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Fragmentation of nanoplastics driven by plant–microbe rhizosphere interaction during abiotic stress combination†

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Large amounts of micro- and nanoplastics, which are released into the environment through environmental weathering of plastic items or sludge disposal/application, can end up in soil, being considered as a new plant abiotic stressor. In nature, plants face a number of abiotic stresses simultaneously. However, it is largely unknown whether and how abiotic stress combination affects the plant uptake of nanoplastics, and how plants tune the rhizosphere interactions to acclimate to a combination of nanoplastic and another abiotic stress. Here we show that smaller, fragmented nanoplastics can accumulate in the root of *Arabidopsis thaliana* under combined nanoplastic and Cd stresses. The specific changes in root exudation of organic acids and bacterial community composition that reveals a metabolic preference for aromatic compounds drive the degradation of nanoplastics in the rhizosphere. Our findings provide critical implications relevant to food security that nanoplastics will contaminate crops as well, and in turn, transfer along the human food chain.

Environmental significance

The ubiquity and rising occurrence of micro- and nanoplastics in soils can be another factor of abiotic stress affecting plant growth and development. Here we report the degradation of nanoplastics in the rhizosphere of *Arabidopsis thaliana* in response to co-occurring nanoplastic and heavy metal stresses, followed by accumulation of the fragmented nanoplastics in the roots. We reveal that according to changes in the root exudate composition, the enrichment of specific soil bacteria that have a distinct preference for aromatic compounds can contribute to the partial degradation of nanoplastics in the rhizosphere, as a part of adaptive strategy to avoid the adverse effects of nanoplastics.

Introduction

Microplastic pollution has been noticed worldwide from inland to polar regions,[1–3] and there are growing concerns of potential risks in biota. While many investigations of the fate of micro- (MPs, <5 mm) and nanoplastics (NPs, <100 nm or 1000 nm) have been carried out in aquatic systems,[4,5] the available data remain limited for soils, despite the increasing evidence that agricultural land is likely a sink for MPs and NPs.[6,7] Emission data show that approximately 63 000–430 000 tons of MPs are added annually to agricultural soil in Europe, which is much higher than the values estimated from surface waters.[8] Moreover, the use of plastic films and sludge-based fertilizer in agricultural practices can introduce a great input of MPs and NPs into farmland.[9,10] Given that once deposited in soils, the MPs and NPs are expected to decompose very slowly,[11] they need to be regarded as a new long-lasting environmental stressor to terrestrial organisms, particularly to plants, which are sessile.

In nature, plants are usually under abiotic stress conditions and must acclimate to diverse stresses to survive and tolerate. The morphological, physiological, biochemical, and molecular responses of plants to abiotic stresses have been the subject of intensive research with much emphasis on individual stress factors.[12,13] However, plants in the field are routinely exposed to combinations of two or more different abiotic stresses, potentially triggering acclimation responses distinct from those for individual stresses.[14] Plant responses to abiotic stress can also be linked with microbiota since plant assembles specific microbial compartments surrounding roots (the rizhosphere). The interplay between

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plant roots and their associated microbes is critical for resilience to abiotic stresses in soil environments.[15,16] Despite the growing number of studies demonstrating the phytotoxicity of MPs or NPs,[17–21] relatively few studies have sought to understand their effects in combination with other abiotic stresses.[22] In addition, whether and how the MP or NP exposure impacts the assembly of the rhizosphere microbiome is still largely unknown.

Stress response in plants occurs at various organ levels among which the root first encountered to many stresses is of particular importance. Previous studies on root–particle interaction demonstrated the size-dependent uptake of nanosized particles by roots.[23] Given recent reports of the plant uptake of NPs and even submicrometer-sized MPs,[18,24] as well as existing information on various nanomaterials,[25,26] original and environmentally-weathered NPs are likely to pass through the plant cell wall and be accumulated in plants, suggesting the potential for transfer of the NPs to higher trophic levels. Here, to address the fate and effects of NPs in the plant–soil system when simultaneously exposed to different abiotic stress, we studied the systemic response of *Arabidopsis thaliana* and its associated bacterial community to a combination of cadmium (Cd) and polystyrene NP (PS NP; ~50 nm in diameter, Fig. S1†). Cd was chosen as a representative of one of the most important abiotic stressors because heavy metal stress is common in both natural and agricultural soils.

Materials and methods

Nanoplastic material

A stock suspension of PS NPs with a diameter of 50 nm was purchased from Polyscience Inc. (USA). Transmission electron microscopy (TEM) images were obtained using a FEI Tecnai G2-F20. Dynamic light scattering analysis of PS NP suspension was performed using a Malvern Zetasizer Nano ZS. PS NPs were 43.6 ± 4.6 nm in diameter (TEM) in the stock suspension and had an average hydrodynamic diameter of 51.1 ± 5.4 nm in deionized water (Fig. S1a. and b†). The PS NPs had a negative zeta potential across the full pH range (pH 3–9) (Fig. S1c†). The composition was confirmed *via* a Fourier transform infrared spectrometer (FTIR; Vertex 80v, Bruker) (Fig. S1d†), with a range of 800–2000 cm^{-1}.

Plant growth and stress treatments

A. thaliana Col-0 (cv. Columbia-0) was used in all experiments. The characteristics of soil (Hungnong Seed Co., Korea) are given in Table S1.† Cd, NPs or Cd/NP were spiked into sterile soils that presented the desired concentrations. In brief, soil was mixed with a diluted Cd stock solution (1 g L^{-1} $Cd(NO_3)_2$). After homogenization, the soil was kept in a glass container for 3 days inside a plant growth chamber to maintain a constant temperature (24 °C). To check the homogeneity of Cd spiking into the soil, the soil samples (triplicates) were analyzed by an inductively coupled plasma optical emission spectrometry (ICP-OES; iCAP6300 DUO, Thermo Scientific). The accuracy of the analytical results was ensured by calibration curves (used by Periodic Table Mix 1 for ICP, Sigma-Aldrich), with a 90–105% recovery rate. After 3 days, the NP stock (25 g L^{-1}) was diluted and suspended in Millipore water by sonication for 3 min and mixed with incubated soil to reach a target concentration of NPs in soil. The soil mixtures were equilibrated at the plant incubator for 1 week before planting.

