Earthworms neutralize the influence of components of particulate pollutants on soil extracellular enzymatic functions in subtropical forests

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Human activities are increasing the input of atmospheric particulate pollutants to forests. The components of particulate pollutants include inorganic anions, base cations and hydrocarbons. Continuous input of particulate pollutants may affect soil functioning in forests, but their effects may be modified by soil fauna. However, studies investigating how soil fauna affect the effects of particulate pollutants on soil functioning are lacking. Here, we investigated how earthworms and the particulate components interact in affecting soil enzymatic functions in a deciduous (*Quercus variabilis*) and a coniferous (*Pinus massoniana*) forest in southeast China. We manipulated the addition of nitrogen (N, ammonium nitrate), sodium (Na, sodium chloride) and polycyclic aromatic hydrocarbons (PAHs, five mixed PAHs) in field mesocosms with and without *Eisenia fetida*, an earthworm species colonizing forests in eastern China. After one year, N and Na addition increased, whereas PAHs decreased soil enzymatic functions (average Z scores of soil extracellular enzyme activities). Earthworms generally stabilized soil enzymatic functions via neutralizing the effects of N, Na and PAHs addition on enzymatic functions in the deciduous but not in the coniferous forest. Specifically, earthworms neutralized the effects of N and Na addition on soil pH and the effects of the addition of PAHs on soil microbial biomass. Further, both particulate components and earthworms changed the correlations among soil enzymatic and other ecosystem functions in the deciduous forest, but the effects depended on the type of particulate components. Generally, the effects of particulate components and earthworms on soil enzymatic functions were less strong in the coniferous than the deciduous forest. Overall, the results indicate that earthworms stabilize soil enzymatic functions in the deciduous but not the coniferous forest irrespective of the type of particulate components. This suggests that earthworms may neutralize the influence of atmospheric particulate pollutants on ecosystem functions, but the neutralization may be restricted to deciduous forests.

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Abstract

Human activities are increasing the input of atmospheric particulate pollutants to forests. The components of particulate pollutants include inorganic anions, base cations and hydrocarbons. Continuous input of particulate pollutants may affect soil functioning in forests, but their effects may be modified by soil fauna. However, studies investigating how soil fauna affect the effects of particulate pollutants on soil functioning are lacking. Here, we investigated how earthworms and the particulate components interact in affecting soil enzymatic functions in a deciduous (*Quercus variabilis*) and a coniferous (*Pinus massoniana*) forest in southeast China. We manipulated the addition of nitrogen (N, ammonium nitrate), sodium (Na, sodium chloride) and polycyclic aromatic hydrocarbons (PAHs, five mixed PAHs) in field mesocosms with and without *Eisenia fetida*, an earthworm species colonizing forests in eastern China. After one year, N and Na addition increased, whereas PAHs decreased soil enzymatic functions (average Z scores of soil extracellular enzyme activities). Earthworms generally stabilized soil enzymatic functions via neutralizing the effects of N, Na and PAHs addition on enzymatic functions in the deciduous but not in the coniferous forest. Specifically, earthworms neutralized the effects of N and Na addition on soil pH and the effects of the addition of PAHs on soil microbial biomass. Further, both particulate components and earthworms changed the correlations among soil enzymatic and other ecosystem functions in the deciduous forest, but the effects depended on the type of particulate components. Generally, the effects of particulate components and earthworms on soil enzymatic functions were less strong in the coniferous than the deciduous forest. Overall, the results indicate that earthworms stabilize soil enzymatic functions in the deciduous but not the coniferous forest irrespective of the type of particulate components. This suggests that earthworms may neutralize the influence of atmospheric particulate pollutants on ecosystem functions, but the neutralization may be restricted to deciduous forests.

Introduction

Secretion of extracellular enzymes is an important means by which microorganisms regulate nutrient cycling in soil (*Luo, Meng & Gu, 2017*), but their secretion is affected by atmospheric pollutants caused by human activities in particular in urban regions (*Bardgett & Wardle, 2010*; *Lin et al., 2017*). In addition, the secretion and activity of extracellular enzymes is also affected by soil fauna such as earthworms (*Hoang et al., 2016*). The European earthworm species *Eisenia fetida*, common in compost heaps, is increasingly colonizing forests of urban regions in eastern China (*Huang, Zhang & Gao, 2003; Aira, Monroy & Domínguez, 2006*). However, the effect of *E. fetida* on soil extracellular enzyme activity in urban forests especially under the addition of atmospheric pollutants remains elusive (*Walker, 1992; Bardgett & Wardle, 2010*; *Blankinship, Niklaus & Hungate, 2011*). This limits our understanding of the role of these earthworms in urban forests under increasing atmospheric pollutants and hampers the development of bioremediation strategies, e.g. the application of earthworms to restore terrestrial ecosystems contaminated by atmospheric pollutants.

Soil extracellular enzymes are key to the functioning of terrestrial ecosystems as they drive nutrient cycling (*Sinsabaugh, 2010*; *Burns et al., 2013*). For example, litter decomposition needs the catalysis of enzymes such as polyphenol oxidase and cellobiohydrolase, two enzymes driving the decomposition of lignin and cellulose, respectively (*Naseby, Pascual & Lynch, 2000*; *Sinsabaugh, Carreiro & Repert, 2002*). Soil extracellular enzymes can be divided into four types, i.e. enzymes involved in carbon (C), nitrogen (N) and phosphorus (P) cycling as well as enzymes such as oxidases (O) involved in the breakdown of complex molecules (*Kizilkaya et al., 2011*; *Xiao et al., 2018*). Since extracellular enzymes are driving different processes in soil they may serve as indicators for the effect of atmospheric pollutants and soil fauna on ecosystem functions (*Maestre et al., 2012*; *Xiao et al., 2018*; *Liu et al., 2019*).

Industrial emission and vehicular exhaust transported by air result in the deposition of a variety of particulate pollutants into terrestrial ecosystems; the components of these pollutants include inorganic anions, base cations and hydrocarbons (*Chan & Yao, 2008*), for example, NO^{3-}, Na^+ and PAHs (*Ye et al., 2003*). Another important source of particulate pollutants is fugitive dust, *Hao et al*. (2005) reported that fugitive dust and industrial dust contributes about 49% and 28% to particulate matters of < 10 µm diameter (PM10) in Beijing. The use of fertilizers in arable systems and salt on roads increased the NO^{3-} and Na^+ contents of soils (*Tiwari & Rachlin, 2018*), adding to the deposition of fugitive dust. Particulate pollutants are transported by air into the surroundings of cities (*Chan & Yao, 2008*) and enter soils by precipitation (*Anderson & Downing, 2006*). In addition, the canopy of forests may collect considerable amounts of pollutants because of their rough surfaces, which later, via shedding of leaves, enter soils where they may accumulate (*Belis, Offenthaler & Weiss, 2011*). The input and accumulation of these pollutants is likely to affect soil functioning. At low rates, however, the components of particulate pollutants may not affect soil enzyme functions (EFs), e.g. Na is limiting microbial activity in soil of inland forests and Na has been shown to increase soil EFs at low concentration (0.39 g Na m^{-2} y^{-1}). However, at high rates each of N, Na and PAHs detrimentally affect soil EFs, although their effects may vary with the type of soil EFs (*Muckian et al., 2007; Lin et al., 2017; Xiao et al., 2018; Ji et al., 2020*). Despite well studied separately, effects of N, Na and PAHs addition on soil EFs have not been investigated in concert with earthworms. This, however, is important for understanding the role of earthworms on soil functions with increasing input of the particulate pollutants.

Earthworms affect belowground processes in multiple ways including digging burrows, fragmenting litter and secreting mucus (*Marhan & Scheu, 2006; Szlavecz et al., 2011; Hoang et al., 2016*). *E. fetida* is well known from compost heaps where it strongly affects enzyme activity resulting in the acceleration of the composting process (*Aira, Monroy & Domínguez, 2006*). This species is very active and reproduces rapidly resulting in fast population growth (*Gunya & Masika, 2022*). However, *E. fetida* also lives in natural habitats in southern Europe and increasingly also in eastern China, in particular close to urban areas, where it lives in forest floors as epigeic species *(Pižl, 2002*; *Huang, Zhang & Gao, 2003*; *Lu & Lu, 2015*; *Koubová et al., 2015*; *Geraskina, 2016*). Epigeic earthworms predominantly live in the litter layer where they regulate biochemical processes (*Mclean & Parkinson, 1997*; *Asshoff, Scheu & Eisenhauer, 2010*). Besides, *E. fetida* is able to live in highly contaminated soil and may even contribute to the decontamination of soils (*Rodriguez-Campos et al., 2014*). However, studies investigating effects of *E. fetida* on soil EFs in urban forests are lacking, this is important for understanding the consequence of *E.fetida* colonizing the urban forests.

Previous studies focused on the toxicological effects of environmental pollutants on earthworms (*Nam et al., 2015*), and the bioaccumulation and bioremediation by earthworms *(Rodriguez-Campos et al., 2014*). Only few laboratory studies investigated the mediation of effects of environmental pollutants on soil enzyme activities by earthworms *(Yin et al., 2003*; *Li et al., 2022*), however, the results of these studies may not show the actual effects of earthworms in forests because of the absence of natural ecosystem process and components, for example, litter decomposition process, various fauna and microorganisms. Field mesocosm experiment may be an appropriate way to reveal the actual effects of earthworms on ecosystem functions in forests (*Shao et al., 2017*). As the abundance of earthworms varies among different types of forests, it may be necessary to investigate their effects at varying density (*Cortez, 1998*; *Szlavecz et al., 2011*). Similarly, the effects of N, Na and PAHs on soil functions may vary with forest types (*Lin et al., 2017*; *Ji et al., 2020*; *Yang et al., 2023*). To understand interactions between earthworms and pollutants in different forests at close to natural settings, experimental studies need to mimic the density of earthworms in the respective forests.

For studying interactions between earthworms and particulate pollutants at high input rates into different forests, we performed a field experiment in a deciduous (*Quercus variabilis*) and a coniferous (*Pinus massoniana*) forest with and without the addition of the earthworm species *E. fetida* in southeast China. We hypothesized that (1) the addition of N, Na and PAHs detrimentally affect soil EFs and (2) earthworms promote soil EFs and counteract negative effects of N, Na and PAHs addition on soil EFs.

Materials & Methods

Study sites

We performed a field experiment in deciduous and coniferous subtropical forests in Zijin Mountain close to the city of Nanjing, Southeast China, from April 2018 to May 2019 (32°4′N, 118°51′E). *Q. variabilis* and *P. massoniana* dominated in the deciduous and coniferous forest, respectively, and *Parthenocissus quinquefolia* and *Carex* spp were the dominant ground floor species (*Tian et al., 2018*). Mean annual air temperature and rainfall are 15.4 °C and 1106 mm, respectively. Soil pH ranges from 5.3 to 5.6. The bedrock is sandstone and shale covered by a humus layer rich in organic matter and nutrients, soils are humic cambisols. The deciduous and coniferous forests were located at similar altitudes (65 and 175 m, respectively) and about 900 m away from each other. For more details on the study sites see Table 1.

