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Delivery rate alters the effects of tire wear particles on soil microbial activities

Yanjie Zhu[1,2], Shin Woong Kim[1,2*], Huiying Li[1,2] and Matthias C. Rillig[1,2]

Abstract

Background Tire wear particles (TWPs) produced by the abrasion between tires and road surfaces have been recognized as an emerging threat to soil health globally in recent years. They can be transported from the road surface to adjacent soil at different delivery rates, with precipitation a main driver underpinning this movement. However, studies typically assume an abrupt exposure of TWPs in their experimental design. In this study, we investigated the impacts of abrupt and gradual delivery of TWPs on soil physicochemical properties and microbial activities. We used two different delivery rates of TWPs (abrupt and gradual) and devised two experimental phases, namely the TWPs-delivery period (phase 1) and the end-of-delivery period (phase 2).

Results We found that the gradual TWPs delivery treatments negatively influenced the activity of carbon cycle-related enzymes (β-glucosidase and β-D-1,4-cellobiosidase). Furthermore, the abrupt treatment highly increased the effects on nitrogen cycle-related enzyme activity (β-1,4-N-acetyl-glucosaminidase). In phase 2 (end-of-delivery period), each enzyme activity was returned to a similar level as the control group, and these changes between phases 1 and 2 depended on the prior delivery rates.

Conclusion Abruptly and gradually delivered TWPs induce different responses to soil microbial activities. Our findings imply that the delivery rate of TWPs could be a key factor changing the effects of TWPs, further enhancing our understanding of the ecological impacts of TWPs.

Keywords Abrupt exposure, Gradual exposure, Microplastics, Enzyme activities, Soil aggregates, Soil pH, Soil respiration

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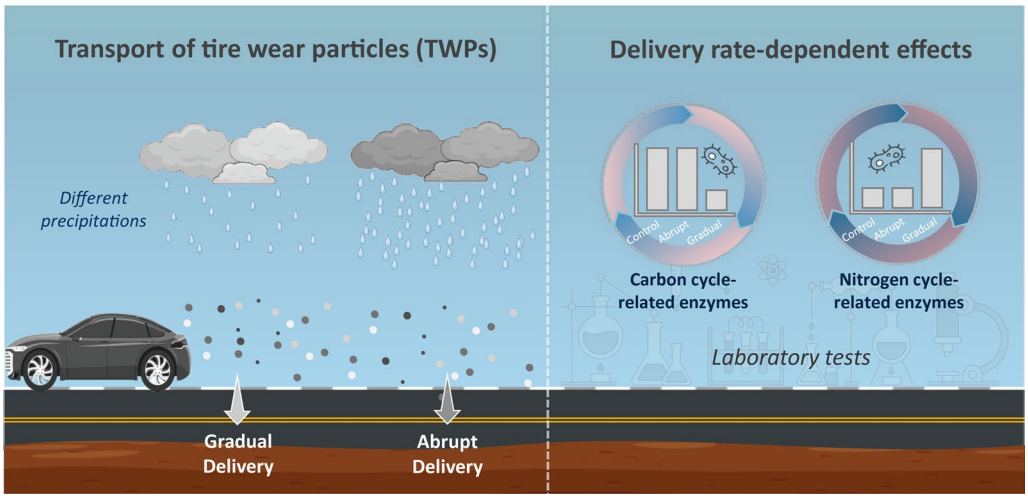
Graphical Abstract



Introduction

Although the first tire was produced in the nineteenth century, tire wear particles (TWPs) pollution in road dust was not highlighted until 1966 [1]. TWPs are usually regarded as microplastics (< 5 mm) due to their size, insolubility, polymer structure, and solid state [2, 3], and have emerged as a main contributor of microplastic pollution [4–7]. The global average emission of TWPs has reached 0.81 kg per year per capita [8], and recent research has reported the potential effects of TWPs on soil ecosystem. TWPs can induce adverse effects on survival and reproduction of soil fauna [4, 9–12], plant growth [13], and microbial activities [13, 14], and these effects have been underpinned with multiple pathways [3]. For instance, TWPs can physically damage the intestine of soil fauna [11], and influence soil microbial activities through changing soil properties and nutrient cycles [3]. Tires consist of rubber, filler, softener, vulcanization agents and various additives [7], including toxic chemicals such as polycyclic aromatic hydrocarbons (PAHs), benzothiazoles, heavy metals (e.g., Cd, Pb, and Zn), and tire rubber antioxidant (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine, 6PPD), and these additives can be released into the environment during the abrasion of tires, contributing to the adverse effects [15–17].