The surface-sterilized seeds of *A. thaliana* were grown in pots (6 cm × 6 cm in size and 8 cm depth) with 100 g soil containing Cd, NP or Cd/NP (n = 10 pots per each treatment) at 22 °C under long-day conditions (16 h photoperiod; 200 μmol m^{-2} s^{-1}), watered every other day, and harvested after 21 days before they flowered to evaluate their dry weight and Cd content. For a single exposure, the concentrations of Cd (5.6 mg kg^{-1} soil) and NPs (0.05, 0.1 and 0.5 g kg^{-1} soil) were chosen on the basis of the levels previously reported in the literature.[27,28] For a combined exposure, the Cd (5.6 mg kg^{-1} soil) and NP (0.1 g kg^{-1} soil) concentrations were applied. The soil samples were taken for analyses of Cd distribution. Soil Cd was fractionated into five operational pools following previous literature,[29] and each digestion extract was analyzed using ICP-OES.

Measurement of Cd and reactive oxygen species (ROS) in plants

To measure Cd content in plant tissues, sampled roots and leaves were dried and digested in concentrated nitric acid at 105 °C overnight. The diluted samples were analyzed by an ICP-OES. $O_2^{\bullet-}$ and H_2O_2 in whole (except roots) 21 day-old plants were determined using nitroblue tetrazolium (NBT) and 3,3-diaminobenzidine (DAB) staining, respectively.[30]

Detection of nanoplastic uptake and degradation

The distribution of NPs in *A. thaliana* grown in soils with NP or Cd/NP for 21 d was analyzed by TEM (JEM-1011, JEOL), as described previously.[18] The roots and leaves were fixed in 2% glutaraldehyde and 2% paraformaldehyde buffer and washed three times with PBS buffer. The samples were stained using 0.5% uranyl acetate, dehydrated in ethanol, and then embedded in Spurr's resin. The samples were sectioned and observed using a TEM.

The degradation byproducts of PS NPs in soils were identified by a GC-MS (HP6890-JMSII, Jeol) equipped with a DB-1 capillary column. Freeze-dried soils (5 g) were ultrasonically extracted for 3 min four times with dichloromethane (DCM), followed by rotary evaporation. The concentrate dissolved in benzene was injected into a gas chromatography-mass spectrometry (GC-MS).[31] Biphenyl and phenanthrene were used as surrogate and internal standard, respectively.

Community-level physiological profiling

Community-level metabolic capabilities of microbes in soil were screened with the Biolog EcoPlates (Biolog Inc.) according to manufacturer's protocol.[32] Each well of the plate was inoculated with 150 μL of soil inoculum and incubated

at 25 °C. Absorbance was measured by a microplate reader (Biochrom EZ Read 400 ELISA) at 590 nm after 3, 5 and 8 days of incubation. The microbial metabolic activity was expressed as AWCD ($\sum OD_i/31$), where OD_i is the optical density of each substrate corrected by subtracting the blank well (without a carbon source) values.

Root bacterial community analysis

DNA in soil samples was extracted using a DNeasy Powersoil Kit (Qiagen) according to the manufacturer's protocol. The universal primer pair (341F and 805R) targeting the V3–V4 regions of bacterial 16S rRNA genes was used for amplification. The amplicons were purified and subjected to Illumina MiSeq 300 bp paired-end sequencing. High-throughput sequencing analysis was performed by Macrogen Inc. (South Korea).

Hydroponic experiments

To directly measure the changes in NP size following the treatment, plants (n = 4 per each treatment) were grown in autoclaved half-strength Murashige and Skoog (MS) media (pH 5.8; adjusted with 0.1 N KOH) with NP (0.1 g L^{-1}) or Cd/NP (5.6 mg L^{-1}/0.1 g L^{-1}) for 21 days. Growth conditions remained same as those of the soil culture. After the plants were removed from the media, the size of residual NPs in the media was characterized using TEM (Tecnai F20 G2, FEI).

Analysis of organic acids released from roots

After plants had been grown in half-strength MS media for 14 days, they were transferred to a 24-well culture plate that contained half-strength MS media with Cd (5.6 mg L^{-1}), NP (0.1 g L^{-1}) or Cd/NP (5.6 mg L^{-1}/0.1 g L^{-1}) and incubated for 1 day. Then, the plants were removed and the remaining solutions were filtered through 0.45 μm filters and stored at −20 °C until analysis. The organic acid analysis were performed using a high performance liquid chromatography (HPLC; Ultimate3000, Thermo Dionex), equipped with an Aminex 87H column (Bio-Rad). The mobile phase was 0.01 N H_2SO_4 at a flow rate of 0.5 ml min^{-1} with the detection wavelength of 210 nm.[33]

Statistical analyses

All experiments were conducted with at least four independent replicates. One-way analysis of variance (ANOVA)