Experimental design

In each forest, we identified a core area of 30 m × 20 m for performing the field experiment. We investigated the effects of particulate components (control, N, Na, PAHs) and earthworms (with and without) on soil EFs. In each forest, each treatment was replicated four times resulting in 32 experimental units (4 particulate components × 2 earthworm treatments × 4 replicates) comprising individual mesocosms. We first dug up a pit of an area of 0.5 m × 0.5 m to a depth of 0.2 m in each unit. Earthworms, herb seedlings and fine roots were hand-sorted from the excavated litter and soil. Thus, the soil EFs investigated were mainly contributed by soil microorganisms (not roots). Then, we placed a square nylon bag (1 m × 1 m, 0.16 mm mesh size) into the pit and filled back the excavated soil to fit the natural layers. Finally, we used a zipper at the top of the bag to close it and covered it with leaf litter. The mesocosms were spaced at least 3 m from each other and set away from thick roots (for details see Fig. S1).

N, Na and PAHs treatments received 500 mL aqueous solutions of NH_4NO_3, NaCl and PAHs every 35 days, the control treatment received 500 mL distilled water. Solutions and distilled water were sprayed onto the soil surface of mesocosms by using bottles with spray head (Amway 00483, USA). The amount of NH_4NO_3 added was equivalent to 47 kg N ha^{-1} y^{-1}, the average amount of N deposited in the urban region of Nanjing (*Lin et al., 2017*). The amount of NaCl added at a Na mass percentage of 0.5% was equivalent to 39 g Na m^{-2} y^{-1}, which is much higher than the natural input, but in the range occurring close to roadsides in the region of Nanjing (*Jia et al., 2015*). In the PAHs treatment, a mixture of five PAHs including fluoranthene, pyrene, chrysene, benzo[a]pyrene and phenanthrene was added equivalent to a total amount of 1.81 μg g^{-1} dry soil y^{-1}, with individual amounts of 519, 429, 328, 278 and 258 μg kg^{-1} dry soil y^{-1}, respectively (Table 1). The added amount of PAHs resembled the amount deposited to soils in the urban region of Nanjing; the five PAHs used account for 54% of the mass of 16 prioritized PAHs in urban soils of Nanjing and Zijin Mountain (Table 1)(*Wang et al., 2015*). The solution of PAHs was prepared by successively dissolving benzo[a]pyrene, phenanthrene, fluoranthene and pyrene in 2.5 mL dimethyl sulfoxide; chrysene was dissolved in 2.5 mL ethanol, then the ethanol was poured into dimethyl sulfoxide and 495 mL distilled water was added. The N and PAHs treatments doubled the rates of natural deposited compounds of N and PAHs in the study region; for the Na treatment we used a higher level of addition to account for extra input of Na by human activities such as road salt (*Li et al., 2016*; *Tiwari & Rachlin, 2018*). The solutions and water were sprayed onto the litterbags (for details see below and Fig. S1) and soil surface prior to closing the zipper of the nylon bags. Based on earlier studies (*Qasemian et al., 2012*; *Jia et al., 2015*; *Lin et al., 2017*), the amount of particulate components added were assumed to affect soil enzyme activities.

To investigate effects of earthworms at densities close to their abundance in the different forests, we added 60 adult individuals *E. fetida* to each mesocosm of the earthworm treatment in the deciduous and 20 in the coniferous forest resembling total earthworm abundance in these forests as investigated by hand-sorting in 2018 (Table 2). The body length of individuals added was 80 – 100 mm. From the litter layer to 20 cm soil depth, *E. fetida* accounted for 52.5 ± 15.1% and 64.4 ± 19.0% of total earthworm abundance, with their biomass averaging 4.7 ± 1.1 and 2.3 ± 1.1 g m^{-1} in the deciduous and coniferous forests, respectively (mean ± SD). We placed 10 litterbags filled with litter of *Q. variabilis* and *P. massoniana* (5 coarse and 5 fine mesh litterbags with 5 mm and 0.2 mm mesh size, respectively, 8 g dry litter for each coarse bags and 4 g litter for each fine bags) on top of the soil surface of each mesocosms in the deciduous and coniferous forest, respectively. Coarse and fine mesh litterbags were used to investigate the contribution of soil fauna and microorganisms to litter decomposition, respectively (for details see Fig. S1). To validate the earthworm treatment, we dug up the soil of mesocosms, picked earthworms from the mesocosms by hand, counted them and then placed them back during the period from May to August 2019. Soil and litter samples were taken at day 70, 140, 210, 280 and 365 resulting in a total of 640 soil (0 – 5 cm depth) and litter samples.

Soil properties and litter mass loss

Soil cores underneath litterbags were taken to measure soil properties at 0 – 5 cm soil depth.

Fresh soil samples were sieved through 1 mm mesh and then stored at 4℃. Soil moisture was determined gravimetrically. Soil pH was measured in a 1 : 2.5 soil to water solution by using a pH meter (Mettler Toledo, Switzerland). Soil microbial biomass was determined by substrate-induced respiration (SIR) following *Bailey et al. (2002)*, for details see *Lin et al. (2017)*. Nine soil enzyme activities were measured including three C related enzymes (β-1,4-glucosidase, E.C. 3.2.1.21; β-1,4-xylosidase, E.C. 3.2.1.37; cellobiohydrolase, E.C. 3.2.1.91), two N related enzymes (nitrate reductase, E.C. 1.7.99.4; urease, E.C. 3.5.1.5), two P related enzymes (acid phosphatase, E.C. 3.1.3.2; alkaline phosphatase, E.C. 3.1.3.1) and two enzymes processing complex C compounds such as lignocellulose (O enzymes; peroxidase, E.C. 1.11.1.7; polyphenol oxidase, E.C. 1.10.3.2) (*Saiya-Cork, Sinsabaugh & Zak, 2002*; *Lin et al., 2017*). Details on the measurements are given in supplemental material.

Since the effects of particulate components and earthworms on soil EFs were investigated at different times of litter decomposition, the rates of litter mass loss were calculated. The litter materials from the litterbags were cleaned using distilled water and then dried at 60°C for 72 h. Total C and N of the litter was measured at days 70, 210 and 365 using an elemental analyzer (Elemental Vario Micro, Germany). Based on these data, we calculated litter mass, C and N loss and expressed them as percentages of initial.

Statistical analyses

All analyses were performed using R v4.0.5 (*https://www.r-project.org/*). Data of the deciduous and coniferous forest were analyzed separately. We used generalized linear models with Poisson distribution to analyze the abundance and biomass of earthworms at the end of the experiment. When the models were over- or under-dispersed, quasi-Poisson distribution was used.

We used average Z scores of enzyme activities to indicate soil EFs (*Maestre et al., 2012*). Five types of soil EFs were calculated, total EF, EF-C, EF-N, EF-P and EF-O referring to the average Z scores of all nine enzyme activities, three C enzyme activities, two N enzyme activities, two P enzyme activities and two O enzyme activities, respectively. We then used permutational multivariate analysis of variance (RERMANOVA) to analyze soil enzyme activities and linear mixed effects models (LMMs) to analyze each of the five soil EFs, soil moisture, pH and microbial biomass. In PERMANOVA and LMM, particulate components (control, N, Na, PAHs), earthworms (with and without), mesh size (fine and coarse) and time (five sampling dates) were treated as fixed factors. In LMM, mesh size was nested in mesocosms and included as random factor to account for non-independence of litterbags within mesocosms and repeated sampling. We used planned contrasts to evaluate the effect sizes of particulate components, earthworms and mesh size with the control as reference. The difference of estimated marginal means of soil EFs resembles Cohen's d as the standard deviation of the z score is 1. Contrasts of soil moisture, pH and microbial biomass are analogous to log response ratios as the response variables were log (x + 1) transformed to improve normality (*Piovia‐Scott et al., 2019*). We used 'nlme' to fit mixed-effects models and 'emmeans' for planned contrasts.

We used one-way ANOVA and unpaired t-test to analyze the difference in enzyme activities between particulate components and earthworm treatments, respectively. Non-parametric tests, i.e. Kruskal-Wallis test and Mann-Whitney test, were used if the data did not fit normality. Percentages of enzyme activity were calculated as $(|t - c|/c) \times 100\%$,, with t and c the enzyme activities of the treatment and control, respectively. Further, we used principal component analysis (PCA) and Pearson correlations to analyze the influence of particulate components and earthworms on soil enzyme activities, and the correlation between soil EFs and other functions including litter decomposition and soil properties.

Results

Earthworm abundance

The addition of *E. fetida* significantly increased the abundance and biomass of total earthworms as well as the abundance and biomass of *E. fetida* in both the deciduous and coniferous forest (P < 0.001, $Chi^2 > 14.14$, df = 1; Table S1). On average the abundance of total earthworms was increased by factors of 2.7 to 7.4 and by factors of 2.3 to 7.5 in the deciduous and coniferous forest, respectively (Table 2). With *E. fetida* addition, the percentage of dry mass of *E. fetida* to total earthworm dry mass ranged from 54.7% to 99.2% except in the Na addition treatment in the coniferous forest (34.1%). With *E. fetida* addition, the survival rates of *E. fetida* in control, N, Na and PAHs treatments were 12.1%, 11.7%, 7.1% and 12.1% in the deciduous forest and 17.5%, 17.5%, 28.8% and 20.0% in the coniferous forest, respectively (Table 2).

Soil enzymatic functions

In the treatments without addition of earthworms, particulate components significantly increased soil total EF, EF-C and EF-N in the deciduous forest after 280 and 365 days, with the increase being highest in the Na and lowest in the PAHs treatments (Table 3, Figs 1, 2). However, at day 70, Na significantly decreased EF-P and EF-O, PAHs significantly decreased soil total EF and soil EF-C in the deciduous forest. In total, Na increased activities of cellobiohydrolase, β-1,4-xylosidase, nitrate reductase and urease by 27.8%, 32.8%, 57.1% and 63.0%, but decreased activities of alkaline phosphatase and peroxidase by 28.0% and 40.0%, respectively (Figs 3, S7). N increased activities of β-1,4-xylosidase and urease by 25.0% and 24.6%, PAHs decreased activities of cellobiohydrolase, β-1,4-glucosidase and β-1,4-xylosidase by 11.1%, 10.2% and 9.4%, respectively.

Factor analysis showed that in the coniferous forest, although N and Na significantly increased EF-N after 280 and 365 days, the effect of particulate components on soil EFs was weaker than in the deciduous forest (Tables 3, S2, Figs 1, 2). In total, N and Na increased urease activities by 10.0% and 13.9%, respectively (Fig. 3).

