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Fate of 2,4,6-Tribromophenol in Soil Under Different Redox Conditions

Xiong Jia[1] · Wenji Wang[1] · Yao Yao[1] · Yujie He[1,2] · Philippe F.-X. Corvini[1,3] · Rong Ji[1,2]

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Abstract

Fate of 2,4,6-tribromophenol (TBP) in environmental matrices is obscure. We used ^{14}C-tracer to investigated mineralization, transformation, and non-extractable residue (NER)-formation of TBP in a soil under continuously oxic, continuously anoxic, and anoxic–oxic alteration conditions. In all cases, TBP rapidly dissipated, mineralized to CO_2, and formed NERs in the soil. Considerable amounts of transformation products (2–12%) were detected during the incubation. Marked mineralization (13–26%) indicated that soil microorganisms used TBP as their energy source. About 62–70% of the initial radioactivity was transformed into NERs, being mainly attributed to binding to humic and fulvic acid fractions. TBP transformation was significantly faster under oxic conditions than under anoxic conditions, and was boosted when the soil redox changed from anoxic to oxic state. The results provide new insights into fate of TBP in soil and suggest the importance to evaluate the stability of NERs for risk assessment of TBP in soil.

Keywords Soil redox · Degradation · Mineralization · Non-extractable residues (NERs) · Bound residues (BRs)

2,4,6-Tribromophenol (TBP) is the most widely brominated phenol used as a fungicide, wood preservative, and brominated flame retardant (BFR) (Aguayo et al. 2009; Koch and Sures 2018). Particularly, TBP was an intermediate for production of conventional (e.g., tetrabromobisphenol A, TBBPA) and novel BFRs (e.g., PolyFR) (Barontini et al. 2004; Koch et al. 2016). TBP has been detected at concentrations from 0.3 to 3690 µg/kg in a variety of environmental matrices such as soil, sediment, aquifer, landfill leachate, and surface water (Han et al. 2013; Howe et al. 2005; Koch and Sures 2018; Xiong et al. 2017; Zhang et al. 2019). Even in the samples of human maternal and cord blood, TBP has been detected at pg/g level (Kawashiro et al. 2008). TBP can cause developmental neurotoxicity, embryotoxicity, fetotoxicity, and reproductive toxicity, and has been added to the list of hazardous wastes by USEPA (Xiong et al. 2017; Zu et al. 2012). Therefore, it is essential to understand the behavior and especially the transformation of TBP in the environment.

To date, studies on transformation of TBP were mainly conducted with microbial pure cultures in medium under oxic and anoxic conditions (Aguayo et al. 2009; Li et al. 2015b; Yamada et al. 2008; Zu et al. 2012), instead of in real environmental matrices. A major sink for TBP contamination in the environment is soil, where the transformation of TBP is largely obscure. Only Nyholm et al. (2010) reported the dissipation of TBP in soil with amendment of sludges under both oxic and anoxic conditions with a half-life of 7–10 days. However, dissipation cannot explain the overall fate of TBP in the complex soil matrix, where degradation, mineralization, and non-extractable residue (NER)-formation may occur as found for another typical BRF, TBBPA (Li et al. 2015a; Liu et al. 2013; Wang et al. 2019). In addition, soil may subject to periods of flooded and drained conditions. The fate of pollutants during this alteration of anoxic and oxic conditions is also of environmental significance.

The objective of this study is to investigate how TBP dissipates in soil under oxic, anoxic, and alterative anoxic–oxic conditions, respectively. By applying ^{14}C-tracer, the fate of TBP in soil including degradation, mineralization, and NER-formation was able to be realized. Our study provides

✉ Yujie He
heyujie@nju.edu.cn

[1] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210023, China

[2] Quanzhou Institute for Environment Protection Industry, Nanjing University, Beifeng Road, Quanzhou 362000, China

[3] Institute for Ecopreneurship, School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, Hofackerstrasse 30, 4132 Muttenz, Switzerland

new insights into the fate of TBP in real soil matrix under different redox conditions, which may help stakeholders to develop sound strategies to remediate TBP-contaminated soils.

