Sherman, A., Peng, R., Zumstein, M.T., Wanek, W., Hüffer, T., Hofmann, T., 2022. Uptake, metabolism, and accumulation of tire wear particle-derived compounds in lettuce. Environ. Sci. Technol. 57, 168–178. https://doi.org/10.1021/acs.est.2c05660.

Creamer, R.E., Bellamy, P., Black, H.I.J., Cameron, C.M., Campbell, C.D., Chamberlain, P., Harris, J., Parekh, N., Pawlett, M., Poskitt, J., Stone, D., Ritz, K., 2009. An inter-laboratory comparison of multi-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring. Biol. Fertil. Soils 6, 623–633. https://doi.org/10.1007/s00374-009-0374-y.

Creamer, R.E., Stone, D., Berry, P., Kuiper, I., 2016. Measuring respiration profiles of soil microbial communities across Europe using MicroResp™ method. Applied Soil Ecology 97, 36–43. https://doi.org/10.1016/j.apsoil.2015.08.004.

DalCorso, G., 2012. Heavy Metal Toxicity in Plants, pp. 1–25. https://doi.org/10.1007/978-94-007-4441-7_1.

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. /ASSET/IMAGES/LARGE/ES-2018-02212S_0007.JPEG.

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.

Delavaux, C.S., Sturmer, S.L., Wagner, M.R., Schütte, U., Morton, J.B., Bever, J.D., 2021. Utility of large subunit for environmental sequencing of arbuscular mycorrhizal fungi. New Phytol. 229, 3048–3052. https://doi.org/10.1111/nph.17080.

Ding, J., Meng, F., Chen, H., Chen, Q., Hu, A., Yu, C.P., Chen, L., Lv, M., 2022. Leachable additives of Tire particles explain the shift in microbial community composition and function in coastal sediments. Environ. Sci. Technol. 56, 12257–12266. https://doi.org/10.1021/ACS.EST.2C02757/SUPPL_FILE/ES2C02757_SI_001.PDF.

Ding, J., Lv, M., Zhu, D., Leifheit, E.F., Chen, Q.L., Wang, Y.Q., Chen, L.X., Rillig, M.C., Zhu, Y.G., 2023. Tire wear particles: an emerging threat to soil health. Crit. Rev. Environ. Sci. Technol. 53, 239–257. https://doi.org/10.1080/10643389.2022.2047581.

Fan, X., Qian, S., Bao, Y., Sha, H., Liu, Y., Cao, B., 2023. Desorption behavior of antibiotics by microplastics (tire wear particles) in simulated gastrointestinal fluids. Environ. Pollut. 323. https://doi.org/10.1016/j.envpol.2023.121252.

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, 123422. https://doi.org/10.1016/j.jhazmat.2020.123422.

Gianfreda, L., Rao, M.A., Piotrowska, A., Palumbo, G., Colombo, C., 2005. Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Sci. Total Environ. 341, 265–279. https://doi.org/10.1016/j.scitotenv.2004.10.005.

Giller, K.E., Witter, E., Mcgrath, S.P., 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem. 30, 1389–1414. https://doi.org/10.1016/S0038-0717(97)00270-8.

Giller, K.E., Witter, E., McGrath, S.P., 2009. Heavy metals and soil microbes. Soil Biol. Biochem. 41, 2031–2037. https://doi.org/10.1016/j.soilbio.2009.04.026.

Glaubitz, F., Rocha Vogel, A., Kolberg, Y., von Tümpling, W., Kahlert, H., 2023. Detailed insights in adsorption process of heavy metals on tire wear particles. Environ. Pollut. 335. https://doi.org/10.1016/j.envpol.2023.122293.

Gong, W., Zhang, W., Jiang, M., Li, S., Liang, G., Bu, Q., Xu, L., Zhu, H., Lu, A., 2021. Species-dependent response of food crops to polystyrene nanoplastics and microplastics. Sci. Total Environ. 796, 148750. https://doi.org/10.1016/j.scitotenv.2021.148750.