In treatments with earthworm addition, both positive and negative effects of particulate components on soil EFs were generally less pronounced in the deciduous than in the coniferous forest (Tables 3, S3, Figs 1, 2). The mitigation of the effect of particulate components by earthworms in the deciduous forest was similar in both coarse and fine mesh bags (Tables 3, S2,

Figs S8, S9). Na increased activities of β-1,4-xylosidase, alkaline phosphatase, cellobiohydrolase, nitrate reductase and urease by 2.7%, 3.4%, 3.8%, 11.9% and 30.0%, respectively, but reduced the activity of peroxidase by 7.6% (Figs 3, S7). N increased activities of urease by 5.5%, but reduced the activity of β-1,4-xylosidase by 6.7%; PAHs increased the activities of β-1,4-xylosidase and β-1,4-glucosidase by 2.7% and 9.6%, respectively. PCA also indicated that earthworms reduced or neutralized the effects of particulate components on total EF irrespective of the type of particulate components in the deciduous forest (Fig. S10).
Generally, the mitigation of effects of particulate components on soil EFs by earthworms was less pronounced in the coniferous than in the deciduous forest (Figs 1, 2, S11). Only in the N and Na treatments earthworms increased urease activity by 7.8% and 9.2%, respectively (Fig. 3).

Soil moisture, pH and microbial biomass

In the treatments without addition of earthworms, the addition of particulate components significantly decreased soil pH in the deciduous forest (Table S5, Fig. 4). Further, the addition of Na and PAHs decreased soil microbial biomass after 70 days in the deciduous forest (Fig. 5).
Soil pH in the control, N, Na and PAHs treatments in the deciduous forest averaged 5.3 ± 0.2, 5.0 ± 0.3, 5.0 ± 0.2 and 4.8 ± 0.2 (means ± SD across mesh size treatments and sampling times, n = 40), respectively (Fig. S12). After 70 days, soil microbial biomass in the control, Na and PAHs treatments averaged 42.8 ± 31.3, 30.5 ± 29.5 and 15.8 ± 15.8 (means ± SD across mesh size treatments, n = 8), respectively (Fig. S13).
In the coniferous forest, the addition of PAHs decreased, but Na addition increased soil pH (Fig. 4). Further, in the coniferous forest the effects of N, Na and PAHs addition on soil microbial biomass were less strong than in the deciduous forest (Fig. 5). Overall, soil pH in the control, Na and PAHs treatments averaged 4.8 ± 0.1, 5.0 ± 0.2 and 4.6 ± 0.2 (means ± SD, n = 40), respectively (Fig. S12).
In the treatments with earthworm addition, the negative effects of N and Na addition on soil pH, and the negative effects of Na and PAHs addition on soil microbial biomass after 70 days were less pronounced in the deciduous than in the coniferous forests (Figs 4, 5). Overall, soil pH in the control, N, Na and PAHs treatments in the deciduous forest averaged 5.2 ± 0.2, 5.1 ± 0.2, 5.2 ± 0.2 and 4.7 ± 0.2 (n = 40) (Fig. S12). After 70 days, soil microbial biomass in the control, Na and PAHs treatments averaged 42.8 ± 31.3, 30.5 ± 29.5 and 15.8 ± 15.8 (means ± SD across mesh size, n = 8), respectively (Fig. S13). In the coniferous forest, soil pH in the control, Na and PAHs treatments averaged 4.8 ± 0.1, 5.0 ± 0.2 and 4.6 ± 0.1 (means ± SD, n = 40), respectively (Fig. S12). In general, the addition of particulate components did not significantly affect soil moisture (Tables S5, S6, Figs. S14, 15).

Correlation between enzymatic functions, litter decomposition and soil properties

In the deciduous forest, particulate components strengthened the correlations between soil EF-C and litter decomposition as well as soil properties, and the same was true for EF-N (Fig. 6). Earthworms decreased the effects of N and Na but increased the effect of PAHs. The addition of

N, Na and PAHs strengthened the correlation between EF-N and litter mass, C and N loss; further, the addition of N strengthened the correlation between EF-C and litter mass, C and N loss. Earthworms weakened the correlations between EF-C and litter mass, C and N loss in the N addition treatment, and the correlations between EF-N and litter mass loss, soil pH, moisture and microbial biomass in the Na addition treatment. Also, earthworms weakened the correlations between EF-N and litter mass loss, C loss and soil microbial biomass in the control without particulate components. Conversely, earthworms strengthened the correlations between EF-N and litter mass, C and N loss in the treatment with addition of PAHs (Fig. 6).
Contrasting the deciduous forest, the effects of particulate components on the correlations between EFs and litter decomposition as well as soil properties were less strong in the coniferous forest (Fig. S16). Although earthworms strengthened the correlations between EF-N and soil pH as well as litter N loss in the Na and PAHs treatments, overall the modification of effects of particulate components on EFs by earthworms was less strong in the coniferous than in the deciduous forest (Fig. S16).

Discussion

We investigated the interactive effects of different particulate components and earthworms on soil EFs in forest ecosystems. To manipulate earthworm treatments, we mixed soil and used nylon bags with 0.16 mm mesh size. Thus, the original soil enzyme hotspots such as worms' burrows and rhizosphere of fine roots were destroyed, and the exchange of nutrients by soil fauna and run-off was partly blocked. This may lead to soil enzyme activity in plots being lower than in natural conditions, but overall the differences in micro-environment between plots were reduced. After one year, N and Na increased, whereas PAHs decreased soil EFs. Earthworms generally stabilized soil EFs and mitigated the detrimental effects of particulate components on EF-C and EF-N irrespective of the type of particulate components in the deciduous forest. Both, effects of particulate components and earthworms on soil EFs in the coniferous were less strong than in the deciduous forest. Earthworms and particulate components changed the correlations between soil EFs and litter decomposition as well as soil parameters with the changes varying with the types of particulate components.

Particulate components significantly affected soil EFs

Without earthworms, PAHs decreased, whereas N and Na increased soil EFs, partly supporting our first hypothesis. Soil EFs are predicted to be sensitive to the addition of particulate components, and benefit, e.g. from increased input of N according to the resource allocation theory (*Sinsabaugh & Moorhead, 1994*; *Allison & Vitousek, 2005*). Conform to these expectations, N and Na increased soil EFs in treatments without earthworms in the deciduous forest. However, the effects of particulate components on soil EFs varied among the types of particulate components studied, with PAHs generally detrimentally affecting soil EFs which is in line with earlier studies (*Klamerus-Iwan et al., 2015*; *Lipińska, Kucharski & Wyszkowska, 2019*). Effects of the addition of N, Na, and PAHs on soil moisture were small in deciduous and

coniferous forests, suggesting that the addition of pollutants may not change the activities of soil macrofauna and therefore also may not affect soil structure (*Brown, 1995*). By contrast, all three particulate components decreased soil pH in the deciduous forest with the effect of PAHs being strongest suggesting that the effects of these components on soil EFs were due to soil acidification. Interestingly, despite decreasing soil pH, N and Na addition increased soil EFs. Previous studies also found N and Na addition to increase soil enzyme activities (*Lin et al., 2017*; *Ji et al., 2020*). Considering the Na limitation in inland forests, Na addition may indirectly increase the energy flow through soil food webs and thereby the activity of soil enzymes (*Kaspari et al., 2009*; *Ji et al., 2020*). The fact that the addition of PAHs did not significantly increase soil EFs may have been due to their low solubility and recalcitrance (*Blakely, Neher & Spongberg, 2002*; *Jonker & van der Heijden, 2007*). It is noteworthy that the three particulate components did not uniformly decrease soil microbial biomass parallel to soil pH suggesting that their effect on EFs was due to modifying extracellular enzymes of specific microorganisms rather than detrimentally affecting the activity of the whole soil microbial community.
Effects of different types of particulate components on ecosystem functions may also vary with the structure of soil food webs (*Berg, Johansson & Meentemeyer, 2000*; *Blakely, Neher & Spongberg, 2002*; *Schwarz, Gocht & Grathwohl, 2011*; *Kaspari et al., 2014*; *Zhang et al., 2016*). Our results indicate that in absence of earthworms, the effects of N, Na and PAHs on soil EF-C and EF-N are stronger than on soil EF-P and EF-O, suggesting that enzymes involved in C and N cycling are more susceptible to particulate components than those involved in EF-P and EF-O. The low response of EF-P to particulate components may have been due to the absence of roots in our mesocosms as rhizosphere bacteria and mycorrhiza are effectively solubilizing inorganic P (*Lladó, López-Mondéjar & Baldrian, 2017*). The observed changes in EF-C and EF-N with addition of particulate components may be mainly due to changes in bacterial activity which have been shown to sensitively respond to particulate components (*Fierer et al., 2012*; *Freedman et al., 2013*; *Frey et al., 2014*). Importantly, the effects of N, Na and PAHs on EFs increased with time of incubation, suggesting that soil microorganisms are resistant against particulate components and only respond if the input of particulate components continues for longer periods of time. However, the increased effects with time may also be related to increased biomass and diversity of bacteria at later stages of litter decomposition (*Voříšková & Baldrian, 2013*; *Purahong et al., 2014*; *Urbanová et al., 2014*; *Tláskal, Voříšková & Baldrian, 2016*).

Earthworms stabilize soil enzymatic functions

Both the beneficial as well as detrimental effects of particulate components on soil EFs diminished or even vanished in presence of earthworms, partly supporting our second hypothesis. The effect of *E. fetida* addition on soil moisture and microbial biomass was small in deciduous and coniferous forests (Figs. 5, S14). As epigeic earthworm species *E. fetida* does not form permanent burrows and therefore its effect on soil porosity is limited (*Brown, 1995*). However, the addition of *E. fetida* increased litter mass loss irrespective of the types of particulate components (*Yang et al., 2023*). Presumably, processing of litter by *E. fetida* facilitated microbial litter decomposition by stimulating the secretion of extracellular enzymes, thereby annihilating beneficial effects of nutrients (*Liu et al., 2019*) and mitigating detrimental effects of PAHs on soil EFs and microbial activity (*Klamerus-Iwan et al., 2015*).

In addition, with the addition of *E. fetida*, the effects of N and Na addition on soil pH were less strong in the deciduous than in the coniferous forest suggesting that earthworms stabilized soil EFs by mitigating effects of N and Na addition. *Wang et al. (2021)* reported that up to 40% of the N deposited in forests may be lost via leaching within three months and this may be promoted by earthworms (*Frelich et al., 2006*). Although *E. fetida* has been shown to promote the binding of toxins such as PAHs to mineral surfaces (*Geissen et al., 2008*; *Rodriguez-Campos et al., 2014*), the addition of *E. fetida* did not mitigate the negative effect of PAHs on soil pH, but the negative effect of PAHs on soil microbial biomass was canceled out after 70 days. This suggests that *E. fetida* in fact mitigated the detrimental effect of PAHs via stimulating the binding of PAHs to mineral surfaces. Our correlation analyses further suggest that earthworms may stabilize soil EFs by affecting multiple ecosystem functions related to litter decomposition, which is consistent with the findings of *Liu et al. (2019)*.