Materials and Methods

Soil collected from a paddy rice field in Jiaxing, Zhejiang was air-dried, sieved through 20-mesh (0.90 mm), and stored at room temperature before use. Incubation of soil was implemented in 25-mL serum bottles sealed with butyl rubber stoppers in dark at 27°C. Three different redox conditions were employed, including continuously oxic, continuously anoxic, and alteration of anoxic to oxic conditions. For the continuously oxic treatment, 2 g of the paddy soil was spiked with uniformly ^{14}C-ring-labeled TBP (^{14}C-TBP) methanolic solution to achieve a final chemical concentration of 5 mg/kg soil dry weight (dw) and a radioactivity concentration of 1×10^5 dpm/g soil dw. ^{14}C-TBP (67 mCi/mmol) with a radiochemical purity of 96% was synthesized in our laboratory from ^{14}C-labeled phenol. The moisture of the soil mixture was adjusted to 70% of its maximal water-holding capacity using distilled water after an overnight evaporation of methanol. For the continuously anoxic treatments, 2 g of the soil was mixed with 4 mL of O_2-free distilled water and incubated under a headspace of N_2/CO_2 (80:20; v:v). Three tubes with the same treatment with addition of resazurin as a redox indicator at 30 mg/L were prepared. ^{14}C-TBP was added when the indicator turned from pink into colorless. After incubation for 30 days, parts of the bottles of the continuously anoxic treatments were altered to oxic condition as following: water layer of the anoxic soil was decanted and the residual soil was re-aerated with air. These bottles underwent anoxic to oxic incubation were the anoxic–oxic alteration treatment. Control treatments with sterile soil, which was prepared by autoclaving the soil three times at 121°C for 20 min on three consecutive days, were conducted the same as the above with ^{14}C-TBP addition in a clean bench. All the active treatments were performed in triplicates and the sterile treatments in duplicates. During the incubation, all bottles incubated under oxic conditions were opened for 10 min every 2 days to allow a headspace exchange with air.

To measure mineralization of TBP in the anoxic treatments, 10 mL of the headspace in the bottles was withdrawn with a syringe at defined sampling time points. The withdrawn gas was injected into 2 mL of alkaline scintillation cocktail (Oxysolve C-400; Zinsser Analytic, Frankfurt, Germany) to absorb $^{14}CO_2$. In the oxic treatments, the generated $^{14}CO_2$ was trapped by 1 mL of NaOH (0.5 M) hold in a vial suspended from the top of the serum bottle and the alkali-trap was mixed with 2 mL of multipurpose scintillation cocktail (Gold Star; Meridian Biotechnologies Ltd., UK). The radioactivity in the cocktails was counted by liquid scintillation counting (LSC) (LS6500; Beckman Coulter, USA).

At each sampling point, a series of bottles were scarified for analysis of extractable residues and NERs of ^{14}C-TBP in soil. The remained radioactivity in the soil after exhaustive extraction was defined as the NERs (or bound residues, BRs). In the anoxic treatments, the water phase was decanted from the soil pellet and determined for radioactivity by LSC. The separated soil pellet was freeze-dried and extracted with 10 mL of methanol three times by repeated shaking (200 rpm, 2 h) and centrifugation (4000 rpm, 10 min). Aliquot (1 mL) of the combined supernatants was used for radioactivity determination by LSC. The organic extract was concentrated to about 1 mL on a rotary evaporator and then to dryness using a gentle nitrogen stream. The dried extract was re-dissolved in 50 µL of methanol for analysis by high-performance liquid chromatography (HPLC) coupled to LSC (HPLC-^{14}C-LSC) to separate TBP and its transformation products in the extract (see below). The soil in the oxic treatments was scarified and extracted using the same method as in the anoxic treatments. The extraction procedure recovered 91 ± 1.3% of TBP in soil.