Gustafsson, M., Blomqvist, G., Gudmundsson, A., Dahl, A., Swietlicki, E., Bohgard, M., Lindbom, J., Ljungman, A., 2008. Properties and toxicological effects of particles from the interaction between tyres, road pavement and winter traction material. Sci. Total Environ. 393, 226–240. https://doi.org/10.1016/J.SCITOTENV.2007.12.030.

Handreck, K.A., 1996. Zinc toxicity from tire rubber in soilless potting media. Commun. Soil Sci. Plant Anal. 27, 2615–2623. https://doi.org/10.1080/00103629609369726.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A. E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N. P., Lusher, A.L., Wagner, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ. Sci. Technol. 53, 1039–1047. https://doi.org/10.1021/acs.est.8b05297.

Ho, J., Tumkaya, T., Aryal, S., Choi, H., Claridge-Chang, A., 2019. Moving beyond P values: data analysis with estimation graphics. Nat. Methods 16, 565–566. https://doi.org/10.1038/s41592-019-0470-3.

Hodenberg, A.V., Finck, A., 1975. Ermittlung von Toxizitäts-Grenzwerten für Zink, Kupfer und Blei in Hafer und Rotklee. Z. Pflanzenernähr. Bodenkd. 138, 489–503.

Hüffer, T., Wagner, S., Reemtsma, T., Hofmann, T., 2019. Sorption of organic substances to tire wear materials: similarities and differences with other types of microplastic. TrAC Trends Anal. Chem. 113, 392–401. https://doi.org/10.1016/J.TRAC.2018.11.029.

Huo, Y., Dijkstra, F.A., Possell, M., Singh, B., 2022. Ecotoxicological effects of plastics on plants, soil fauna and microorganisms: a meta-analysis. Environ. Pollut. 310, 119892. https://doi.org/10.1016/J.ENVPOL.2022.119892.

Joner, E.J., Leyval, C., 2003. Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. Environ. Sci. Technol. 37, 2371–2375. https://doi.org/10.1021/ES020196Y.

Kandeler, E., Kampichler, C., Horak, O., 1996. Influence of heavy metals on the functional diversity of soil microbial communities. Biol. Fertil. Soils 23, 299–306. https://doi.org/10.1007/BF00335958.

Kim, L., Lee, T.Y., Kim, H., An, Y.J., 2022a. Toxicity assessment of tire particles released from personal mobilities (bicycles, cars, and electric scooters) on soil organisms. J. Hazard. Mater. 437, 129362. https://doi.org/10.1016/J.JHAZMAT.2022.129362.

Kim, S.W., Xu, Y., Meidl, P., Bi, M., Zhu, Y., Rillig, M.C., 2022b. Soil storage conditions alter the effects of tire wear particles on microbial activities in laboratory tests. Environ. Sci. Technol. Lett. 9, 1037–1043. https://doi.org/10.1021/acs.estlett.2c00585.

Kole, J.P., Löhr, A.J., Van Belleghem, F.G.A.J., Ragas, A.M.J., 2017. Wear and tear of tyres: a stealthy source of microplastics in the environment. Int. J. Environ. Res. Public Health 14, 1265. https://doi.org/10.3390/IJERPH14101265.

Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, L.I., Finley, B.L., 2010. Physical and chemical characterization of tire-related particles: comparison of particles generated using different methodologies. Sci. Total Environ. 408, 652–659. https://doi.org/10.1016/J.SCITOTENV.2009.10.016.

Künzli, M., Dürr-Auster, T., Bracher, C., Zhao, Y., Bachelder, J., Emmanuel, F., Wiggenhauser, M., 2024. 67Zn and 111Cd labelled green manure to determine the fate and dynamics of zinc and cadmium in soil–fertilizer–crop systems. Isot. Environ. Health Stud. 60 (3), 286–308.

Lassen, C., Hansen, S.F., Magnusson, K., Hartmann, N.B., Jensen, P.R., Nielsen, T.G., Brinch, A., 2015. Microplastics: Occurrence, Effects and Sources of Releases to the Environment in Denmark. Danish Environmental Protection Agency. Ministry of Environment and Food.