Once TWPs are generated on the road surface, they can be delivered into the soil environment either through road runoff or airborne transport [6]. Rainfall is considered as a main factor influencing the transport of TWPs [18], and the intensity of precipitation is directly linked with TWPs delivery rates [6]. For instance, during a strong rainfall event, TWPs can be abruptly washed from the air or road surfaces to roadside soils [6, 19], while no precipitation or weak/steady rainfall leads to a more gradual delivery of TWPs. Although the TWPs could already exist in soil, the different delivery rates of TWPs have been never discussed in previous research. This is important since there is still a knowledge gap regarding the different delivery rates of TWPs due to the lack of empirical data [20]. The rate of changes in environmental (e.g., drought) or anthropogenic (e.g., chemical pollutants) factors have been well established in global change research [21], and different effects under different rates of changes have been observed frequently [21, 22].

Soil is a main sink of contaminants emitted into the environment, and 67% of TWPs are delivered to the soil environment eventually. In this study, we investigated whether the delivery rate influences the impacts of TWPs on soil microbial activities and physicochemical properties. We established the different delivery rates of TWPs (abrupt and gradual), and designed two phases that capture the TWPs-delivery period (phase 1) and the end-of-delivery period (phase 2). We hypothesized that (1) TWPs pollution affects soil microbial activities and physicochemical properties, (2) delivery rate alters the effects of TWPs, and (3) these effects will be changed at the end-of-delivery period, depending on the prior delivery rates. We assumed that the abruptly delivered TWPs would induce stronger effects on soil microbial activities and physicochemical properties than the gradually delivered TWPs.

Materials and methods

Test soil and TWPs

Test soil (loamy sandy; Albic Luvisol) was collected from a grassland site of the Institute of Biology of Freie Universität Berlin (52°28' N, 13°17' E) [23]. Soil bulk density and water holding capacity (WHC) were 1.2 g cm^{-3} and 39.5%, and pH and EC were 5.8 and 58.8 µS cm^{-1}. The soil was air-dried for one week at room temperature and passed through a 2 mm-sieve for further use. TWPs (< 500 µm) were obtained from the company KURZ-Karkassenhandel (Landau, Germany) (https://kurz-karkassenhandel.de/en/rubber-products/). The waste tires of cars, trucks, motorcycles, and bicycles were collected, and crushed by ambient grinding. We sieved the purchased TWPs through a 250 µm mesh for further use. A light microscope (MDG41, Leica, Wetzlar, Germany) equipped with a camera (Flexacam C1, Leica, Wetzlar, Germany) was used to measure the average size of particles (88.4 µm, 4.5–276.8 µm) (Fig. S1), and this particle size is within a range of TWPs produced during driving (4–350 µm) [16].

Experiment set-up

The experiment was established in a greenhouse (22 ± 2/18 ± 2 °C, day/night; daylight period, 16:8; relative humidity, 40%). We placed 200 g of test soil in 210 mL round plastic containers (diameter, 80 mm; height, 48 mm; RPC superfos a/s, Denmark; soil layer thickness, 30 mm) covered with nylon mesh (pore size, 38 µm), and pre-incubated with 60% of WHC for a week to achieve a steady state of soil microbial metabolism (n = 12 for each three treatment). After the pre-incubation, we divided the experiment into two different phases, the TWPs-delivery period (phase 1; duration of 9 weeks) and end-of-delivery period (phase 2; duration of 4 weeks). During phase 1, we treated soils with TWPs at three different delivery rates including control (no TWPs addition), gradual delivery, and abrupt delivery. The abrupt and gradual delivery treatments had the same overall dose by the addition of TWPs according to a "week-dose" principle (Fig. S2). Briefly, 0.2 g of TWPs (0.1%, total dry soil mass) was added weekly in the gradual delivery treatment, and 1.8 g of TWPs (0.9%, total dry soil mass) was added at the fifth week for the abrupt treatment. These concentrations were based on the measurements (0.2 to 2%, dry weight) of TWPs in roadside soils according to previous studies [6]. At the beginning of phase 1, TWPs were suspended in deionized water with a volume matching 100% of the soil WHC, and we evenly poured this onto the soil surface for the gradual delivery treatment. The control and abrupt delivery treatments were treated with only deionized water (no TWPs addition). The water contents in each container were checked and replenished with deionized water (35 to 40 mL) every week to keep uniform moisture (100% of WHC). This water maintenance was accompanied with TWPs addition for the gradual delivery treatment, and the same TWPs addition method was applied for the abrupt delivery treatment in the fifth week (Fig. S3). We applied TWPs to the soil surface (soil depth, 30 mm) without subsequent mixing to prevent artificial disturbance, and saturated the soil with 100% of WHC to simulate rainy season conditions and to facilitate the particle penetration into the soil. At the end of phase 1, 6 units of each treatment were harvested, and the rest of experimental units (n = 6) were incubated for 4 more weeks with no further TWPs addition (phase 2) (Fig. S3). After harvest, each soil was carefully homogenized using a spatula, and fresh soil samples were stored at 4 °C for the subsequent measurements. The rest of the soil samples were air dried and then stored at room temperature.