After the exhaustive extraction by methanol, the soil was air-dried for NER measurement by a biological oxidizer (OX-500, Zinsser Analytic, Germany). The NERs were fractionated into humic acid (HA)-, fulvic acid (FA)- and humin-bound residues (Shan et al. 2010). Briefly, about 1 g of the air-dried soil was extracted with 5 mL of O_2-free NaOH (0.1 M) by 24 h horizontal shaking at 200 rpm and 20 min centrifugation at 11,000×g. The pellet was combusted by the biological oxidizer to determine the radioactivity of humin-bound NERs. The supernatant was separated into FAs (supernatant) and HAs (precipitate) by acidification with HCl (1 M) to pH 1. Radioactivity in FAs and HAs was determined by LSC, representing the radioactivity of FA-bound NERs and HA-bound NERs, respectively.

Separation of TBP and its metabolites was performed on an Eclipse XBD-C18 column (5 µm, 3.5 mm × 250 mm; Agilent, USA) at 30°C using a HPLC system (Agilent 1100) equipped with a diode array detector. The mobile phase was a mixture of 72% methanol and 28% water at a flow rate of 1 mL/min. UV adsorption wavelength was set at 296 nm. Sample injection volume was 35 µL. The eluent was collected every minute and determined for radioactivity by LSC (using 2 mL of the scintillation cocktail Gold Star).

Dissipation of TBP in soil was fitted to an exponential model using the software Sigmaplot (SigmaPlot 10.0, USA). Half-lives of TBP were calculated only if the simulation was of statistical significance ($p < 0.001$).

Results and Discussion

During an incubation of 60 days, the radioactivity of TBP distributed mainly in the gas, organic extractable and non-extractable phases in the three treatments, i.e., incubation under continuously anoxic, continuously oxic, and the alteration of anoxic and oxic conditions (Figs. 1, 2). The total recovery of the applied radioactivity (91.7–105%) indicated a well-obtained mass balance of TBP in the

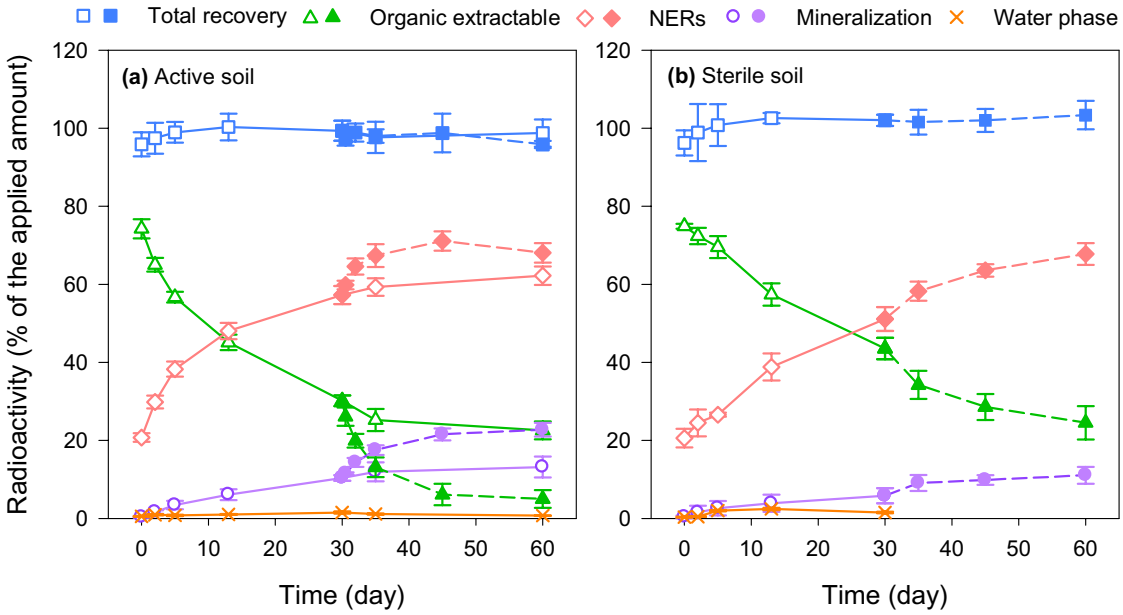

Fig. 1 Distribution of residual radioactivity of ^{14}C-labeled 2,4,6-tribromophenol (^{14}C-TBP) during incubation in **a** active soil and **b** sterile soil under continuously anoxic and anoxic–oxic alteration conditions. The results are presented as the average ± standard deviation (active treatment, $n = 3$) or mean deviation (sterile treatment, $n = 2$). The solid symbols represent for the oxic condition and the empty symbols for the anoxic condition