Although not replicated, our results suggest that the effects of earthworms on soil EFs differ between deciduous and coniferous forests. The addition of particulate components changed soil pH rather than soil microbial biomass and soil EFs in the coniferous forest. In the coniferous forest, the content of soil organic matter is low and litter decomposition is slower than in the deciduous forest (*Yang et al., 2023*). Further, correlations between soil EFs and litter decomposition were also less strong in the coniferous than in the deciduous forest. Therefore, the addition of N and Na in the coniferous forest may not have satisfied the nutrient demand of microorganisms to secrete extra extracellular enzymes. Soil microorganisms of coniferous forests have been suggested to secrete more extracellular oxidases such as polyphenol oxidase due to high lignin content of coniferous litter (*Ji et al., 2020*). Thus, the negative effect of PAHs on soil EFs in the coniferous forest may have been less strong because of the PAH-decomposing ability of enzymes related to EF-O (*Hammel, Kalyanaraman & Kirk, 1986*). However, the less pronounced effect of earthworms on soil EFs in the coniferous forest also is related to the fact that particulate components little affected soil EFs in this forest. By contrast, the effect of earthworms on soil EFs in the deciduous forest was mainly in the treatments where particulate components modified EFs. Further, we added a lower number of earthworms to the coniferous than to the deciduous forest which also may have contributed to the less pronounced effect of earthworms in the coniferous compared to the deciduous forest. Previous studies suggested that the effect of earthworms on ecosystem functions weakens with lower earthworm abundance (*Cortez, 1998*; *Szlavecz et al., 2011*). Overall, our results suggest that for stabilizing the functioning of microorganisms, a minimum number of earthworms might be needed.

Conclusions

This study provided novel and detailed insight into how particulate components and soil fauna affect ecosystem functions in interactive ways, although the animal species, i.e. *E. fetida* was suggested not abundant in natural ecosystems such as forests. Our findings suggest that earthworms stabilize soil EFs irrespective of the type of particulate components, but that the mechanisms responsible for this stabilization vary among the types of particulate components. In addition to accelerating litter decomposition, earthworms also neutralized the effects of N and Na addition on soil EFs by affecting soil pH but neutralized the effect of PAHs by affecting soil microbial biomass. The results further suggest that differences in soil microbial composition and earthworm abundance are responsible for the differential effects of particulate components and earthworms on soil EFs in deciduous and coniferous forests. The results highlight the importance of studying the effects of soil fauna and particulate components on ecosystem functions in concert as earthworms may diminish or even cancel out the influence of atmospheric pollutants and stabilize ecosystem functions.

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References

Aira M, Monroy F, Domínguez J. 2006. *Eisenia fetida* (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting. *Microbial Ecology* 52:738–747. DOI: 10.1007/s00248-006-9109-x.

Allison SD, Vitousek PM. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry* 37:937–944. DOI: 10.1016/j.soilbio.2004.09.014.

Anderson KA, Downing JA. 2006. Dry and wet atmospheric deposition of nitrogen, phosphorus and silicon in an agricultural region. *Water, Air, and Soil Pollution* 176:351–374. DOI: 10.1007/s11270-006-9172-4.

Asshoff R, Scheu S, Eisenhauer N. 2010. Different earthworm ecological groups interactively impact seedling establishment. *European Journal of Soil Biology* 46:330–334. DOI: 10.1016/j.ejsobi.2010.06.005.

Bailey VL, Peacock AD, Smith JL, Bolton H. 2002. Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipid fatty acid analysis. *Soil Biology & Biochemistry* 34:1385–1389. DOI: 10.1016/S0038-0717(02)00070-6.

Bardgett RD, Wardle DA. 2010. *Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change*. Oxford University Press.

Belis CA, Offenthaler I, Weiss P. 2011. Semivolatiles in the forest environment: the case of PAHs. *Organic Xenobiotics and Plants: From Mode of Action to Ecophysiology*:47–73.

Berg B, Johansson MB, Meentemeyer V. 2000. Litter decomposition in a transect of Norway spruce forests: substrate quality and climate control. *Canadian Journal of Forest Research* 30:1136–1147. DOI: 10.1139/x00-044.

Blakely JK, Neher DA, Spongberg AL. 2002. Soil invertebrate and microbial communities, and decomposition as indicators of polycyclic aromatic hydrocarbon contamination. *Applied Soil Ecology* 21:71–88. DOI: 10.1016/S0929-1393(02)00023-9.

Blankinship JC, Niklaus PA, Hungate BA. 2011. A meta-analysis of responses of soil biota to global change. *Oecologia* 165:553–565. DOI: 10.1007/s00442-011-1909-0.

Brown GG. 1995. How do earthworms affect microfloral and faunal community diversity? *Plant and Soil* 170:209–231. DOI: 10.1007/BF02183068.

Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A. 2013. Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biology and Biochemistry* 58:216–234. DOI: 10.1016/j.soilbio.2012.11.009.

Chan CK, Yao X. 2008. Air pollution in mega cities in China. *Atmospheric Environment* 42:1–42. DOI: 10.1016/j.atmosenv.2007.09.003.

Cortez J. 1998. Field decomposition of leaf litters: Relationships between decomposition rates and soil moisture, soil temperature and earthworm activity. *Soil Biology & Biochemistry* 30:783–793. DOI: 10.1016/S0038-0717(97)00163-6.

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R. 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal* 6:1007–1017. DOI: 10.1038/ismej.2011.159.
Freedman Z, Eisenlord SD, Zak DR, Xue K, He Z, Zhou J. 2013. Towards a molecular understanding of N cycling in northern hardwood forests under future rates of N deposition. *Soil Biology and Biochemistry* 66:130–138. DOI: 10.1016/j.soilbio.2013.07.010.
Frelich LE, Hale CM, Scheu S, Holdsworth AR, Heneghan L, Bohlen PJ, Reich PB. 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. *Biological Invasions* 8:1235–1245. DOI: 10.1007/s10530-006-9019-3.
Frey SD, Ollinger S, Nadelhoffer K, Bowden R, Brzostek E, Burton A, Caldwell BA, Crow S, Goodale CL, Grandy AS, Finzi A, Kramer MG, Lajtha K, LeMoine J, Martin M, McDowell WH, Minocha R, Sadowsky JJ, Templer PH, Wickings K. 2014. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. *Biogeochemistry* 121. DOI: 10.1007/s10533-014-0004-0.
Geissen V, Gomez-Rivera P, Huerta Lwanga E, Bello Mendoza R, Trujillo Narcias A, Barba Marcias E. 2008. Using earthworms to test the efficiency of remediation of oil-polluted soil in tropical Mexico. *Ecotoxicology and Environmental Safety* 71:638–642. DOI: 10.1016/j.ecoenv.2008.02.015.
Geraskina AP. 2016. The population of earthworms (Lumbricidae) in the main types of dark coniferous forests in Pechora-Ilych Nature Reserve. *Biology Bulletin* 43:819–830. DOI: 10.1134/S1062359016080082.
Gunya B, Masika PJ. 2022. *Eisenia fetida* worm as an alternative source of protein for poultry: a review. *International Journal of Tropical Insect Science* 42:1–8. DOI: 10.1007/s42690-021-00531-6.
Hammel KE, Kalyanaraman B, Kirk TK. 1986. Oxidation of polycyclic aromatic hydrocarbons and dibenzo [p]-dioxins by Phanerochaete chrysosporium ligninase. *Journal of Biological Chemistry* 261:16948–16952. DOI: 10.1016/S0021-9258(19)75982-1.
Hao J, Wang L, Li L, Hu J, Yu X. 2005. Air pollutants contribution and control strategies of energy-use related sources in Beijing. *Science in China, Series D: Earth Sciences* 48:138–146. DOI: 10.1360/05yd0403.
Hoang DTT, Pausch J, Razavi BS, Kuzyakova I, Banfield CC, Kuzyakov Y. 2016. Hotspots of microbial activity induced by earthworm burrows, old root channels, and their combination in subsoil. *Biology and Fertility of Soils* 52:1105–1119. DOI: 10.1007/s00374-016-1148-y.
Huang C, Zhang X, Gao H. 2003. The ecological distribution of earthworms in Maoer Mount forest ecosystem. *Natural Sciences Journal of Harbin Normal University* 19:92–95. [In Chinese]

Ji Y, Li Q, Tian K, Yang J, Hu H, Yuan L, Lu W, Yao B, Tian X. 2020. Effect of sodium amendments on the home-field advantage of litter decomposition in a subtropical forest of China. *Forest Ecology and Management* 468:118148. DOI: 10.1016/j.foreco.2020.118148.

Jia Y, Kong X, Weiser MD, Lv Y, Akbar S, Jia X, Tian K, He Z, Lin H, Bei Z, Tian X. 2015. Sodium limits litter decomposition rates in a subtropical forest: Additional tests of the sodium ecosystem respiration hypothesis. *Applied Soil Ecology* 93:98–104. DOI: 10.1016/j.apsoil.2015.04.012.

Jonker MTO, van der Heijden SA. 2007. Bioconcentration factor hydrophobicity cutoff: An artificial phenomenon reconstructed. *Environmental Science & Technology* 41:7363–7369. DOI: 10.1021/es0709977.

Kaspari M, Clay NA, Donoso DA, Yanoviak SP. 2014. Sodium fertilization increases termites and enhances decomposition in an Amazonian forest. *Ecology* 95:795–800. DOI: 10.1890/13-1274.1.

Kaspari M, Yanoviak SP, Dudley R, Yuan M, Clay NA. 2009. Sodium shortage as a constraint on the carbon cycle in an inland tropical rainforest. *Proceedings of the National Academy of Sciences of the United States of America* 106:19405–19409. DOI: 10.1073/pnas.0906448106.

Kizilkaya R, Karaca A, Turgay OC, Cetin SC. 2011. Earthworm interactions with soil enzymes. In: Karaca A ed. *Biology of Earthworms*. Berlin, Heidelberg: Springer, 141–158. DOI: 10.1007/978-3-642-14636-7_9.

Klamerus-Iwan A, Błońska E, Lasota J, Kalandyk A, Waligórski P. 2015. Influence of oil contamination on physical and biological properties of forest soil after chainsaw use. *Water, Air, & Soil Pollution* 226:389. DOI: 10.1007/s11270-015-2649-2.

Koubová A, Chroňáková A, Pižl V, Sánchez-Monedero MA, Elhottová D. 2015. The effects of earthworms *Eisenia* spp. on microbial community are habitat dependent. *European Journal of Soil Biology* 68:42–55. DOI: 10.1016/j.ejsobi.2015.03.004.

Li H, Wang Q, Yang M, Li F, Wang J, Sun Y, Wang C, Wu H, Qian X. 2016. Chemical characterization and source apportionment of PM2.5 aerosols in a megacity of Southeast China. *Atmospheric Research* 181:288–299. DOI: 10.1016/j.atmosres.2016.07.005.

Li W, Zhang P, Qiu H, Van Gestel CAM, Peijnenburg WJGM, Cao X, Zhao L, Xu X, He E. 2022. Commonwealth of Soil Health: How Do Earthworms Modify the Soil Microbial Responses to CeO2 Nanoparticles? *Environmental Science & Technology* 56:1138–1148. DOI: 10.1021/acs.est.1c06592.