Leifheit, E.F., Lehmann, A., Rillig, M.C., 2021. Potential effects of microplastic on arbuscular mycorrhizal fungi. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.626709.

Leifheit, E.F., Kissener, H.L., Faltin, E., Ryo, M., Rillig, M.C., 2022. Tire abrasion particles negatively affect plant growth even at low concentrations and alter soil biogeochemical cycling. Soil Ecology Letters 4, 409–415. https://doi.org/10.1007/s42832-021-0114-2.

Liu, Yan, Zhou, H., Yan, M., Liu, Yang, Ni, X., Song, J., Yi, X., 2022. Toxicity of tire wear particles and the leachates to microorganisms in marine sediments. Environ. Pollut. 309, 119744. https://doi.org/10.1016/j.envpol.2022.119744.

Liu, X., Li, Y., Yu, Y., Yao, H., 2023a. Effect of nonbiodegradable microplastics on soil respiration and enzyme activity: a meta-analysis. Applied Soil Ecology 184, 104770. https://doi.org/10.1016/J.APSOIL.2022.104770.

Liu, Y., Xu, F., Ding, L., Zhang, G., Bai, B., Han, Y., Xiao, L., Song, Y., Li, Y., Wan, S., Li, G., 2023b. Microplastics reduce nitrogen uptake in peanut plants by damaging root cells and impairing soil nitrogen cycling. J. Hazard. Mater. 443, 130384. https://doi.org/10.1016/J.JHAZMAT.2022.130384.

Lushchak, V.I., 2014. Dissection of the hormetic curve: analysis of components and mechanisms. Dose-Response 12, 466–479. https://doi.org/10.2203/dose-response.13-051.Lushchak.

Lv, M., Meng, F., Man, M., Lu, S., Ren, S., Yang, X., Wang, Q., Chen, L., Ding, J., 2024. Aging increases the particulate- and leachate-induced toxicity of tire wear particles to microalgae. Water Res. 256, 121653. https://doi.org/10.1016/J.WATRES.2024.121653.

Marschner, H., 2011. Marschner's Mineral Nutrition of Higher Plants. Academic press.

Martinez Arbizu, P., 2020. pairwiseAdonis: pairwise multilevel comparison using adonis. R package version 0.4 1.

Marx, M.C., Wood, M., Jarvis, S.C., 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol. Biochem. 33, 1633–1640. https://doi.org/10.1016/S0038-0717(01)00079-7.

McGonigle, T.P.T., Miller, M.H.H., Evans, D.G., Fairchild, L.G., Swan, J.A., Fairchild, G. L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol. 115, 495–501. https://doi.org/10.1111/j.1469-8137.1990.tb00476.x.

Mennekes, D., Nowack, B., 2022. Tire wear particle emissions: measurement data where are you? Sci. Total Environ. 830, 154655. https://doi.org/10.1016/J.SCITOTENV.2022.154655.

Müller, A., Kocher, B., Altmann, K., Braun, U., 2022. Determination of tire wear markers in soil samples and their distribution in a roadside soil. Chemosphere 294, 133653. https://doi.org/10.1016/j.chemosphere.2022.133653.

O'Brien, A.M., Lins, T.F., Yang, Y., Frederickson, M.E., Sinton, D., Rochman, C.M., 2022. Microplastics shift impacts of climate change on a plant-microbe mutualism: temperature, CO2, and tire wear particles. Environ. Res. 203, 111727. https://doi.org/10.1016/J.ENVRES.2021.111727.

Poschenrieder, C., Bech, J., Llugany, M., Tobías, F.J., Barceló, J., 1995. Availability of Cu and Zn to plants growing on and off a malachite site. Toxicol. Environ. Chem. 52, 143–151.

Posit team, 2023. RStudio: Integrated Development Environment for R.

Powell, J.R., Rillig, M.C., 2018. Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. New Phytol. 220, 1059–1075. https://doi.org/10.1111/nph.15119.

R Core Team, 2023. R: A Language and Environment for Statistical Computing.