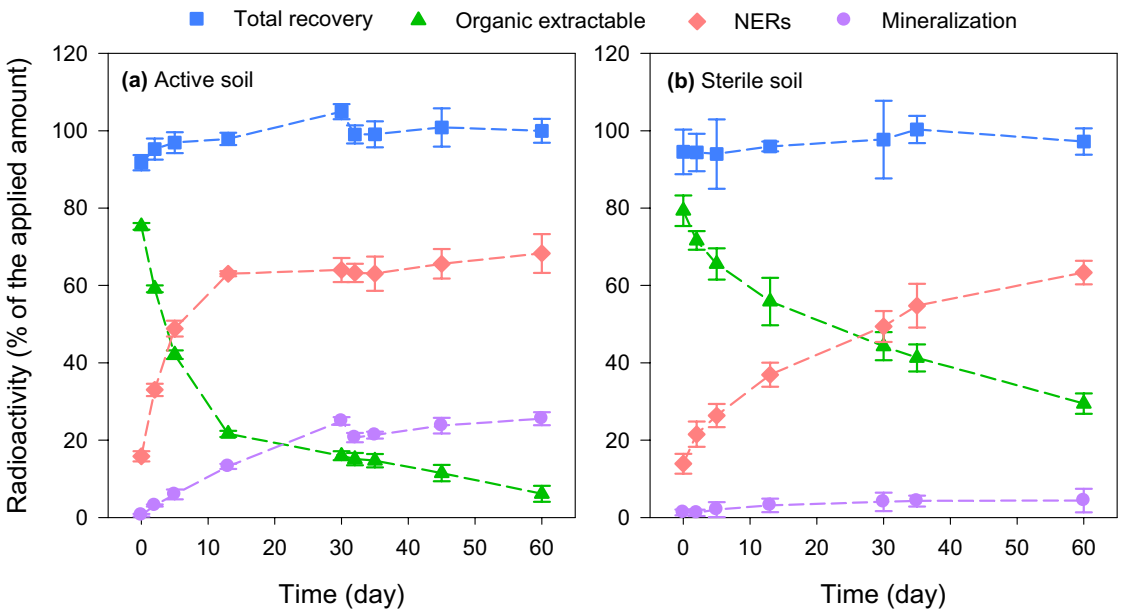

Fig. 2 Distribution of residual radioactivity of ^{14}C-TBP during incubation in **a** active soil and **b** sterile soil under continuously oxic conditions. The results are presented as average ± standard deviation (active treatment, $n = 3$) or mean deviation (sterile treatment, $n = 2$)

batch systems. In the active soil, TBP rapidly dissipated from the organic extractable phase to generate CO_2 and form NERs (Figs. 1a, 2a). Transformation of TBP into CO_2 and NERs was also observed in the sterile treatments, but the mineralization was significantly less compared to the active treatments and the NER transformation was obviously slower (Figs. 1b, 2b). This phenomenon may be resulted from abiotic physical–chemical aging processes that sequester xenobiotics in soil (Gevao et al. 2000) and/or fungi degradation (under oxic conditions). Autoclaving sometimes are not completely fatal for fungi (Nowak and Wronkowska 1987), which may be capable to grow and degrade TBP during the incubation, considering that several types of fungi were found facilitate degradation of TBP under oxic conditions and were recommended for remediation purpose (Donoso et al. 2008; Monrroy et al. 2007).

Under the simulated flooded condition (anoxic), bioaccessibility of TBP was low during the whole incubation as indicated by the less than 2.5% of radioactivity in the water phase (Fig. 1a, b). Similarly, Nyholm et al. (2010) also found a very low amount of TBP (< 2%) remained in the water phase in anoxic soil microcosms. Normally, brominated phenols like TBP with low carbon contents (21.6% carbon, 72% bromide of the compound weight) would display a limited transformation (Ronen et al. 2000). Still, TBP was found degraded by various bacterial and fungal species isolated from TBP-contaminated sites or even natural habitats such as desert soil, forest, and estuarine sediment (Koch and Sures 2018). Li et al. (2015b) found that 32% of TBP was mineralized after 15 days of incubation by applying a synthetic bacterial community comprising of two isolated strains (*Clostridium* sp. strain Ma13, *Desulfatiglans parachlorophenolica* strain DS) and one enriched culture containing dehalogenating species, which is the first work confirmed TBP mineralization under anoxic conditions. In our soil from paddy rice field, marked mineralization was found under both oxic and anoxic conditions (Figs. 1a, 2a), indicating that the microbial community in the soil was able to use TBP as their energy source.