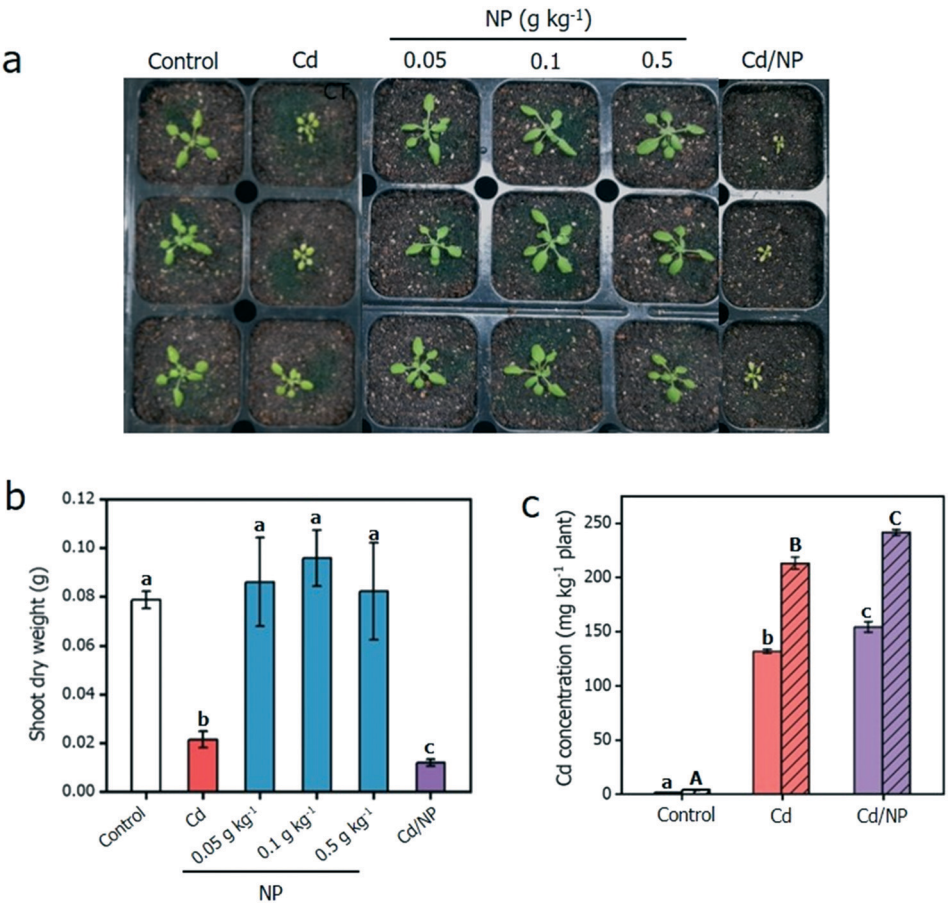

Fig. 1 (a) Phenotypes of 21 day-old *A. thaliana* exposed to Cd (5.6 mg kg^{-1}), NP (0.05, 0.1 or 0.5 g kg^{-1}) or a combination of Cd and NP (Cd/NP; 5.6 mg kg^{-1}/0.1 g kg^{-1}) in soil. Photographs are from three representative plants out of ten. (b) Shoot dry weight. (c) Cd concentrations in roots (filled bars) and shoots (diagonal bars) of plants subjected to Cd or Cd/NP treatments. Averages and standard deviations are shown; different letters indicate significant differences according to Duncan's test (n = 10, $p < 0.05$).

with Tukey HSD test (IBM SPSS statistics 20) or Student's *t*-test (two-tailed) was used to determine if differences were significant (*i.e.*, $p < 0.05$). Data were presented as mean ± standard deviation (S. D.).

Results and discussion

Physiological responses of *A. thaliana* to individual and combined stresses

We grew *A. thaliana* in soil pots mixed with Cd, NPs or a combination of Cd and NP (Cd/NP). *A. thaliana* exposed to NPs exhibited similar appearances and shoot dry weights as the control plant, regardless of the NP concentration applied (0.05, 0.1 and 0.5 g kg^{-1} soil) (Fig. 1a and b), suggesting that the NP exposure alone did not affect the plant physiology under those particular conditions. The phytotoxicity of NPs remains controversial so far. For example, the treatment of NPs reduced root elongation in onion (*Allium cepa*) and decreased the growth of cucumber (*Cucumis sativus*),[19,20] while it had no discernible effect on wheat (*Triticum aestivum*).[34] This suggests that the toxic effect of NPs on plants depends on the physicochemical properties (*i.e.*, size, surface charge) and concentration of NPs, and test plant species. By contrast, more severe growth inhibition with decreased shoot dry weight was observed in the plants stressed by Cd/NP compared to Cd alone (Fig. 1a and b).

To explore the possible involvement of intracellular reactive oxygen species (ROS) in the joint effects of Cd and NP, the ROS levels in 21 day-old plants were determined by using two histochemical probes, DAB for H_2O_2 and NBT for $O_2^{\bullet-}$, finding elevated ROS levels in the Cd/NP-treated sample (Fig. S2†). Given that NPs which adsorb heavy metals, might affect the metal bioavailability in soil and uptake by plants, we also analyzed the Cd contents in the roots and shoots (Fig. 1c). The Cd/NP treatment promoted Cd accumulations in roots by 16% and in shoots by 13.7% over those of the Cd-treated plant. This was linked to changes in Cd speciation in NP-amended soil (Fig. S3†), with a shift from residual to organically-bound phases that was likely due to Cd sorption onto NPs. The observation of increased uptake of Cd by *Arabidopsis* plants in NP-amended soil is consistent with previous studies,[35,36] demonstrating that carbon materials (*i.e.*, graphene oxide and carbon dot) addition could increase the exchangeable level of heavy metals in soils, which then enhanced plant availability. Thus, the overall effect of NP and Cd stress combination was found to be additive, resulting in enhanced growth inhibition by increased ROS generation and bioavailable Cd accumulation in plants.