Lin H, He Z, Hao J, Tian K, Jia X, Kong X, Akbar S, Bei Z, Tian X. 2017. Effect of N addition on home-field advantage of litter decomposition in subtropical forests. *Forest Ecology and Management* 398:216–225. DOI: 10.1016/j.foreco.2017.05.015.

Lipińska A, Kucharski J, Wyszkowska J. 2019. Activity of Phosphatases in Soil Contaminated with PAHs. *Water, Air, & Soil Pollution* 230:298. DOI: 10.1007/s11270-019-4344-1.

Liu T, Chen X, Gong X, Lubbers IM, Jiang Y, Feng W, Li X, Whalen JK, Bonkowski M, Griffiths BS, Hu F, Liu M. 2019. Earthworms coordinate soil biota to improve multiple ecosystem functions. *Current Biology* 29:3420-3429.e5. DOI: 10.1016/j.cub.2019.08.045.

Lladó S, López-Mondéjar R, Baldrian P. 2017. Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. *Microbiology and Molecular Biology Reviews* 81:e00063-16. DOI: 10.1128/MMBR.00063-16.

Lu Y-F, Lu M. 2015. Remediation of PAH-contaminated soil by the combination of tall fescue, arbuscular mycorrhizal fungus and epigeic earthworms. *Journal of Hazardous Materials* 285:535–541. DOI: 10.1016/j.jhazmat.2014.07.021.

Luo L, Meng H, Gu J-D. 2017. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. *Journal of Environmental Management* 197:539–549. DOI: 10.1016/j.jenvman.2017.04.023.

Maestre FT, Quero JL, Gotelli NJ, Escudero A, Ochoa V, Delgado-Baquerizo M, García-Gómez M, Bowker MA, Soliveres S, Escolar C. 2012. Plant species richness and ecosystem multifunctionality in global drylands. *Science* 335:214–218. DOI: 10.1126/science.1215442.

Manning P, Saunders M, Bardgett RD, Bonkowski M, Bradford MA, Ellis RJ, Kandeler E, Marhan S, Tscherko D. 2008. Direct and indirect effects of nitrogen deposition on litter decomposition. *Soil Biology and Biochemistry* 40:688–698. DOI: 10.1016/j.soilbio.2007.08.023.

Marhan S, Scheu S. 2006. Mixing of different mineral soil layers by endogeic earthworms affects carbon and nitrogen mineralization. *Biology and Fertility of Soils* 42:308–314. DOI: 10.1007/s00374-005-0028-7.

Mclean MA, Parkinson D. 1997. Changes in structure, organic matter and microbial activity in pine forest soil following the introduction of *Dendrobaena octaedra* (Oligochaeta, Lumbricidae). *Soil Biology and Biochemistry* 29:537–540. DOI: 10.1016/S0038-0717(96)00178-2.

Muckian L, Grant R, Doyle E, Clipson N. 2007. Bacterial community structure in soils contaminated by polycyclic aromatic hydrocarbons. *Chemosphere* 68:1535–1541. DOI: 10.1016/j.chemosphere.2007.03.029.

Nam T-H, Jeon H-J, Mo H, Cho K, Ok Y-S, Lee S-E. 2015. Determination of biomarkers for polycyclic aromatic hydrocarbons (PAHs) toxicity to earthworm (*Eisenia fetida*). *Environmental Geochemistry and Health* 37:943–951. DOI: 10.1007/s10653-015-9706-z.

Naseby DC, Pascual JA, Lynch JM. 2000. Effect of biocontrol strains of Trichoderma on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities. *Journal of Applied Microbiology* 88:161–169. DOI: 10.1046/j.1365-2672.2000.00939.x.

Piovia-Scott J, Yang LH, Wright AN, Spiller DA, Schoener TW. 2019. Pulsed seaweed subsidies drive sequential shifts in the effects of lizard predators on island food webs. *Ecology Letters* 22:1850–1859. DOI: 10.1111/ele.13377.

Pižl V. 2002. Earthworms of the Czech Republic. *Sborník Přírodovědného klubu v U. Hradišti (in Czech) Suppl* 9:1–154.

Purahong W, Schloter M, Pecyna MJ, Kapturska D, Däumlich V, Mital S, Buscot F, Hofrichter M, Gutknecht JLM, Krüger D. 2014. Uncoupling of microbial community structure and function in decomposing litter across beech forest ecosystems in Central Europe. *Scientific Reports* 4:7014. DOI: 10.1038/srep07014.

Qasemian L, Guiral D, Ziarelli F, Van Dang TK, Farnet A-M. 2012. Effects of anthracene on microbial activities and organic matter decomposition in a *Pinus halepensis* litter from a Mediterranean coastal area. *Soil Biology and Biochemistry* 46:148–154. DOI: 10.1016/j.soilbio.2011.12.002.

Rodriguez-Campos J, Dendooven L, Alvarez-Bernal D, Contreras-Ramos SM. 2014. Potential of earthworms to accelerate removal of organic contaminants from soil: A review. *Applied Soil Ecology* 79:10–25. DOI: 10.1016/j.apsoil.2014.02.010.

Saiya-Cork KR, Sinsabaugh RL, Zak DR. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology and Biochemistry* 34:1309–1315. DOI: 10.1016/S0038-0717(02)00074-3.

Schwarz K, Gocht T, Grathwohl P. 2011. Transport of polycyclic aromatic hydrocarbons in highly vulnerable karst systems. *Environmental Pollution* 159:133–139. DOI: 10.1016/j.envpol.2010.09.026.

Shao Y, Zhang W, Eisenhauer N, Liu T, Xiong Y, Liang C, Fu S. 2017. Nitrogen deposition cancels out exotic earthworm effects on plant-feeding nematode communities. *Journal of Animal Ecology* 86:708–717. DOI: 10.1111/1365-2656.12660.

Sinsabaugh RL. 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology and Biochemistry* 42:391–404. DOI: 10.1016/j.soilbio.2009.10.014.

Sinsabaugh R, Carreiro M, Repert D. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry* 60:1–24. DOI: 10.1023/A:1016541114786.

Sinsabaugh R, Moorhead D. 1994. Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. *Soil biology and biochemistry* 26:1305–1311. DOI: 10.1016/0038-0717(94)90211-9.

Szlavecz K, McCormick M, Xia L, Saunders J, Morcol T, Whigham D, Filley T, Csuzdi C. 2011. Ecosystem effects of non-native earthworms in Mid-Atlantic deciduous forests. *Biological Invasions* 13:1165–1182. DOI: 10.1007/s10530-011-9959-0.

Tian K, Kong X, Gao J, Jia Y, Lin H, He Z, Ji Y, Bei Z, Tian X. 2018. Local root status: a neglected bio-factor that regulates the home-field advantage of leaf litter decomposition. *Plant and Soil* 431:175–189. DOI: 10.1007/s11104-018-3757-8.

Tiwari A, Rachlin JW. 2018. A review of road salt ecological impacts. *Northeastern Naturalist* 25:123–142. DOI: 10.1656/045.025.0110.
Tláskal V, Voříšková J, Baldrian P. 2016. Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. *FEMS Microbiology Ecology* 92:fiw177. DOI: 10.1093/femsec/fiw177.
Urbanová M, Šnajdr J, Brabcová V, Merhautová V, Dobiášová P, Cajthaml T, Vaněk D, Frouz J, Šantrůčková H, Baldrian P. 2014. Litter decomposition along a primary post-mining chronosequence. *Biology and Fertility of Soils* 50:827–837. DOI: 10.1007/s00374-014-0905-z.
Voříšková J, Baldrian P. 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. *The ISME Journal* 7:477–486. DOI: 10.1038/ismej.2012.116.
Walker BH. 1992. Biodiversity and ecological redundancy. *Conservation biology* 6:18–23. DOI: 10.1046/j.1523-1739.1992.610018.x.
Wang A, Chen D, Phillips OL, Gundersen P, Zhou X, Gurmesa GA, Li S, Zhu W, Hobbie EA, Wang X, Fang Y. 2021. Dynamics and multi-annual fate of atmospherically deposited nitrogen in montane tropical forests. *Global Change Biology* 27:2076–2087. DOI: 10.1111/gcb.15526.
Wang C, Wu S, Zhou S, Wang H, Li B, Chen H, Yu Y, Shi Y. 2015. Polycyclic aromatic hydrocarbons in soils from urban to rural areas in Nanjing: Concentration, source, spatial distribution, and potential human health risk. *Science of the Total Environment* 527:375–383. DOI: 10.1016/j.scitotenv.2015.05.025.
Xiao W, Chen X, Jing X, Zhu B. 2018. A meta-analysis of soil extracellular enzyme activities in response to global change. *Soil Biology and Biochemistry* 123:21–32. DOI: 10.1016/j.soilbio.2018.05.001.
Yang J, Tian K, Lu J-Z, Kong X, Li Q, Ye R, Zeng X, Cao T, Hu H, Ji Y, Tian X, Scheu S. 2023. *Earthworms increase forest litter mass loss irrespective of deposited compounds -- A field manipulation experiment in subtropical forests*. Preprints. DOI: 10.22541/au.167325400.00229268/v1.
Ye B, Ji X, Yang H, Yao X, Chan CK, Cadle SH, Chan T, Mulawa PA. 2003. Concentration and chemical composition of PM2.5 in Shanghai for a 1-year period. *Atmospheric Environment* 37:499–510. DOI: 10.1016/S1352-2310(02)00918-4.
Yin S, Yang L, Yin B, Mei L. 2003. Nitrification and denitrification activities of zinc-treated soils worked by the earthworm *Pheretima* sp. *Biology and Fertility of Soils* 38:176–180.
Zhang W, Chao L, Yang Q, Wang Q, Fang Y, Wang S. 2016. Litter quality mediated nitrogen effect on plant litter decomposition regardless of soil fauna presence. *Ecology* 97:2834–2843. DOI: 10.1002/ecy.1515.

Table 1(on next page)

Site conditions and litter traits of the deciduous and coniferous forests.

means ± SD. Different letters indicate significant differences; t-test ($p < 0.05$, $n = 5$). Values of soil C, soil N and soil C/N ratio were taken from Tian et al. (2018); values of concentrations of PAHs in soil were taken from Wang et al. (2015).

Table 1

Site conditions	Deciduous	Coniferous
Latitude (°N)	32.0555	32.0635
Longitude (°E)	118.8736	118.8727
Elevation (m a.s.l.)	65	175
Slope (°)	5	15
Soil pH	5.30 ± 0.19a	5.62 ± 0.48a
Soil moisture (%)	24.25 ± 1.86a	21.48 ± 0.40b
Soil C (g kg^{-1})	20.30a	15.40b
Soil N (g kg^{-1})	1.30a	1.10a
Soil C: N	15.52a	14.39a
Soil organic matter (g kg^{-1})	35.00a	26.55b
PAH concentrations (μg kg^{-1})		
Fluoranthene (Flu)	519 ± 726	
Pyrene (Pyr)	429 ± 596	
Chrysene (Chr)	328 ± 395	
Benzo[a]pyrene (BaP)	278 ± 379	
Phenanthrene (Phe)	259 ± 314	
∑16PAHs	3330 ± 4250	
Litter traits	*Quercus variabilis*	*Pinus massoniana*
Lignin (%)	31.20 ± 1.08b	40.60 ± 0.77a
Total C (%)	49.50 ± 0.83b	51.30 ± 1.26a
Total N (%)	1.27 ± 0.07a	0.85 ± 0.08b
Lignin: N	33.31 ± 1.77b	60.53 ± 5.77a
C: N	24.63 ± 2.68b	48.47 ± 6.86a

Table 2(on next page)

Abundance and biomass of earthworms in mesocosms of the deciduous and coniferous forest with and without addition of *Eisenia fetida*.