Measurement of soil properties

Soil respiration rate was indicated by the CO_2 concentration produced per hour (µM M^{-1} h^{-1}) [24]. Thirty gram of fresh soil was placed in a 50 mL falcon tube equipped with a rubber stopper on the lid. The tubes were flushed with CO_2-free air for 5 min to eliminate background CO_2, and then incubated for 4 h at 20 °C. We sampled 1 mL of air in the headspace of the tube using a syringe and injected this sample into an infrared gas analyzer (LI-6400XT, LI-COR Inc., Bad Homburg, Germany). The activities of C-related enzymes β-glucosidase (EC3.2.1.21) and β-D-1,4-cellobiosidase (EC3.2.1.91), N-related enzyme β-1,4-N-acetyl-glucosaminidase (EC3.2.1.52), and P-related enzyme phosphatase (EC3.1.3.2) were measured with artificial *p*-nitrophenyl (*p*NP) linked substrates, and quantified by a microplate reader [25]. Briefly, 10 mL of 50 mM acetate buffer (pH, 5.0 − 5.5) was added into 50 mL-test tubes containing 5.0 g of each soil sample. The tubes were vortexed for 5 secs, and each soil slurry (150 µL) into each well (6 wells per sample) in the 96-well plate. Then, 150 µL of each substrate was added: *p*NP-β-D-glucopyranoside (Sigma no. N7006), *p*NP-β-D-cellobioside (Sigma no. N5759), *p*NP-N-acetyl-β-D-glucosaminide (Sigma no. N9376), *p*NP-phosphate disodium salt hexahydrate (Sigma no. 71,768). After 2 h (β-glucosidase and phosphatase) and 4 h (β-D-1,4-cellobiosidase and β-1,4-N-acetyl-glucosaminidase) incubations at 20 °C under dark conditions, each plate was centrifuged at 3,000 rpm for 5 min, and 100 µL of supernatant was transferred into a new plate and mixed with 200 µL of 0.1 M NaOH. Absorbances were determined at 410 nm using a microplate reader (Benchmark Plus, BioRad Laboratories GmbH, Hercules, CA, USA), and each enzyme activity was calculated as µmol

p-nitrophenol g soil dry mass^{-1} h^{-1}. Water stable aggregates (WSA) content was measured following the wet sieving method of Kemper and Rosenau [26] with a slight modification. Four gram of air-dried soil was put in a small sieve with a mesh size of 0.25 mm, re-wetted by capillarity with deionized water and inserted into a sieving machine (Agrisearch Equipment, Royal Eijkelkamp B.V., Giesbeek, Netherlands) to be wet-sieved for 3 min. The soil left on the sieve (fraction 1: stable soil aggregates and coarse matter) was dried at 60 °C and weighed, and then crushed manually in the wet sieve to obtain the coarse matter (fraction 2). Calculations of the percentage of WSA were according to: WSA (%) = (fraction 1 − fraction 2)/(4.0 −fraction 2) × 100%. [27]. To measure soil pH, 25 mL of distilled water was added to 5.0 g of air-dried soil in a falcon tube. The tube was shaken in 250 rpm for 30 min and then centrifuged in 3000 rpm for 5 min. The supernatant was filtered, and the pH was determined by a pH-meter (Hanna Instruments GmbH, Smithfield, USA).