Uptake of fragmented NPs in plants under combined stress

To study NP distribution in *A. thaliana*, we subjected plants to a combination of Cd and NP stresses and analyzed the

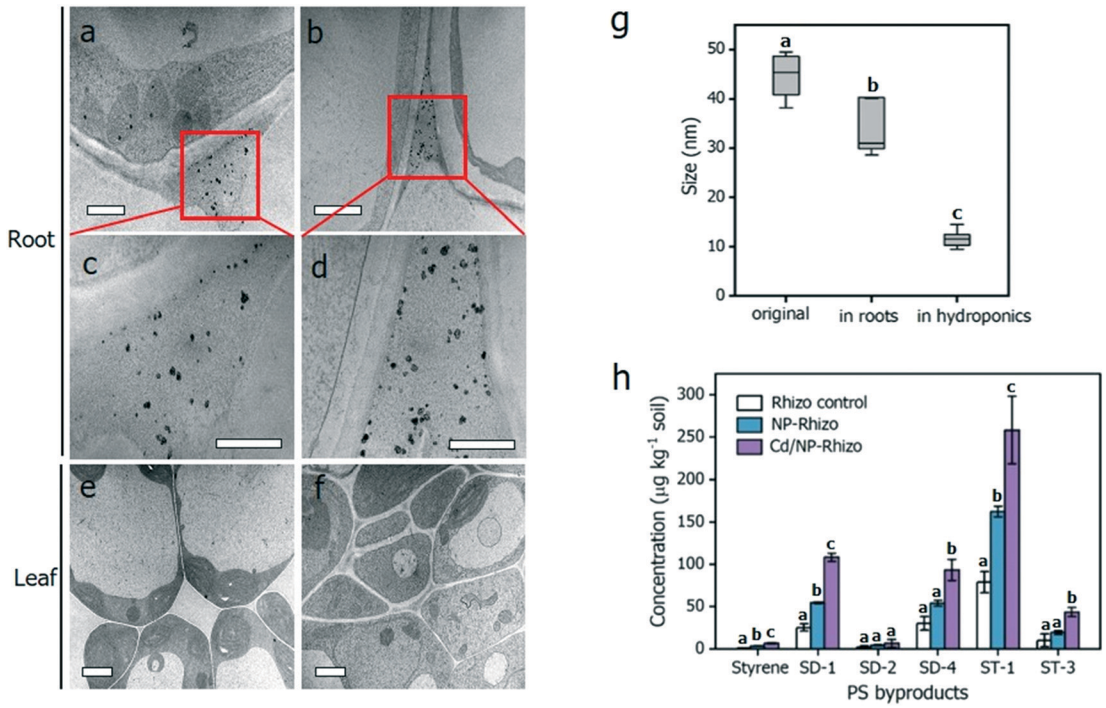

Fig. 2 (a–f) TEM images of transverse sections of 21 day-old roots and leaves subjected to Cd/NP. (c and d) Enlarged images of red squares in upper panels. Scale bars are 1 μm in a, b, e, and f, 500 nm in c and d. (g) NP particle size in exposure stock suspension, roots and MS media. For a size measurement in hydroponics, plants were grown in half-strength MS media with Cd and NPs for 21 days. Averages and standard deviations are shown; different letters indicate significant differences according to Duncan's test ($n = 100$, $p < 0.05$). (h) PS byproduct detection in treated and control rhizosphere soils. Averages and standard deviations are shown; different letters indicate significant differences according to Duncan's test ($n = 3$, $p < 0.05$).

plant tissues by TEM. Most NPs accumulated in the intercellular regions of roots (Fig. 2a–d), suggesting the existence of an apoplastic pathway for NPs in plant roots. A large number of studies have proposed the apoplastic pathway in which NPs first contact the root surface and then pass through the intercellular space associated with the outer apoplast without crossing the cell membrane.[23] The lack of detection of NPs in the leaves (Fig. 2e and f) implies that NP translocation from roots to leaves was likely limited.

Interestingly, the size of NPs in the roots was smaller than in the stock suspension (Fig. 2g). The roots that had been exposed to NP alone were also analyzed for a comparison, and there was no statistically significant difference in the particle size between NP-treated roots and stock suspension (Fig. S4a and b†), which is consistent with a previous study that demonstrated the plant accumulation of NPs.[18] Therefore, the reduced particle size within roots of Cd/NP-treated plant provided evidence of NP degradation under combined stress conditions. It is important to note that due to technical limitations (*i.e.*, practical difficulties to separate the NPs from soil), the size of the residual NPs was measured in MS media after removing plants, while the uptake experiment was performed with plants grown in soils. We discovered the presence of much smaller NPs (by 60–80% of their original diameter) in hydroponics compared to in roots (Fig. 2g and S5†), because NP degradation can be additionally accelerated by the radicals generated from photolysis of organic acid exudates in a hydroponic system.[37]

Further evidence that NP degradation could be occurring in planted soil system was provided by the identification of PS degradation byproducts in soil samples using GC-MS (Table S2†). The untreated rhizosphere contained the low levels of styrene derivatives that occurred naturally.[38] The concentrations of PS-derived oligomers were higher in the Cd/NP-treated rhizosphere than in the NP only-treated rhizosphere (Fig. 2h), which indicates that NP degradation was specifically promoted in the rhizosphere exposed to a combination of NP and Cd. These findings provide a plausible explanation for the observed higher accumulation of Cd in the plants treated with Cd/NP than in those treated with Cd alone, as aging has been noted as an important factor enhancing the heavy metal adsorption capacity of MPs.[39] Together, the altered NPs can be taken up by plants after partial degradation in the rooting zone of *A. thaliana* during stress combination.