Means ± SD, n = 4 (n = 5 for initial values).

Table 2

Treatments	Deciduous - Earthworms	Deciduous + Earthworms	Coniferous - Earthworms	Coniferous + Earthworms
Total abundance (number)				
Initial	60.60 ± 9.71		19.60 ± 8.08	
Control	3.67 ± 6.35	10.00 ± 4.69	0.50 ± 0.58	3.75 ± 3.20
N addition	3.75 ± 4.86	15.75 ± 8.73	1.75 ± 2.36	4.00 ± 0.82
Na addition	1.25 ± 1.26	8.00 ± 4.24	4.00 ± 4.62	9.50 ± 5.00
PAHs addition	2.00 ± 2.16	14.75 ± 14.08	1.25 ± 1.50	4.75 ± 1.26
Total biomass (dry mass, g)				
Initial	1.69 ± 0.41		0.81 ± 0.25	
Control	0.04 ± 0.06	0.50 ± 0.37	0.01 ± 0.01	0.08 ± 0.05
N addition	0.07 ± 0.05	0.95 ± 1.02	0.05 ± 0.06	0.11 ± 0.03
Na addition	0.03 ± 0.03	0.30 ± 0.08	0.06 ± 0.07	0.41 ± 0.28
PAHs addition	0.04 ± 0.06	0.97 ± 1.13	0.04 ± 0.07	0.12 ± 0.07
***Eisenia* abundance** (number)				
Initial	31.00 ± 7.18		12.20 ± 5.07	
Control	0	7.25 ± 2.75	0	3.50 ± 2.89
N addition	1.50 ± 1.73	7.00 ± 10.80	1.75 ± 2.36	3.50 ± 1.29
Na addition	0.25 ± 0.50	4.25 ± 3.40	2.00 ± 2.45	5.75 ± 2.63
PAHs addition	0.25 ± 0.50	7.25 ± 9.98	1.00 ± 1.41	4.00 ± 1.41
***Eisenia* biomass** (dry mass, g)				
Initial	1.18 ± 0.28		0.59 ± 0.27	
Control	0	0.36 ± 0.17	0	0.08 ± 0.05
N addition	0.05 ± 0.05	0.52 ± 0.95	0.05 ± 0.06	0.09 ± 0.05
Na addition	0.01 ± 0.02	0.22 ± 0.13	0.04 ± 0.05	0.14 ± 0.07
PAHs addition	0.01 ± 0.01	0.72 ± 0.99	0.04 ± 0.07	0.11 ± 0.07

Table 3 (on next page)

Results of linear mixed-effects models on the effects of particulate components, earthworms, mesh size and time on Z scores of total soil enzymatic activities in deciduous and coniferous forests.

For the F- and P-values of linear mixed-effects models on the effects of the factors studied on Z scores of individual soil enzymatic activities of C, N, P and O in the deciduous and coniferous forests see Table S3 and Table S4, respectively; df, numerator and denominator degrees of freedom.

Table 3

	Deciduous			Coniferous		
	df	F	P	df	F	P
Particulate components (PC)	*3,23*	4.35	**0.014**	*3,24*	1.85	0.166
Earthworms (E)	*1,23*	1.67	0.209	*1,24*	0.58	0.454
Mesh size (M)	*1,23*	11.56	**0.002**	*1,24*	16.96	**<0.001**
Time (T)	*4,184*	30.28	**<0.001**	*4,192*	323.43	**<0.001**
PC × E	*3,23*	5.08	**0.008**	*3,24*	0.29	0.835
PC × M	*3,23*	1.88	0.161	*3,24*	0.26	0.854
E × M	*1,23*	0.02	0.884	*1,24*	0.72	0.404
PC × T	*12,184*	7.94	**<0.001**	*12,192*	4.48	**<0.001**
E × T	*4,184*	3.79	**0.005**	*4,192*	0.98	0.421
M × T	*4,184*	25.18	**<0.001**	*4,192*	16.09	**<0.001**
PC × E × M	*3,23*	6.11	**0.003**	*3,24*	0.70	0.559
PC × E × T	*12,184*	2.38	**0.007**	*12,192*	1.67	0.077
PC × M × T	*12,184*	2.30	**0.009**	*12,192*	3.91	**<0.001**
E × M × T	*4,184*	2.41	0.051	*4,192*	0.37	0.829
PC × E × M × T	*12,184*	1.36	0.190	*12,192*	0.86	0.589

Figure 1

Changes in estimates of Z scores of soil total enzyme activities with time as affected by different types of particulate components and earthworms in deciduous and coniferous forests.

Means with 95% confidence intervals; effect sizes were averaged across mesh size (coarse and fine), n = 8; asterisks indicate significant differences to the control ($P < 0.05$). For the original values of Z scores of soil total enzyme activities see Figure S2.

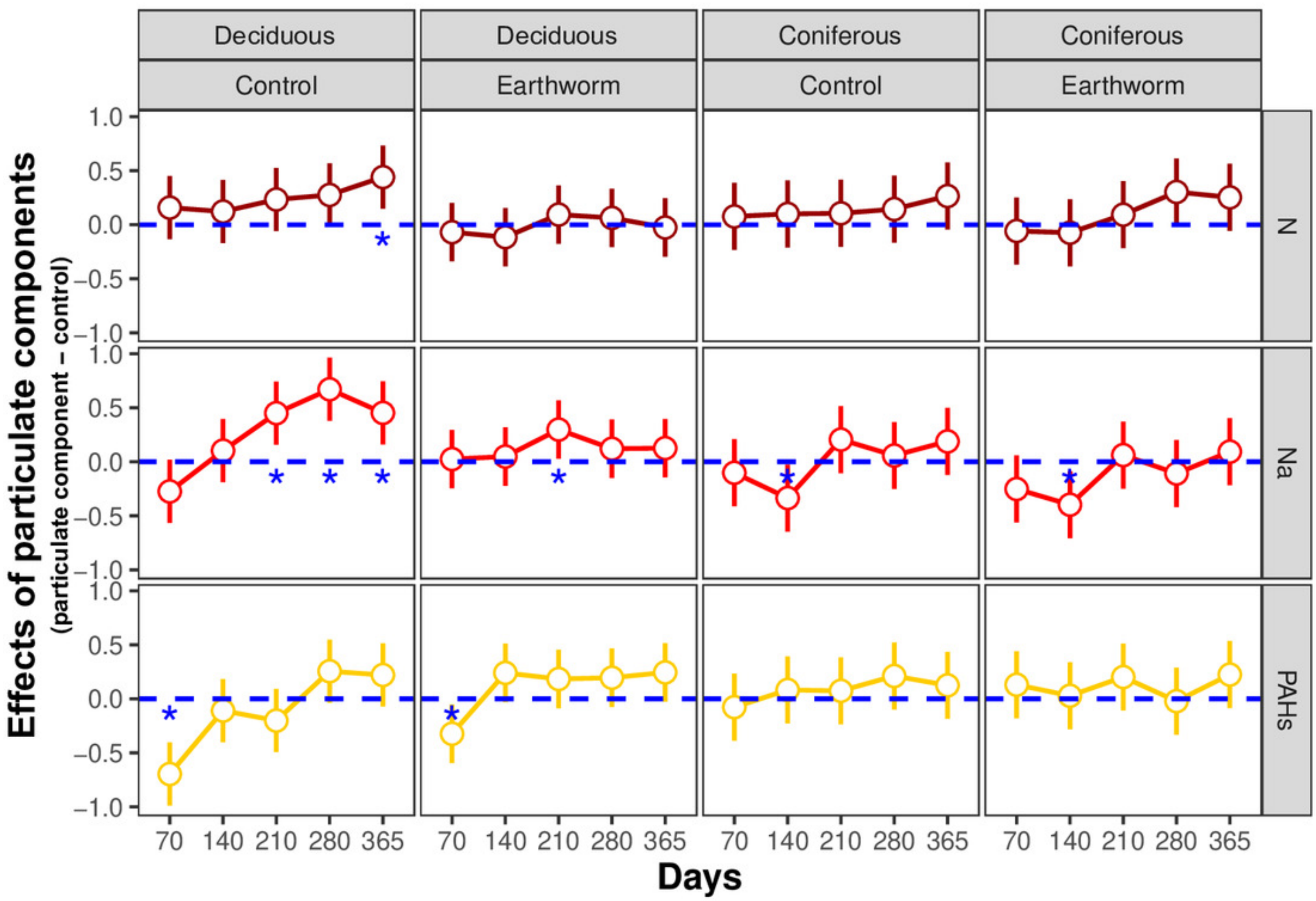


Figure 2

Changes in estimates of Z scores of soil C, N, P enzymes and oxidases activities with time as affected by different types of particulate components and earthworms in deciduous and coniferous forests.

EF-C refers to three soil carbon enzyme activities, i.e. β-1,4-glucosidase, β-1,4-xylosidase and cellobiohydrolase; EF-N refers to two soil nitrogen enzyme activities, i.e. nitrate reductase and urease; EF-P refers to two soil phosphorus enzyme activities, i.e. acid and alkaline phosphatases; EF-O refers to two soil oxidases activities, i.e. peroxidase and polyphenol oxidase; means with 95% confidence intervals; effect sizes were averaged across mesh size (coarse and fine), n = 8; asterisks indicate significant differences to the control ($P < 0.05$). For original values of Z scores of soil carbon, nitrogen, phosphorus and oxidase enzyme activities see Figs S3, S4, S5, S6, respectively.