Dissipation and transformation of TBP was significantly faster under oxic conditions than under anoxic conditions. After 30 days of incubation, 30.3% of the initial radioactivity remained in the organic extractable fraction in the anoxic soil (Fig. 1a) while only 16% remained in the oxic soil (Fig. 2a); in the anoxic treatment 10.4% of TBP was mineralized and 57.3% was transformed to NERs while 25% of mineralization and 64% of NER formation was observed in the oxic treatment (Figs. 1a, 2a). Half-lives of TBP was calculated as 17 days and 5.2 days under anoxic and oxic conditions, respectively (Fig. 3). In the study of Nyholm et al. (2010), similar half-lives of TBP (7–8 days) were found in oxic and anoxic soils amended with 0.5% activated sludge,

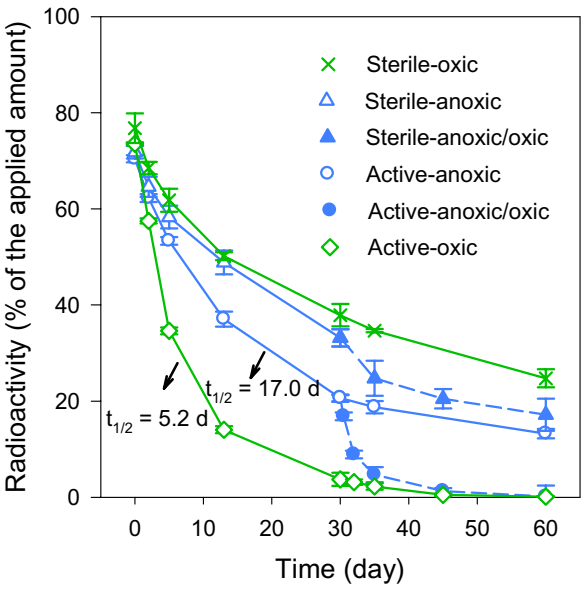

Fig. 3 Dissipation of TBP in the organic solvent-extractable fraction in active and sterile soils under continuously oxic, continuously anoxic and of anoxic–oxic alteration conditions. The results are presented as average ± standard deviation (active treatments, $n = 3$) or mean deviation (sterile treatments, $n = 2$)

instead of an oxic dissipation preference. In contrast, in the same study other four degradable BFRs (TBBPA, 2,4,4′-tribromodiphenyl ether, hexabromobenzene, and 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane) dissipated slower under anoxic conditions than under oxic conditions (Nyholm et al. 2010). The distinct findings between TBP and other BFRs may not only be attributed to chemical structural difference, but also to occurrence of specific anoxic microbial degraders in the applied soil (Nyholm et al. 2010), which may lead to the incomparable conclusions observed in our study.

Dissipation and transformation of TBP obviously slowed down after 30 days of incubation under both anoxic and oxic conditions (Figs. 1a, 2a). After a redox alteration from anoxic to oxic state on day 30, TBP transformation was boosted and even reached a level comparable to the treatment under continuously oxic incubation after another 30 days of incubation (i.e., on day 60) (Figs. 1a, 2a). So far there was no study exploring transformation of TBP under the redox alteration conditions. Referring to a similar study, TBBPA was effectively debrominated to bisphenol A (BPA) during anoxic incubation and then BPA rapidly degraded during subsequent oxic incubation (Liu et al. 2013). Namely, oxic conditions favored biodegradation of TBBPA metabolites generated during anoxic incubation and the redox state alteration promoted transformation of TBBPA. Nevertheless, in this study formation of TBP metabolites (2–12%) followed a similar tendency that was accumulating in the first 30 days and dissipating thereafter under the continuously