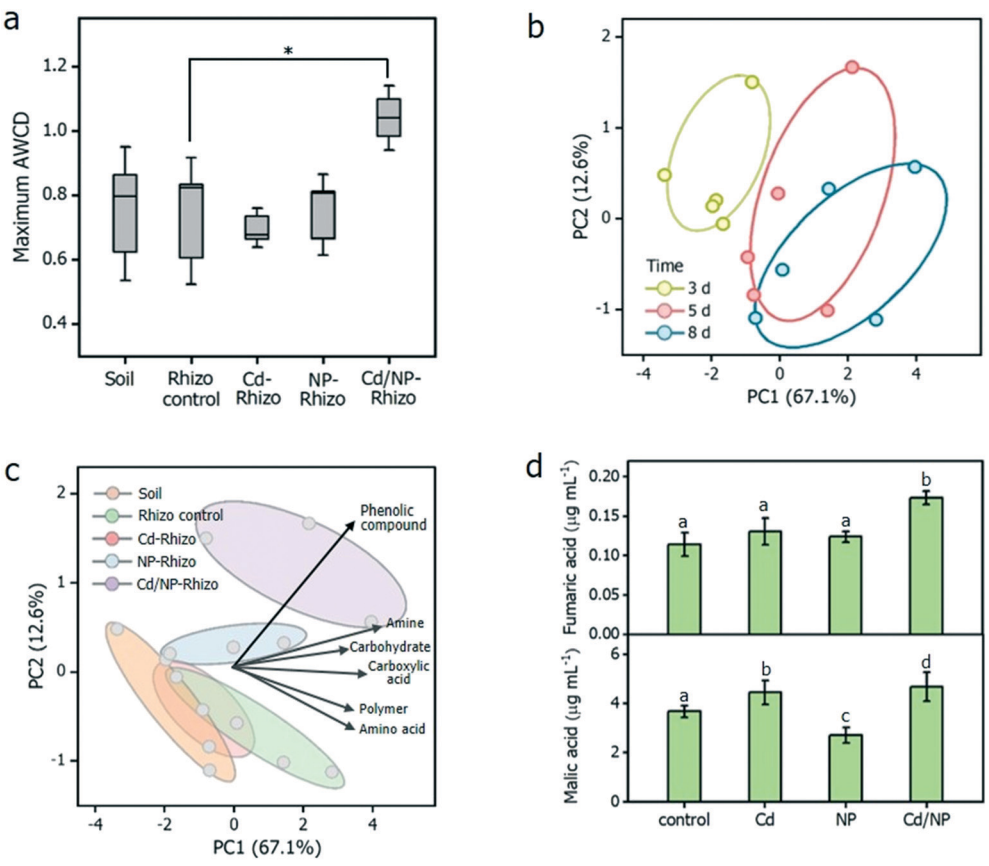

Fig. 3 (a) Carbon metabolic activities reflected by maximum AWCD (*$p < 0.05$). (b) PCA based on carbon source utilization of bacterial communities at each incubation time point. (c) PCA based on the carbon source utilization of bacterial communities under different stress treatments. (d) Quantification of fumaric and malic acids in root exudates of control and treated plants. Averages and standard deviations are shown ($n = 3$); significant differences are indicated by different letters ($p < 0.05$).

Abiotic stress-induced variations in metabolic traits and structure of root-inhabiting bacterial community

If the rhizosphere bacterial community plays a role in degrading NPs, a treatment of Cd and NP stresses might affect both metabolic function and structure of bacterial community assembly. To address this possibility, we sought to quantify the metabolic potentials of control and treated rhizosphere microbiome in respect to carbon metabolism using Biolog EcoPlates. No significant differences were observed in the microbial metabolic activities reflected by the maximum average substrate utilization (maximum average well color development (AWCD)) between control and Cd or NP-exposed groups (Fig. 3a and S6a†). Counter to our expectation, however, the community treated with Cd/NP exhibited the higher maximum AWCD compared to control, indicating that a combined exposure to Cd and NP boosts the overall metabolic activity of rhizosphere bacteria.

The substrates in the EcoPlates represented six carbon-based components, including carbohydrates, carboxylic acids, amino acids, amines, polymers, and phenolic compounds (Table S3 and Fig. S6b†). A principal component analysis (PCA) of rhizosphere metabolic capacity on the basis of exposure period and different stress treatment revealed a clear segregation of bacterial communities in treated and control groups (Fig. 3b and c). After NP and/or Cd exposure, the metabolic profile after 3 days of incubation of the Ecoplates was distinct from those after 5 and 8 days of incubation, as the bacterial community in the early stage of adapting to stress exhibits a trade-off between maintenance energy requirement and metabolic efficiency.[40] At which saturation of carbon utilization was reached (5 and 8 days), we found that phenolic compounds were more preferentially consumed by the community, especially subjected to Cd/NP exposure. Some phenolic compounds such as benzoic acid and 3-vinylcatechol, are known products of the styrene oxidation pathway in soils.[41] This observation of phenolic substrate preference corroborates our finding that PS degradation products such as styrene dimers and trimers, are identified in Cd/NP-exposed rhizosphere soil of *A. thaliana* (Fig. 2h).

Plant roots exude various metabolites (*e.g.* sugars, amino acids and organic acids) as a defense mechanism against external stimuli, and the composition and extent of plant exudates may induce the metabolic reprogramming of rhizosphere bacterial communities;[42] thus, we determined the root exudation of low-molecular-weight organic acids (LMWOAs) from 1 day-treated *A. thaliana* with NP and/or Cd. Malic and fumaric acids were dominant OAs in the root exudates of treated plants, and these were significantly enriched in the Cd/NP-treated sample (Fig. 3d and S7†). Although we were able to detect a notable increase in particular OAs in our study, it should be noted that it represents only a small fraction of the total exudates. These results support our hypothesis that greater exudation of organic acids from the Cd/NP-treated plants may alter the metabolic activity of rhizosphere microbiome that are able to degrade and/or consume NPs and their degradation products as carbon sources, prompting us to explore whether a