Reuter, D.J., Robson, A.D., Loneragan, J.F., Tranthim-Fryer, D.J., 1981. Copper nutrition of subterranean clover (Trifolium subterraneum L. cv. Seaton Park). II. Effects of copper supply on distribution of copper and the diagnosis of copper deficiency by plant analysis. Aust. J. Agr. Res. 32, 267–282. https://doi.org/10.1071/AR9810267.

Rillig, M.C., Leifheit, E., Lehmann, J., 2021. Microplastic effects on carbon cycling processes in soils. PLoS Biol. 19, e3001130. https://doi.org/10.1371/JOURNAL.PBIO.3001130.

Rillig, M.C., Kim, S.W., Zhu, Y.-G., 2024. The soil plastisphere. Nat. Rev. Microbiol. 2, 64. https://doi.org/10.1038/s41579-023-00967-2.

Riveros, G., Urrutia, H., Araya, J., Zagal, E., Schoebitz, M., 2021. 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, 2021 29:6.

Rogge, W.F., Hildemann, L.M., Mazurek, M.A., Cass, G.R., Simoneit, B.R.T., 1993. Sources of fine organic aerosol. 3. Road dust, Tire debris, and organometallic brake lining dust: roads as sources and sinks. Environ. Sci. Technol. 27 (9), 1892–1904. https://doi.org/10.1021/ES00046A019.

Schlaeppi, K., Bender, S.F., Mascher, F., Russo, G., Patrignani, A., Camenzind, T., Hempel, S., Rillig, M.C., van der Heijden, M.G.A.A., 2016. High-resolution community profiling of arbuscular mycorrhizal fungi. New Phytol. 212, 780–791. https://doi.org/10.1111/nph.14070.

Schulz, M., 1987. Wirkung von Gummimehl auf Phaseolus vulgaris. Zeitschrift für Pflanzenernährung und Bodenkunde 150, 37–41. https://doi.org/10.1002/JPLN.19871500108.

Sieber, R., Kawecki, D., Nowack, B., 2020. Dynamic probabilistic material flow analysis of rubber release from tires into the environment. Environ. Pollut. 258, 113573. https://doi.org/10.1016/J.ENVPOL.2019.113573.

Smolders, E., Degryse, F., 2002. Fate and effect of zinc from tire debris in soil. Environ. Sci. Technol. 36, 3706–3710. https://doi.org/10.1021/ES025567P.

Smreczak, B., Maliszewska-Kordybach, B., Martyniuk, S., 1999. Effect of PAHs and heavy metals on activity of soil microflora. In: Bioavailability of Organic Xenobiotics in the Environment, pp. 377–380. https://doi.org/10.1007/978-94-015-9235-2_19.

Sommer, F., Dietze, V., Baum, A., Sauer, J., Gilge, S., Maschowski, C., Gieré, R., 2018. Tire abrasion as a major source of microplastics in the environment. Aerosol Air Qual. Res. 18, 2014–2028. https://doi.org/10.4209/AAQR.2018.03.0099.

Tedersoo, L., Tooming-klunderud, A., Anslan, S., 2018. PacBio metabarcoding of Fungi and other eukaryotes: errors, biases and perspectives. New Phytol. 217, 1370–1385. https://doi.org/10.1111/nph.14776.

Thonar, C., Erb, A., Jansa, J., 2012. Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities—marker design, verification, calibration and field validation. Mol. Ecol. Resour. 12, 219–232. https://doi.org/10.1111/j.1755-0998.2011.03086.x.

Van Eynde, E., Fendrich, A.N., Ballabio, C., Panagos, P., 2023. Spatial assessment of topsoil zinc concentrations in Europe. Sci. Total Environ. 892, 164512. https://doi.org/10.1016/J.SCITOTENV.2023.164512.

Vierheilig, H., Coughlan, A.P., Wyss, U., Piche, Y., 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl. Envir. Microbiol. 64, 5004–5007. https://doi.org/10.1128/aem.64.12.5004-5007.1998.

Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T., Reemtsma, T., 2018. Tire wear particles in the aquatic environment - a review on generation, analysis, occurrence, fate and effects. Water Res. 139, 83–100. https://doi.org/10.1016/j.watres.2018.03.051.