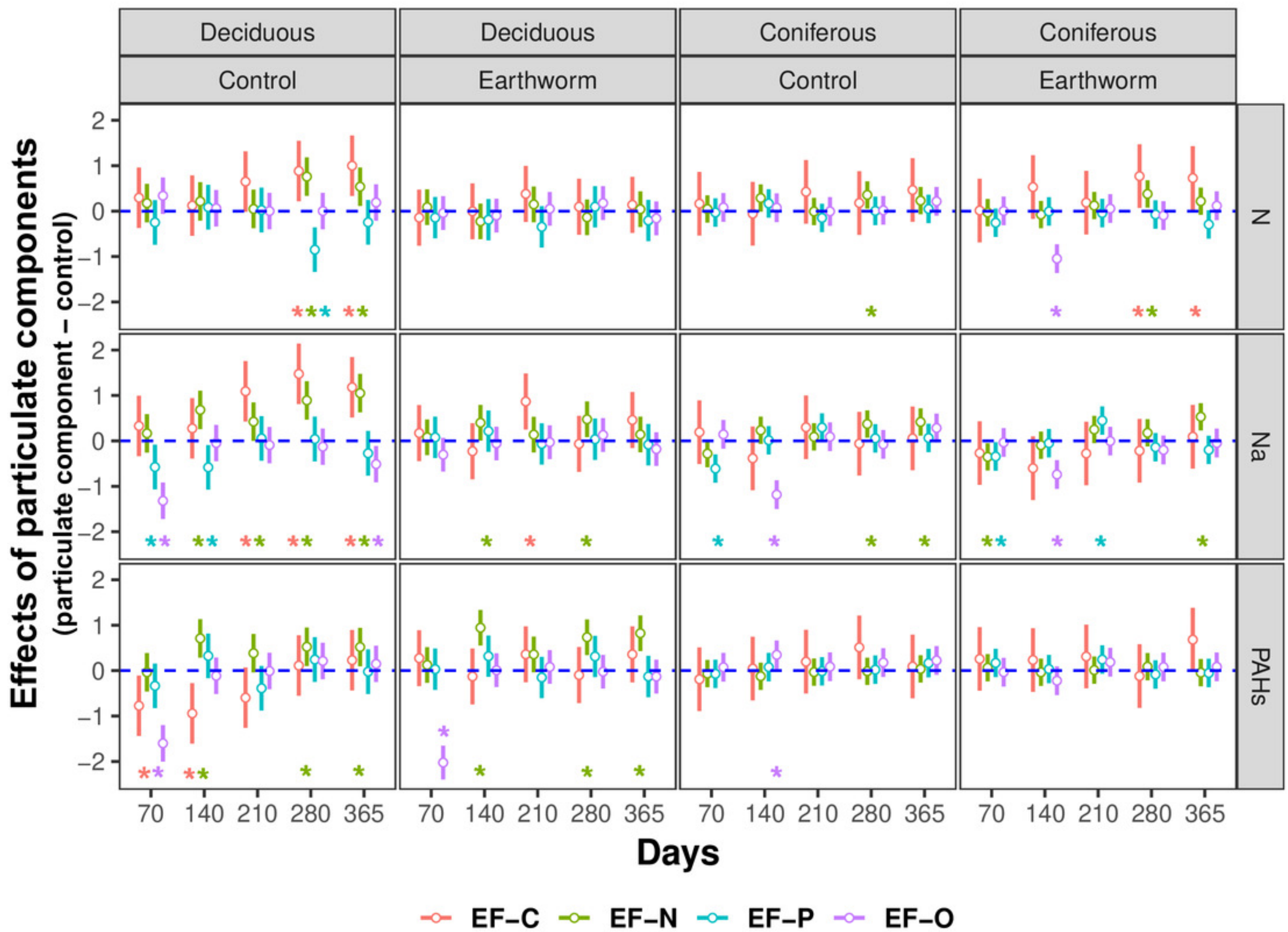

Deciduous
Control
Deciduous
Earthworm
Coniferous
Control
Coniferous
Earthworm
N
Na
PAHs
Effects of particulate components
(particulate component – control)
Days
70 140 210 280 365
EF−C
EF−N
EF−P
EF−O

Figure 3

Changes in carbon and nitrogen enzyme activities as affected by different types of particulate components and earthworms in the soil underneath litterbags in deciduous and coniferous forests.

Means ± SE, values were averaged across sampling dates (70, 140, 210, 280, 365 day) and mesh size (coarse and fine), n = 40; lower case and capital letters indicated the difference in enzyme activities in deposited compound treatments (Control, N, Na and PAHs) and in earthworm treatments (without, with), respectively. For original values of soil phosphorus and oxidase enzyme activities see Fig. S7.

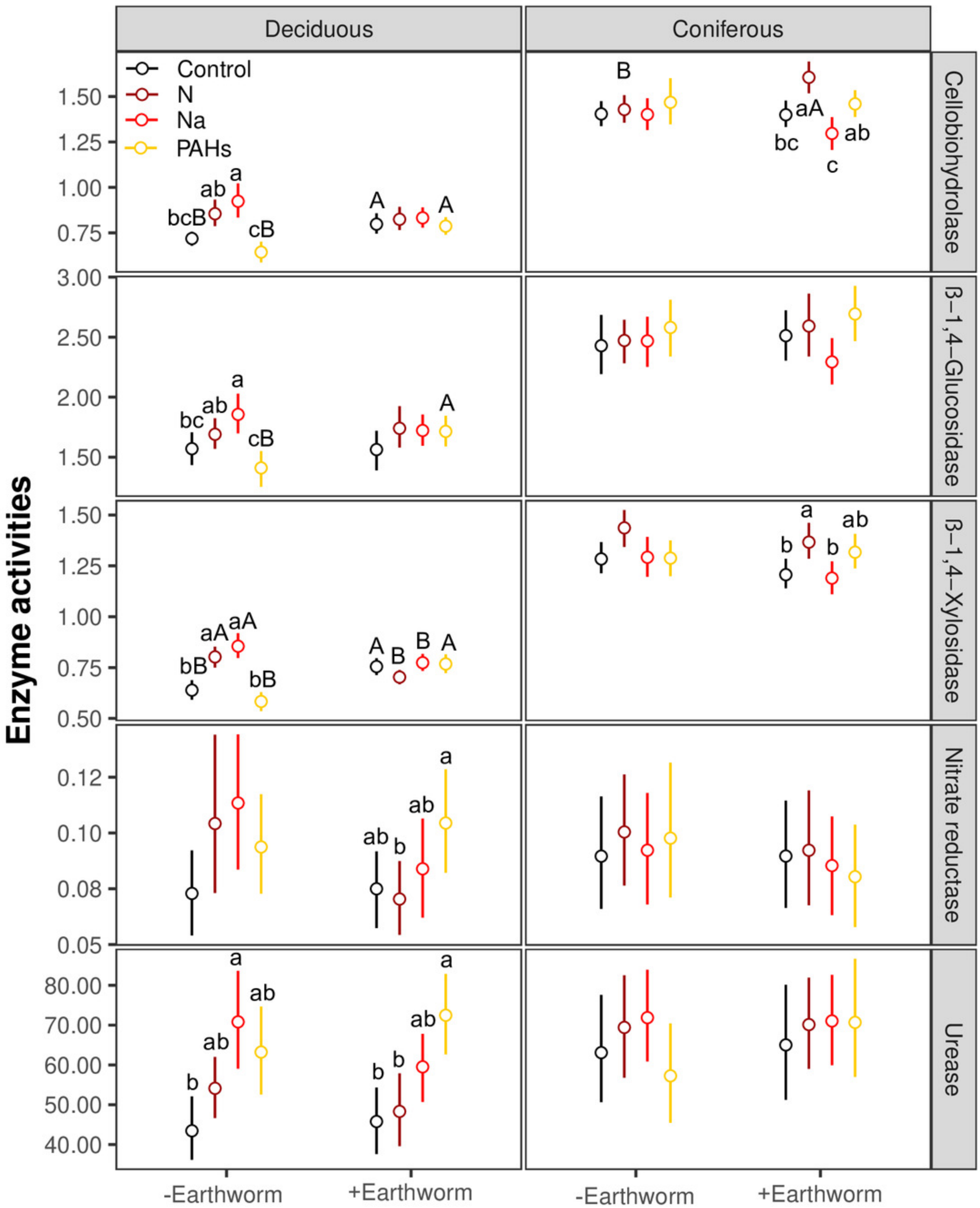

Deciduous
Coniferous
Control
N
Na
PAHs
Cellobiohydrolase
β−1,4−Glucosidase
β−1,4−Xylosidase
Nitrate reductase
Urease
Enzyme activities
-Earthworm
+Earthworm

Figure 4

Changes in estimates of soil pH with time as affected by different types of particulate components and earthworms in deciduous and coniferous forests.

Means with 95% confidence intervals; effect sizes were averaged across mesh size (coarse and fine), n = 8; asterisks indicate significant differences to the control (P < 0.05). For the original values of soil pH see Figure S12.

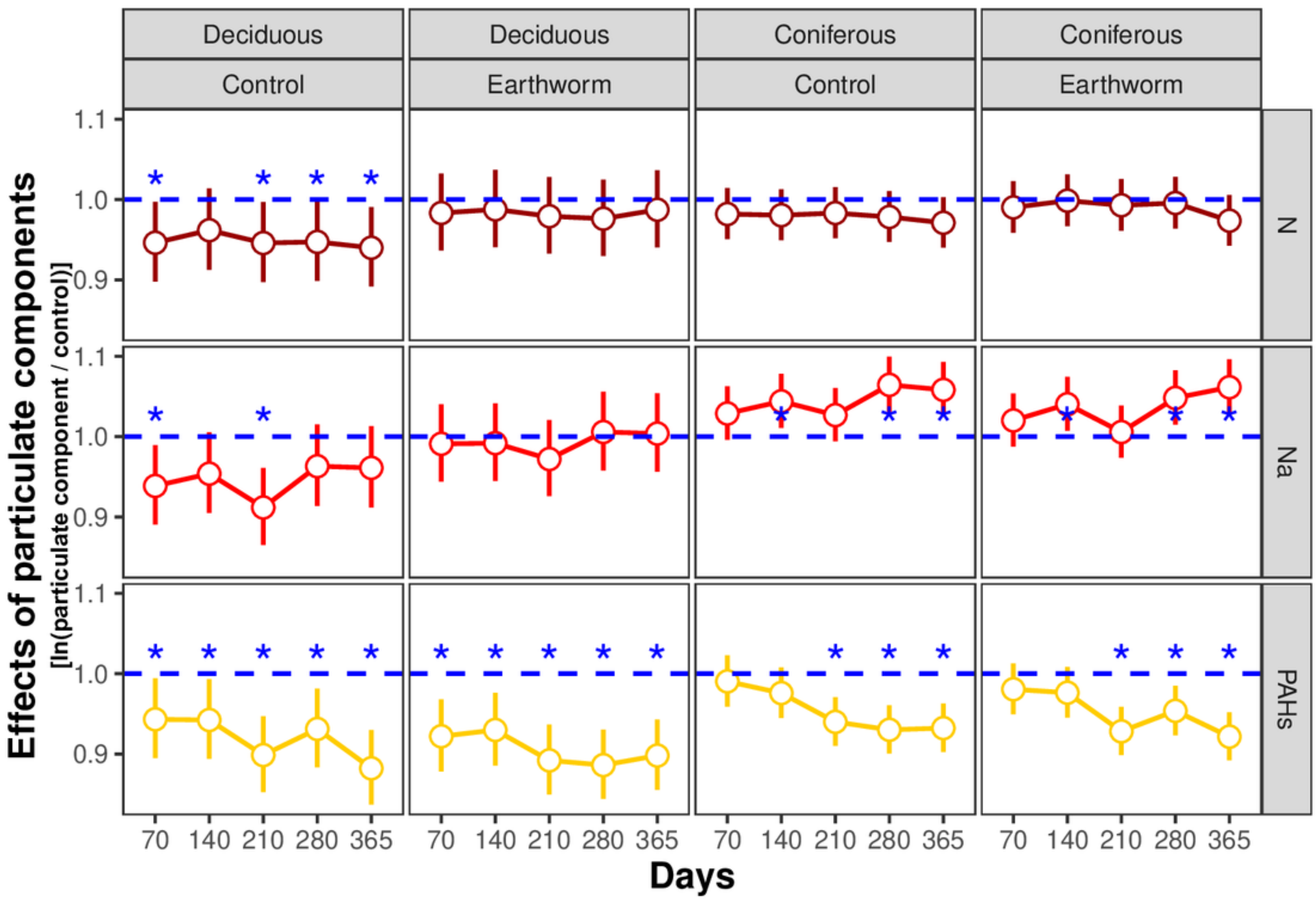


Figure 5

Changes in estimates of soil microbial biomass with time as affected by different types of particulate components and earthworms in deciduous and coniferous forests.