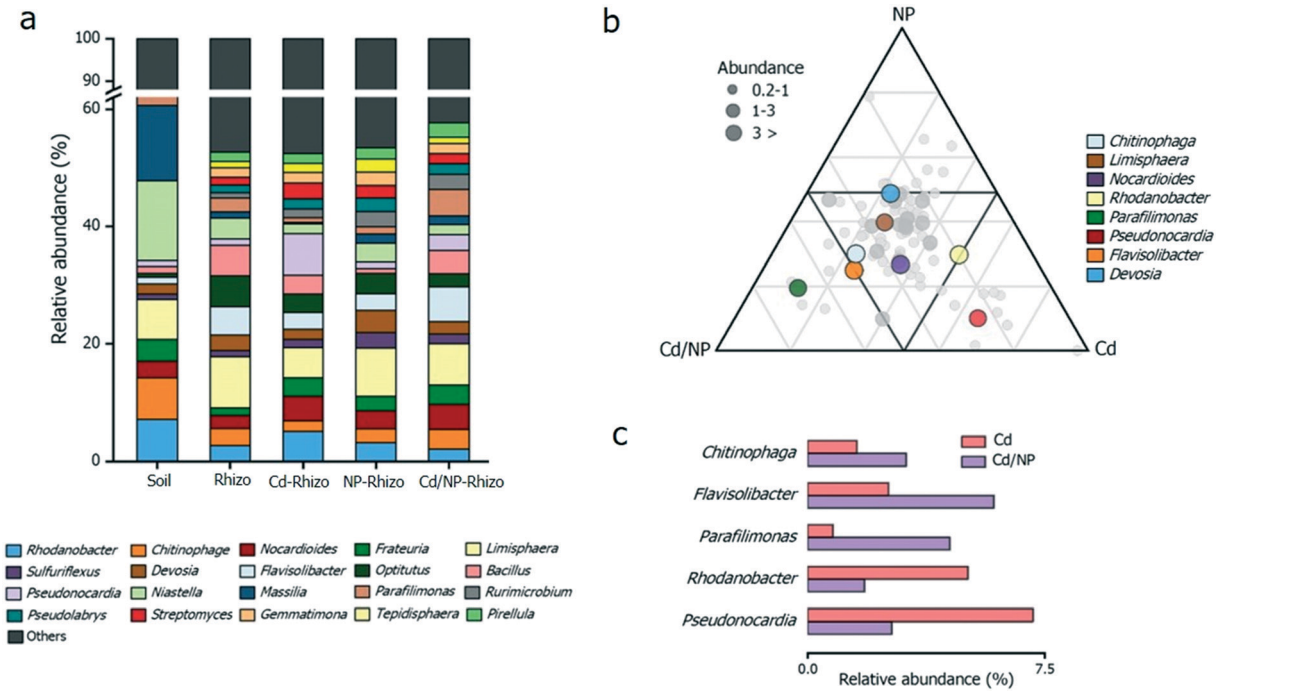

Fig. 4 (a) Average relative abundance in each root microbiome at genus level. Classified genera with relative abundances >0.2% are indicated. (b) Ternary plot of genera with average relative abundance >0.2% enriched in the rhizosphere soils exposed to Cd, NP and Cd/NP. The size of the circle indicates the relative abundance for that genus. Genera with relative abundance >3% are colored. (c) Relative abundance of selected genera in bacterial community subjected to Cd or Cd/NP treatments.

relationship between carbon substrate uptake traits and bacterial community shifts existed.

The bacterial community structure in control and NP- and/or Cd-treated rhizosphere soils was characterized through 16S rRNA gene sequencing. Although treated and control rhizosphere soils had similar numbers of operational taxonomic units (OTUs) (669–679 *vs.* 688), the bacterial diversity within the samples was different, disclosing an increase in Shannon index upon treatment (Fig. S8†). All samples were dominated by bacteria belonging to the phyla Bacteroidetes and Proteobacteria (Fig. S9†). We found that many species at genus level were shared among the samples, but several genera were specifically enriched in Cd- or Cd/NP-treated samples (Fig. 4a and b). For example, Cd-resistant genera such as *Rhodanobacter* and *Pseudonocardia* were found in larger abundances in the Cd-exposed community.[43,44] Compared to control, the Cd/NP-exposed bacterial community showed notable increases in relative abundance in the genera *Chitinophaga*, *Parafilimonas*, and *Flavisolibacter* that have been found in aromatic-impacted environments or described to be able to degrade aromatic compounds.[45–47] Notably, the relative abundances of two major genera observed in the Cd-exposed community were significantly depleted in the Cd/NP-exposed community (Fig. 4c), indicating that combined stress treatment induces a unique pattern of gene expression that is not a simple sum of each stress response. These changes in bacterial community compositions, particularly through the niche partitioning of aromatic-degrading bacterial taxa, provide insight into how plant–microbe interactions in the rhizosphere contribute to NP degradation under combined stress condition.

Conclusions

Plants can shape their root microbiome towards a better adaptation to various stress conditions. This study demonstrates the plant uptake of fragmented NPs that are generated by biologically facilitated degradation in the rhizosphere. We suggest that organic acid exudate-induced enrichment/selection of root-specific bacteria with metabolic preferences for aromatic compounds may facilitate the NP degradation. Our finding that much smaller NPs and more Cd are accumulated in the plants grown in co-contaminated soils with Cd and NP implies the potential for higher-level human health and ecological effects afforded by ingesting them. Given the widespread distribution of NPs and heavy metals in soils, the NP degradation could be common in agricultural systems. Thus, further research is recommended to examine the transformation of NPs under different stress combinations and its effects on the biological activity of some terrestrial species.

Author contributions

Hakwon Yoon: Investigation, methodology, writing-original draft; Jun-Tae Kim: investigation, methodology; Yoon-Seok Chang: supervision, writing-review & editing; Eun-Ju Kim: conceptualization, supervision, funding acquisition, writing-review & editing.

Conflicts of interest

There are no conflicts of interest to declare.