Data analysis

We performed all the statistical analyses and plotting in R (v.4.1.2). Unpaired t-tests were performed by the t.test() function with a confidence interval of 95% to compare the difference between each treatment and phase. The variance was estimated separately for each group and the Welch modification to the degrees of freedom was used. Boxplots were drawn using the ggplot2 package. The changes of enzyme activities between phase 1 and phase 2 were calculated by the percentage (%) of the changes in phase 2 compared to phase 1.

Results

We evaluated the activities of four soil enzymes at the end of two different phases (phase 1 and 2), and found that their activities were dependent on TWPs delivery rates (abrupt and gradual) (Fig. 1). In phase 1, the activities of β-glucosidase and β-D-1,4-cellobiosidase were significantly reduced in the gradual treatment compared to the control (Fig. 1a, b). The activity of β-1,4-N-acetyl-glucosaminidase increased in the abrupt treatment (Fig. 1c), but there was no significant change in phosphatase activity in both abrupt and gradual treatments (Fig. 1d). We found no significant effect on pH, soil respiration, and WSA in both abrupt and gradual treatments (Fig. 2).

After phase 1, each soil was incubated for 4 weeks with no additional TWPs addition (phase 2). The activities of none of the enzymes were significantly different in both delivery treatments, compared to control (Fig. 1). There was no significant effect on WSA, soil respiration, and pH (Fig. 2). Through a comparison between phase 1 and

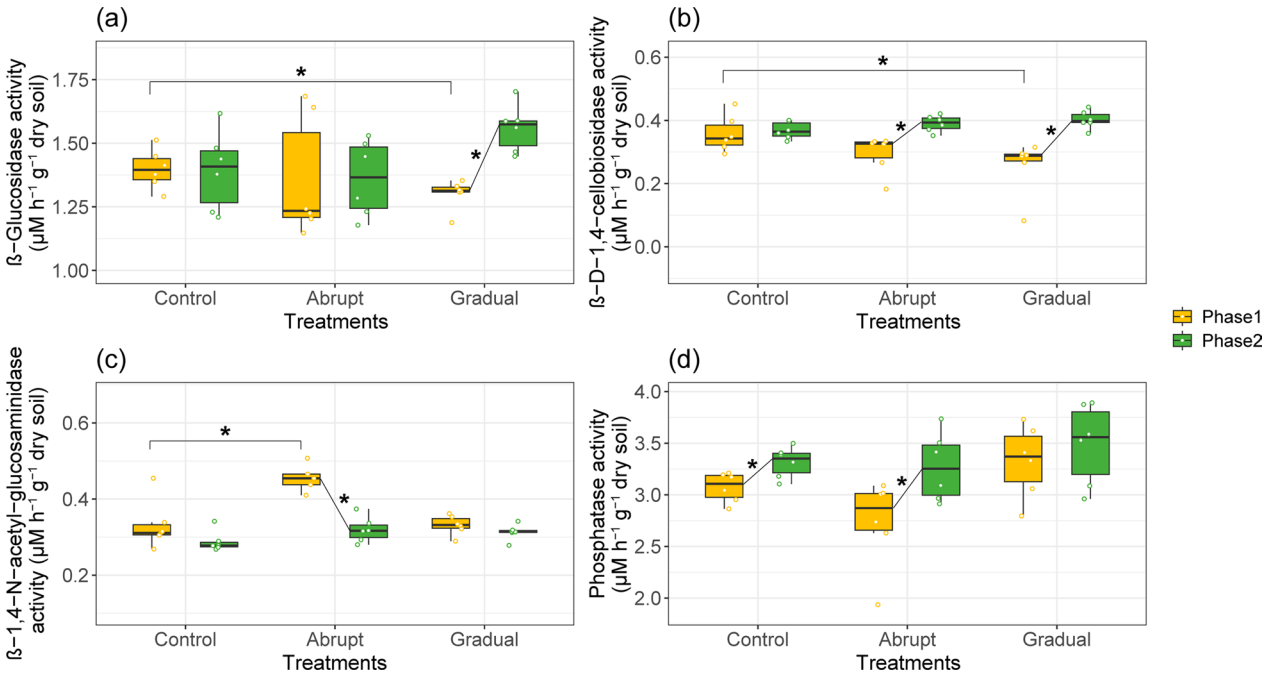


Fig. 1 Effects of TWPs delivery rates (abrupt and gradual) on the activities of **a** β-glucosidase, **b** β-D-1,4-cellobiosidase, **c** β-1,4-N-acetyl-glucosaminidase and **d** phosphatase. Boxplot indicates the median (thick black line), first quartile (bottom line), third quartile (top line), upper and lower limits (whiskers) for each group. Asterisks (*) indicate significant differences ($p < 0.05$) compared to the control or phase 1 according to unpaired t-tests