Wan, Y., Wu, C., Xue, Q., Hui, X., 2019. Effects of plastic contamination on water evaporation and desiccation cracking in soil. Sci. Total Environ. 654, 576–582. https://doi.org/10.1016/J.SCITOTENV.2018.11.123.

Wang, F., Zhang, X., Zhang, Shuqi, Zhang, Shuwu, Sun, Y., 2020. Interactions of microplastics and cadmium on plant growth and arbuscular mycorrhizal fungal communities in an agricultural soil. Chemosphere 254, 126791. https://doi.org/10.1016/J.CHEMOSPHERE.2020.126791.

Wang, C., Wang, L., Ok, Y.S., Tsang, D.C.W., Hou, D., 2022. Soil plastisphere: exploration methods, influencing factors, and ecological insights. J. Hazard. Mater. 430. https://doi.org/10.1016/J.JHAZMAT.2022.128503.

Wei, Z., Ma, X., Chai, Y., Senbayram, M., Wang, X., Wu, M., Zhang, G., Cai, S., Ma, J., Xu, H., Bol, R., Rillig, M.C., Ji, R., Yan, X., Shan, J., 2025. Tire wear particles exposure enhances denitrification in soil by enriching labile DOM and shaping the microbial community. Environ. Sci. Technol. 59, 1209–1221. https://doi.org/10.1021/acs.est.4c09766.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer.

Wik, A., Dave, G., 2009. Occurrence and effects of tire wear particles in the environment - a critical review and an initial risk assessment. Environ. Pollut. 157, 1–11. https://doi.org/10.1016/j.envpol.2008.09.028.

Xu, Q., Wu, Z., Xu, Z., Li, G., 2024. Soil moisture-dependent tire wear particles aging processes shift soil microbial communities and elevated nitrous oxide emission on drylands. Sci. Total Environ. 952, 175948. https://doi.org/10.1016/J.SCITOTENV.2024.175948.

Yan, B.F., Dürr-Auster, T., Frossard, E., Wiggenhauser, M., 2021. The use of stable zinc isotope soil labeling to assess the contribution of complex organic fertilizers to the zinc nutrition of ryegrass. Front. Plant Sci. 12, 730679.

Zeb, A., Liu, W., Ali, N., Shi, R., Lian, Y., Wang, Q., Wang, J., Li, J., Zheng, Z., Liu, Jinzheng, Yu, M., Liu, Jianv, 2024a. Integrating metabolomics and high-throughput sequencing to investigate the effects of tire wear particles on mung bean plants and soil microbial communities. Environ. Pollut. 340. https://doi.org/10.1016/j.envpol.2023.122872.

Zeb, A., Liu, W., Ali, N., Shi, R., Zhao, Y., Wang, J., Wang, Q., Khan, S., Baig, A.M., Liu, J., Khan, A.A., Ge, Y., Li, X., Yin, C., 2024b. Impact of pristine and aged tire wear particles on *Ipomoea aquatica* and rhizospheric microbial communities: insights from a long-term exposure study. Environ. Sci. Technol. https://doi.org/10.1021/ACS.EST.4C07188. /SUPPL_FILE/ES4C07188_SI_001.PDF.

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “plastisphere”: microbial communities on plastic marine debris. Environ. Sci. Technol. 47 (13), 7137–7146. https://doi.org/10.1021/ES401288X.

Zhang, Z., Zhao, J., Li, K., Wang, X., Xu, H., Mao, D., Liu, S., 2025. “Tire plastisphere” in aquatic ecosystems: biofilms colonizing on tire particles exhibiting a distinct community structure and assembly compared to conventional plastisphere. J. Hazard. Mater. 483, 136660. https://doi.org/10.1016/J.JHAZMAT.2024.136660.

Zhu, Y., Kim, S.W., Li, H., Rillig, M.C., 2024. Delivery rate alters the effects of tire wear particles on soil microbial activities. Environ. Sci. Eur. 36, 1–8. https://doi.org/10.1186/S12302-024-00918-5/FIGURES/3.