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References

1 R. Hurley, J. Woodward and J. J. Rothwell, Microplastic contamination of river beds significantly reduced by catchment-wide flooding, *Nat. Geosci.*, 2018, **11**, 251–257.
2 J. A. Ivar do Sul and M. F. Costa, The present and future of microplastic pollution in the marine environment, *Environ. Pollut.*, 2014, **185**, 352–364.
3 M. Bergmann, S. Mützel, S. Primpke, M. B. Tekman, J. Jürg Trachsel and G. Gerdts, White and wonderful? Microplastics prevail in snow from the Alps to the Arctic, *Sci. Adv.*, 2019, **5**, eaax1157.
4 E. Besseling, J. T. K. Quik, M. Sun and A. A. Koelmans, Fate of nano- and microplastic in freshwater systems: A modeling study, *Environ. Pollut.*, 2017, **220**, 540–548.
5 H. S. Auta, C. U. Emenike and S. H. Fauziah, Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions, *Environ. Int.*, 2017, **102**, 165–176.
6 M. C. Rillig, Microplastic in terrestrial ecosystems and the soil?, *Environ. Sci. Technol.*, 2012, **46**, 6453–6454.
7 M. Bläsing and W. Amelung, Plastics in soil: analytical methods and possible sources, *Sci. Total Environ.*, 2018, **612**, 422–435.
8 L. Nizzetto, S. Langaas and M. Futter, Pollution: do microplastics spill on to farm soils?, *Nature*, 2016, **537**, 488.
9 N. Weithmann, J. N. Möller, M. G. J. Löder, S. Piehl, C. Laforsch and R. Freitag, Organic fertilizer as a vehicle for the entry of microplastic into the environment, *Sci. Adv.*, 2018, **4**, 8060.
10 Y. Huang, Q. Liu, W. Jia, C. Yan and J. Wang, Agricultural plastic mulching as a source of microplastics in the terrestrial environment, *Environ. Pollut.*, 2020, **260**, 114096.
11 S. Zhang, J. Wang, X. Liu, F. Qu, W. Wang, X. Wang, Y. Li and Y. Sun, Microplastics in the environment: A review of

analytical methods, distribution, and biological Effects, *TrAC, Trends Anal. Chem.*, 2019, **111**, 62–72.

12 N. Altangerel, G. O. Ariunbold, C. Gorman, M. H. Alkahtani, E. J. Borrego, D. Bohlmeyer, P. Hemmer, M. V. Kolomiets, J. S. Yuan and M. O. Scully, In vivo diagnostics of early abiotic plant stress response via Raman spectroscopy, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, 3393–3396.

13 P. Ahmad and M. N. V. Prasad, *Abiotic Stress Responses in Plants*, Springer-Verlag New York, New York, 2012.

14 S. I. Zandalinas, Y. Fichman, A. R. Devireddy, S. Sengupta, R. K. Azad and R. Mittler, Systemic signaling during abiotic stress combination in plants, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 13810–13820.

15 C. H. Haney, B. S. Samuel, J. Bush and F. M. Ausubel, Associations with rhizosphere bacteria can confer an adaptive advantage to plants, *Nat. Plants*, 2015, **1**, 15051.

16 L. Philippot, J. M. Raaijmakers, P. Lemanceau and W. H. van der Putten, Going back to the roots: the microbial ecology of the rhizosphere, *Nat. Rev. Microbiol.*, 2013, **11**, 789–799.

17 B. Boots, C. W. Russell and D. S. Green, Effects of microplastics in soil ecosystems: Above and below ground, *Environ. Sci. Technol.*, 2019, **53**, 11496–11506.

18 X.-D. Sun, X.-Z. Yuan, Y. Jia, L.-J. Feng, F.-P. Zhu, S.-S. Dong, J. Liu, X. Kong, H. Tian, J.-L. Duan, Z. Ding, S.-G. Wang and B. Xing, Differentially charged nanoplastics demonstrate distinct accumulation in *Arabidopsis thaliana*, *Nat. Nanotechnol.*, 2020, **15**, 755–760.

19 Y. Qi, X. Yang, A. M. Pelaez, E. H. Lwanga, N. Beriot, H. Gertsen, P. Garbeva and V. Geissen, Macro- and micro-plastics in soil-plant system: Effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth, *Sci. Total Environ.*, 2018, **645**, 1048–1056.

20 L. Giorgetti, C. Spanò, S. Muccifora, S. Bottega, F. Barbieri, L. Bellani and M. R. Castiglione, Exploring the interaction between polystyrene nanoplastics and *Allium cepa* during germination: Internalization in root cells, induction of toxicity and oxidative stress, *Plant Physiol. Biochem.*, 2020, **149**, 170–177.

21 Z. Li, R. Li, Q. Li, J. Zhou and G. Wang, Physiological response of cucumber (*Cucumis sativus L.*) leaves to polystyrene nanoplastics pollution, *Chemosphere*, 2020, **255**, 127041.

22 Y. M. Lozano and M. C. Rillig, Effects of microplastic fibers and drought on plant communities, *Environ. Sci. Technol.*, 2020, **54**, 6166–6173.

23 J. Lv, P. Christie and S. Zhang, Uptake, translocation, and transformation of metal-based nanoparticles in plants: Recent advances and methodological challenges, *Environ. Sci.: Nano*, 2019, **6**, 41–59.

24 L. Li, Y. Luo, R. Li, Q. Zhou, W. J. G. M. Peijnenburg, N. Yin, J. Yang, C. Tu and Y. Zhang, Effective uptake of submicrometre plastics by crop plants *via* a crack-entry mode, *Nat. Sustain.*, 2020, **3**, 927–937.

25 A. D. Dwivedi, H. Yoon, J. P. Singh, K. H. Chae, D. S. Hwang and Y.-S. Chang, Uptake, distribution, and transformation of zerovalent iron aanoparticles in the edible plant *Cucumis sativus*, *Environ. Sci. Technol.*, 2018, **52**, 10057–10066.

26 C. Huang, T. Xia, J. Niu, Y. Yang, S. Lin, X. Wang, G. Yang, L. Mao and B. Xing, Transformation of ^{14}C-labeled graphene to $^{14}CO_2$ in the shoots of a rice plant, *Angew. Chem.*, 2018, **130**, 9907–9911.

27 A. A. de Souza Machado, W. Kloas, C. Zarfl, S. Hempel and M. C. Rillig, Microplastics as an emerging threat to terrestrial ecosystems, *Glob. Chang. Biol.*, 2018, **24**, 1405–1416.