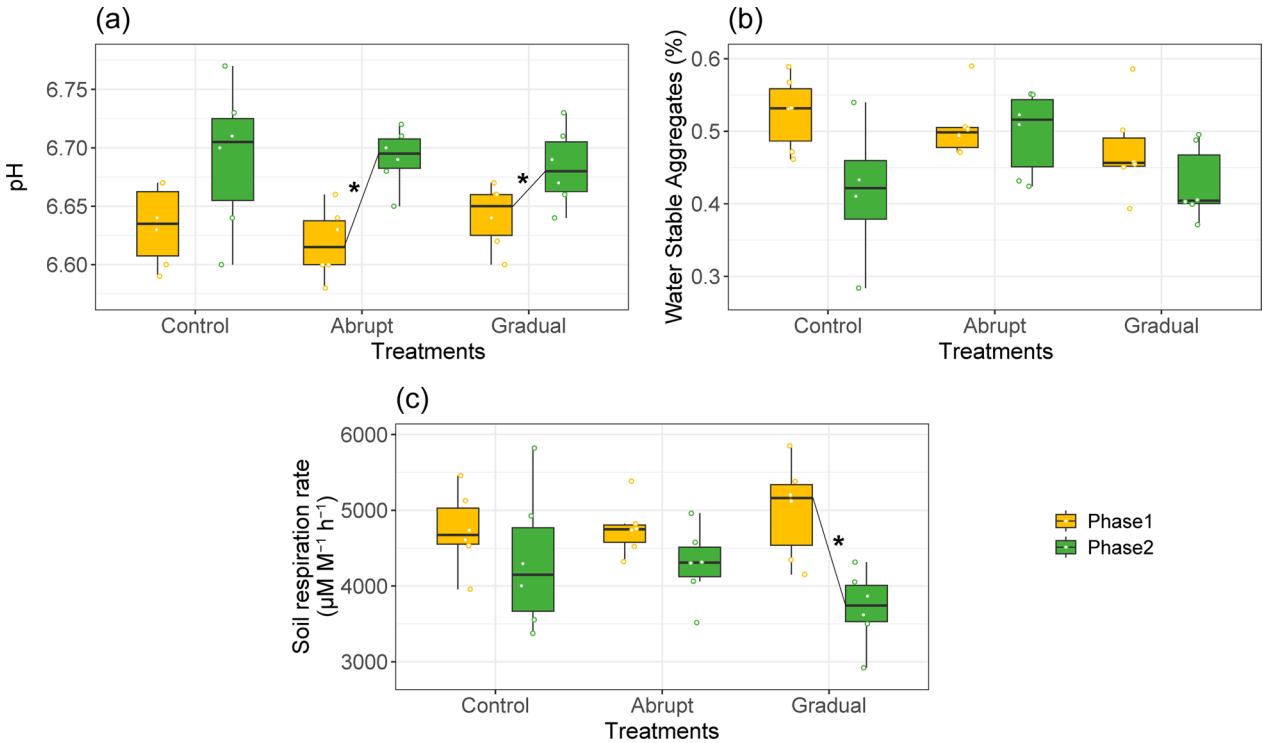


Fig. 2 Effects of TWPs delivery rate (abrupt and gradual) on **a** soil pH, **b** the percentage of soil water stable aggregates (WSA), and **c** soil respiration rate. Boxplot indicates the median (thick black line), first quartile (bottom line), third quartile (top line), upper and lower limits (whiskers) for each group. Asterisks (*) indicate significant differences ($p < 0.05$) compared to the control or phase 1 according to unpaired t-tests

2, we found that the activities of β-glucosidase (gradual) and β-D-1,4-cellobiosidase (abrupt and gradual) notably increased over time to a similar level as those observed in the control group (Fig. 1a, b). The β-1,4-N-acetyl-glucosaminidase activity significantly decreased in the abrupt treatment (Fig. 1c), and phosphatase activity increased in the control and the abrupt treatment (Fig. 1d). In addition, soil pH significantly increased over time in both of the TWPs treatments (gradual and abrupt) (Fig. 2a).