Means with 95% confidence intervals; effect sizes were averaged across mesh size (coarse and fine), n = 8; asterisks indicate significant differences to the control (P < 0.05). For the original values of soil microbial biomass see Figure S13.

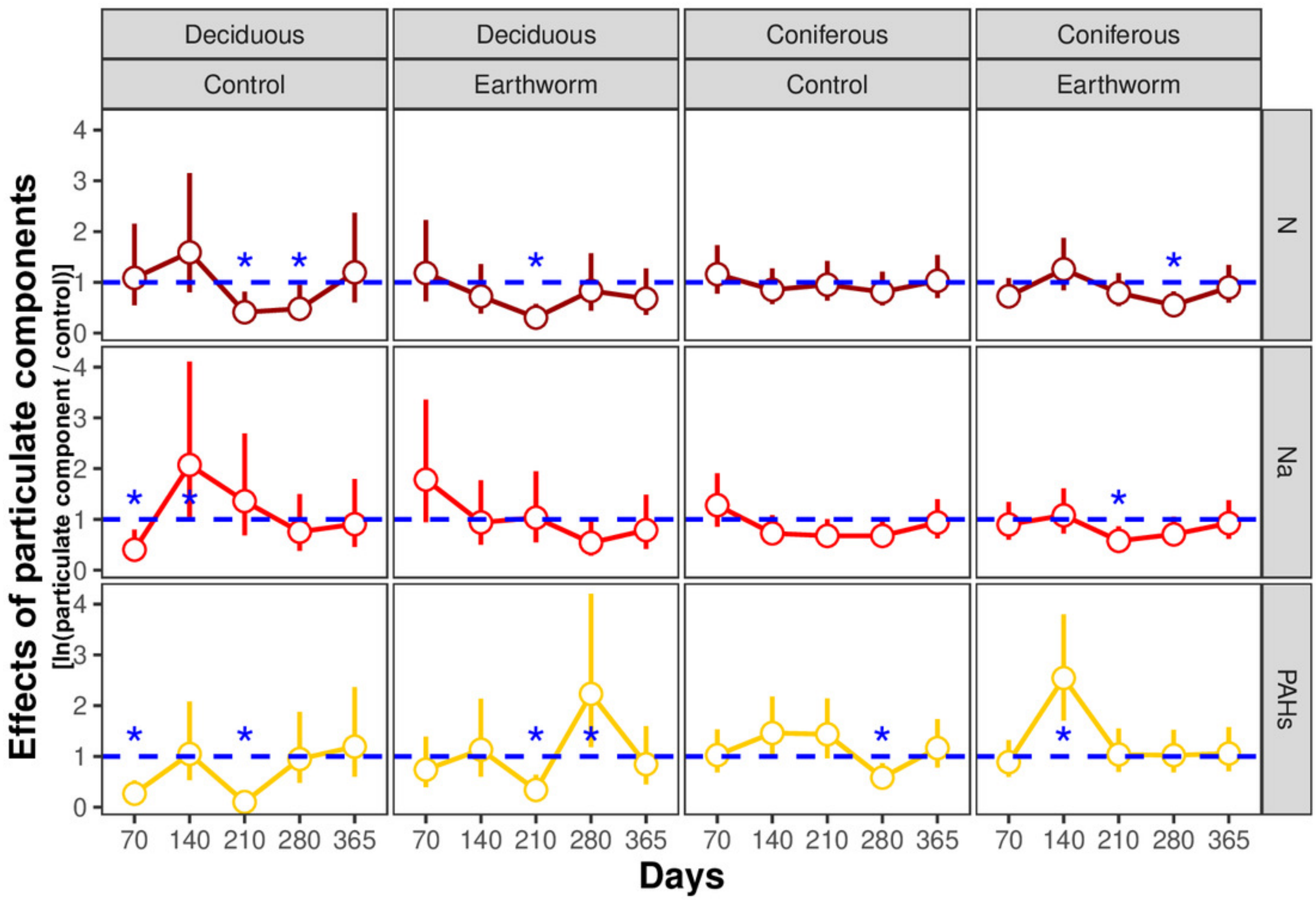
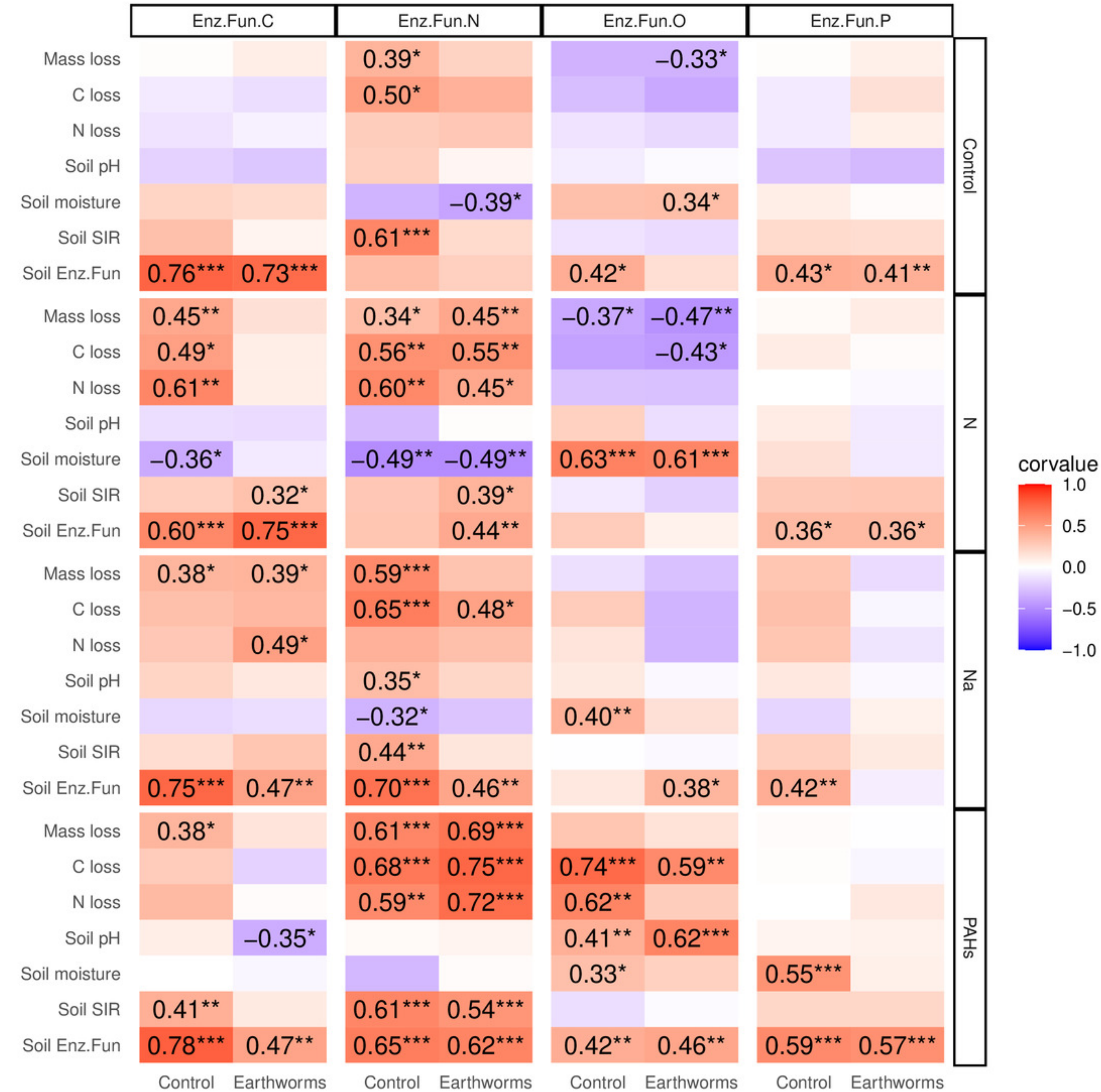


Figure 6

Correlation matrix between different soil enzymatic functions and litter decomposition parameters as well as soil properties in particulate components and earthworm treatments in the deciduous forest.

Enz.Fun.C, Enz.Fun.N, Enz.Fun.O, Enz.Fun.P, Soil Enz.Fun refer to enzymatic functions of carbon, nitrogen, oxidase, phosphorus and total, respectively. Mass loss, C loss and N loss refer to percentage changes during decomposition of *Quercus variabilis* litter. Corvalue refers to the Pearson correlation coefficient and is given for correlations with $P < 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.


Enz.Fun.C
Enz.Fun.N
Enz.Fun.O
Enz.Fun.P
Control
Mass loss 0.39* −0.33*
C loss 0.50*
N loss
Soil pH
Soil moisture −0.39* 0.34*
Soil SIR 0.61***
Soil Enz.Fun 0.76*** 0.73*** 0.42* 0.43* 0.41**
N
Mass loss 0.45** 0.34* 0.45** −0.37* −0.47**
C loss 0.49* 0.56** 0.55** −0.43*
N loss 0.61** 0.60** 0.45*
Soil pH
Soil moisture −0.36* −0.49** −0.49** 0.63*** 0.61***
Soil SIR 0.32* 0.39*
Soil Enz.Fun 0.60*** 0.75*** 0.44** 0.36* 0.36*
Na
Mass loss 0.38* 0.39* 0.59***
C loss 0.65*** 0.48*
N loss 0.49*
Soil pH 0.35*
Soil moisture −0.32* 0.40**
Soil SIR 0.44**
Soil Enz.Fun 0.75*** 0.47** 0.70*** 0.46** 0.38* 0.42**
PAHs
Mass loss 0.38* 0.61*** 0.69***
C loss 0.68*** 0.75*** 0.74*** 0.59**
N loss 0.59** 0.72*** 0.62**
Soil pH −0.35* 0.41** 0.62***
Soil moisture 0.33* 0.55***
Soil SIR 0.41** 0.61*** 0.54***
Soil Enz.Fun 0.78*** 0.47** 0.65*** 0.62*** 0.42** 0.46** 0.59*** 0.57***
Control Earthworms Control Earthworms Control Earthworms Control Earthworms
corvalue
1.0
0.5
0.0
−0.5
−1.0