28 E.-L. Ng, E. H. Lwanga, S. M. Eldridge, P. Johnston, H.-W. Hu, V. Geissen and D. Chen, An overview of microplastic and nanoplastic pollution in agroecosystems, *Sci. Total Environ.*, 2018, **627**, 1377–1388.

29 E. Chavez, Z. L. He, P. J. Stoffella, R. S. Mylavarapu, Y. C. Li and V. C. Baligar, Chemical speciation of cadmium: An approach to evaluate plant-available cadmium in Ecuadorian soils under cacao production, *Chemosphere*, 2016, **150**, 57–62.

30 D. Kumar, M. A. Yusuf, P. Singh, M. Sardar and N. B. Sarin, Histochemical detection of superoxide and H_2O_2 accumulation in *Brassica juncea* seedlings, *Bio-Protoc.*, 2014, **4**, e1108.

31 B. G. Kwon, K. Koizumi, S.-Y. Chung, Y. Kodera, J.-O. Kim and K. Saido, Global styrene oligomers monitoring as new chemical contamination from polystyrene plastic marine pollution, *J. Hazard. Mater.*, 2015, **300**, 359–367.

32 C. Yang, W. Zhang, R. Liu, Q. Li, B. Li, S. Wang, C. Song, C. Qiao and A. Mulchandani, Phylogenetic diversity and metabolic potential of activated sludge microbial communities in full-scale wastewater treatment plants, *Environ. Sci. Technol.*, 2011, **45**, 7408–7415.

33 R. A. Narang, A. Bruene and T. Altmann, Analysis of phosphate acquisition efficiency in different *Arabidopsis* accessions, *Plant Physiol.*, 2000, **124**, 1786–1799.

34 J. Lian, J. Wu, H. Xiong, A. Zeb, T. Yang, X. Su and W. Liu, Impact of polystyrene nanoplastics (PS NPs) on seed germination and seedling growth of wheat (*Triticum aestivum L.*), *J. Hazard. Mater.*, 2020, **385**, 121620.

35 Y. He, L. Qian, K. Zhou, R. Hu, M. Huang, M. Wang, G. Zhao, Y. Liu, Z. Xu and H. Zhu, Graphene oxide promoted cadmium uptake by rice in soil, *ACS Sustainable Chem. Eng.*, 2019, **7**, 10283–10292.

36 Q. Chen, B. Liu, H. Man, L. Chen, X. Wang, J. Tu, Z. Guo, G. Jin, J. Lou and L. Ci, Enhanced bioaccumulation efficiency and tolerance for Cd(II) in *Arabidopsis thaliana* by amphoteric nitrogen-doped carbon dots, *Ecotoxicol. Environ. Saf.*, 2020, **190**, 110108.

37 C. Wang, Z. Xian, X. Jin, S. Liang, Z. Chen, B. Pan, B. Wu, Y. S. Ok and C. Gu, Photo-aging of polyvinyl chloride microplastic in the presence of natural organic acids, *Water Res.*, 2020, **183**, 116082.

38 B. J. Finkle, Soil humic acid as a hydroxypolystyrene: A biochemical hypothesis, *Nature*, 1965, **207**, 604–605.

39 R. Mao, M. Lang, X. Yu, R. Wu, X. Yang and X. Guo, Aging mechanism of microplastics with UV irradiation and its effects on the adsorption of heavy metals, *J. Hazard. Mater.*, 2020, **393**, 122515.

40 A. A. Malik, J. Puissant, K. M. Buckeridge, T. Goodall, N. Jehmlich, S. Chowdhury, H. S. Gweon, J. M. Peyton, K. E. Mason, M. van Agtmaal, A. Blaud, I. M. Clark, J. Whitaker, R. F. Pywell, N. Ostle, G. Gleixner and R. I. Griffiths, Land use driven change in soil pH affects microbial carbon cycling processes, *Nat. Commun.*, 2018, **9**, 3591.

41 D. Tischler, *Microbial Styrene degradation*, Springer International Publishing, New York, 2015.

42 K. Zhalnina, K. B. Louie, Z. Hao, N. Mansoori, U. N. da Rocha, S. Shengjing Shi, H. Cho, U. Karaoz, D. Loqué, B. P. Bowen, M. K. Firestone, T. R. Northen and E. L. Brodie, Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly, *Nat. Microbiol.*, 2018, **3**, 470–480.

43 S. Ishikawa, Mechanisms of cadmium accumulation in rice grains and molecular breeding for its reduction, *J. Soil Sci. Plant Nutr.*, 2020, **66**, 28–33.

44 S. Truyens, B. Beckers, S. Thijs, N. Weyens, A. Cuypers and J. Vangronsveld, Cadmium-induced and trans-generational changes in the cultivable and total seed endophytic community of *Arabidopsis thaliana*, *Plant Biol.*, 2018, **18**, 376–381.

45 M. Wang, C. Sha, J. Wu, J. Su, J. Wu, Q. Wang, J. Tan and S. Huang, Bacterial community response to petroleum contamination in brackish tidal marsh sediments in the Yangtze River Estuary, China, *J. Environ. Sci.*, 2021, **99**, 160–167.

46 X. Su, S. Zhang, R. Mei, Y. Zhang, M. Z. Hashmi, J. Liu, H. Lin, L. Ding and F. Sun, Resuscitation of viable but non-culturable bacteria to enhance the cellulose-degrading capability of bacterial community in composting, *Microb. Biotechnol.*, 2018, **11**, 527–536.

47 Z. Lin, Z. Zhen, L. Ren, J. Yang, C. Luo, L. Zhong, H. Hu, Y. Liang, Y. Li and D. Zhang, Effects of two ecological earthworm species on atrazine degradation performance and bacterial community structure in red soil, *Chemosphere*, 2018, **196**, 467–475.