Discussion

The effects of TWPs on extracellular enzyme activities have been explored in previous studies [28, 29], and our results showed general agreement with these studies, such as the increase of β-1,4-N-acetyl-glucosaminidase activity [29], the decrease of β-glucosidase activity [29], and no significant change in phosphatase activity [28]. However, the impacts of TWPs delivery rates have not been reported before. Our findings indicated that the gradual delivery treatment induced a significant decrease in the activity of carbon cycle-related enzymes including β-glucosidase and β-D-1,4-cellobiosidase, while there was no effect in the abrupt treatment. TWPs can be presumably classified as an inert (persistent) carbon source, similar to microplastics and charcoal [30], and the fraction of persistent carbon is barely utilized by microbes through the production of hydrolytic enzymes [31, 32]. The increase of β-1,4-N-acetyl-glucosaminidase activity (Fig. 1c) was observed in the abrupt treatment, and this may be abruptly caused by the release of chemical additives from TWPs such as nitro-compounds (vulcanization agent) and PAHs, and PAHs can increase nitrogen mineralization/transcripts with the changes in the functional gene abundance [15, 33–35].

TWPs negatively affected the carbon cycle-related enzymes during phase 1 (TWPs-delivery period), and these effects disappeared in phase 2 (end-of-delivery period). This may be linked to previous studies that reported higher abundance of carbon cycle-related functional genes than before TWPs exposure [36]. The soil microbial community has the capacity to establish a new equilibrium in an altered environment, which is defined as the adaptation/adjustment capacity [37], and this capacity seems to be shown in both delivery treatments. However, we assumed that abruptly and gradually delivered TWPs have elicited different adaptation/adjustment of carbon cycle-related microorganisms to TWPs pollution, since the changes from phase 1 to phase 2 of both β-glucosidase and

β-D-1,4-cellobiosidase activities were more remarkable in the gradual treatment (19.82% and 59.29%) than those in the abrupt treatment (0.31% and 32.34%) (Table 1) [38–41].

In the results of β-1,4-N-acetyl-glucosaminidase, we found that the enzyme activity dramatically decreased in the abrupt treatment in phase 2 compared to phase 1. Although the activities of most soil extracellular enzymes generally decrease over time under normal conditions [42, 43], this reduction was more remarkable in the abrupt treatment (29.80%) compared to other treatments (control and gradual, 13.16% and 5.59%) (Table 1). We assumed that these differences were influenced by the prior delivery rates. According to a previous study, abrupt TWPs pollution can positively affect the abundance of bacterial genes related to nitrogen metabolism [36], which may potentially reduce the activities of nitrogen cycle-related enzymes in a long run, and this may cause the decrease in β-1,4-N-acetyl-glucosaminidase activity over time in the abrupt delivery treatment. We also assumed that the highly increased enzyme activity in phase 1 could be related to this gap between phases 1 and 2. In addition, TWPs generally contain $CaCO_3$ as filler [44] and thus increase the soil pH, and we assumed that the delayed changes in soil pH were found in our experimental conditions.

Although we attempted to explore the delivery-rate-dependent effects of TWPs, our study still has several limitations. The target TWPs in this study are distinguished from tire and road wear particles (TRWPs), which contain the environmental elements (e.g., minerals and exogenous material) from the road. In addition, our experiment was designed to start with TWPs-free soil, which is likely never the case in the environment. These differences can change the results, and the adaptation/adjustment of microbial community would be

Table 1 The changes of enzyme activities from phase 1 to phase 2 in different treatments. Each value indicates the percent (%) change of each enzyme activity in phase 2 compared to the activity in phase 1

Treatment	β-glucosidase (%)	β-D-1,4-cellobiosidase (%)	β-1,4-N-acetyl-glucosaminidase (%)	Phosphatase (%)
Control	− 0.47 ± 12.41	2.96 ± 18.27	− 13.16 ± 18.75	7.89 ± 6.95*
Abrupt	0.31 ± 20.81	32.34 ± 28.59*	− 29.80 ± 9.16*	19.57 ± 22.35*
Gradual	19.82 ± 8.91*	59.29 ± 54.28*	− 5.59 ± 9.51	4.926 ± 16.12

means ± standard deviations

* Significant differences between phase 1 and phase 2 ($p < 0.05$)

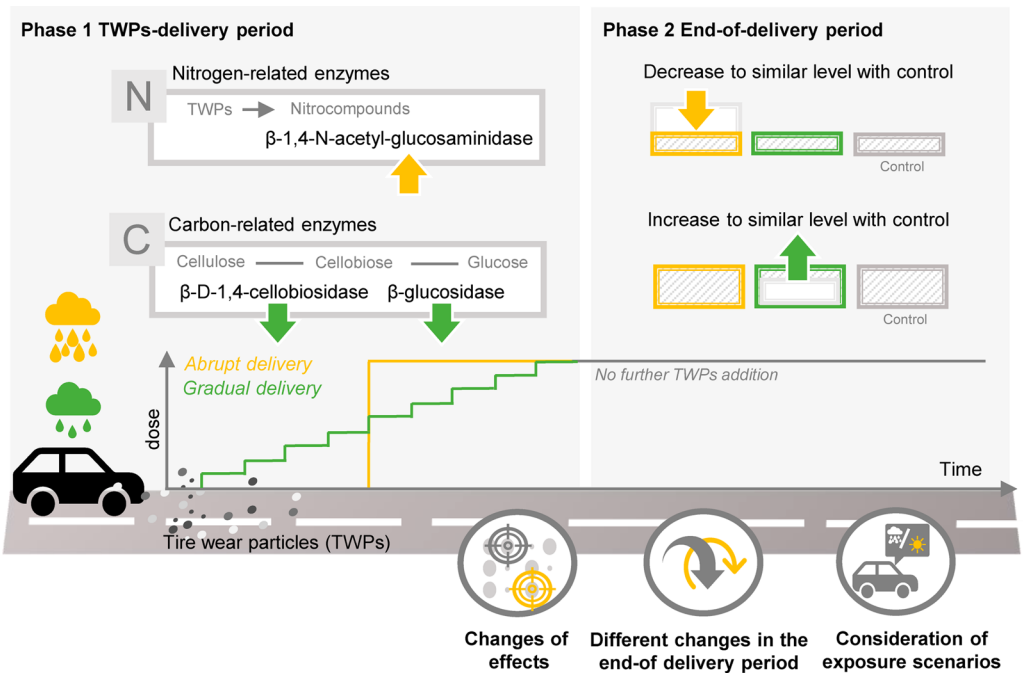


Fig. 3 Conceptual interpretation of the effects of TWPs at different delivery rates

different in situ, compared to our study. We designed only two phases for this study, but more observation points would be needed to understand the effects of different delivery rates.

Conclusions

Given the mode of arrival of TWPs in soil, it is important to carefully consider their transport from the road surface into the environment in order to understand their effects. Unfortunately, this aspect has been overlooked in previous experiments. These findings indicated that the delivery rate of TWPs may represent another key factor (exposure scenario) with physicochemical characteristics (e.g., size and shape) of TWPs and environmental factors (e.g., soil properties), which can alter the effect of TWPs. It is thus imperative to consider exposure scenarios when interpreting the impacts of TWPs in soil systems (Fig. 3), and this study enhances our understanding of the effects of TWPs on microbial activities. Further research is warranted to investigate the responses at the microbial community level, with the use of different test soils and a variety of delivery rates, to gain a comprehensive understanding of how TWPs delivery rates influence soil ecosystems. The shifts in TWPs effects under different delivery rates are important for deciphering the true extent of TWPs effects in the field, and likely are also relevant for other types of microplastics.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-024-00918-5.

Supplementary Material 1. Supplementary figure of photographic processing of tire wear particle sizes to determine average diameter and size range (Fig. S1), scheme of the gradual and abrupt TWPs treatments based on the "week-dose" principle (Fig. S2), experimental design (Fig. S3).

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Author contributions
YZ: conceptualization, design of the study, experiment setup, analysis of data, and writing; SWK: conceptualization, design of the study, writing, review and editing; HL: experiment setup. M.C.R.: design of the study, review and editing. All authors contributed to the article and approved the submitted version.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and the supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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