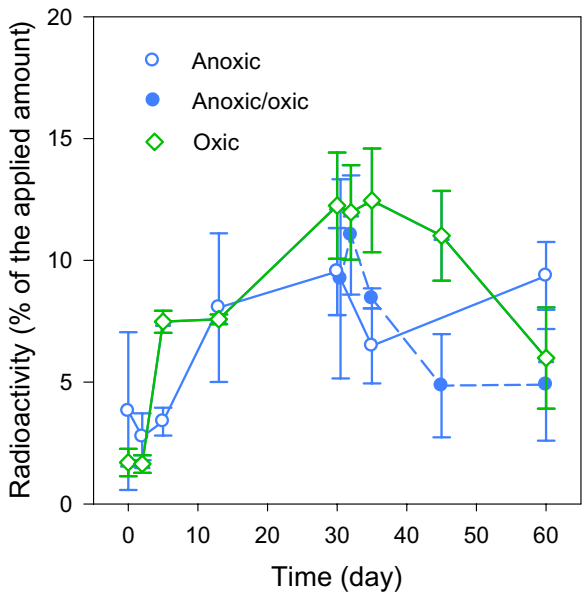

Fig. 4 Occurrence of TBP transformation products during incubation of TBP in the active soil under continuously oxic, continuously anoxic, and anoxic–oxic alteration conditions. The results are presented as the average ± standard deviation ($n = 3$)

anoxic, continuously oxic, and anoxic–oxic alteration conditions (Fig. 4). Therefore, the promoted transformation of TBP after alternation to oxic conditions was probably because the soil aerobic microorganism had a higher activity to degrade TBP than the anaerobes. Analysis of microbial communities is needed to further understand the mechanism of the transformation preference of TBP under the different redox conditions.

Once exposed, approximately 20% of TBP was bound to the soil and became organic solvent non-extractable (Figs. 1, 2). NER formation is a significant mechanism for xenobiotics to detoxify in the environment, via physical entrapment/occlusion, chemical bonding, and biological assimilation (Gevao et al. 2000; Kästner et al. 2014). After 60 days of incubation, about 62–70% of the initial radioactivity was transformed into NERs under all the redox conditions investigated (Figs. 1a, 2a). Production of NERs achieved similar yields at day 60 in the sterile and active treatments, while the production rates were higher in the beginning of the incubation in the active treatments than in the sterile control (Figs. 1, 2). Therefore, both biotic and abiotic reactions are significant for NER formation, and the formation would be speeded up with biotic reaction present. Substances with phenolic structure likely react with humic substances in soil via coupling reactions and thus have a great tendency to form NERs (Kästner et al. 1999). NER formation has pronounced impacts on environmental behavior of xenobiotics by influencing their bioavailability, mobility, toxicity, and volatility (Liu et al. 2014). However, NER formation of TBP in soil has not been reported before. The formation of NERs was positively correlated to the dissipation of TBP in the soil (Fig. 5), indicating that the hydrophobic TBP likely bounded to humic substances during its dissipation and transformation.

Humic substances differ in their carbon, hydrogen, and oxygen content as well as in their functional groups, especially the carboxylic and phenolic functions (Riefer et al. 2011). TBP-derived NERs are mainly bound to the HA and FA fractions under all incubation conditions (Fig. 6). HAs could effectively sorb and bind TBP (Xiong et al. 2017) while sorption is the primary step for the formation of NERs via covalent linkages (Kästner et al. 1999; Riefer et al. 2011). HA-, FA- and humin-bound NERs were continuously formed with the rates slowing down after 13 days under continuously oxic conditions (Fig. 6a) and 30 days under continuously anoxic conditions (Fig. 6b). When the redox state altered from anoxic to oxic, the HA-, FA- and humin-bound NERs increased to an amount comparable to those under continuously oxic conditions (Fig. 5). Although TBP in soil mainly ended up with the NERs during its dissipation, the formed NERs still had a potential to be released, due to changes of soil conditions, including pH, redox potential, humidity, aeration, temperature, carbon source, microbial activity, and humification of soil organic matter (Wang et al. 2019). Humin consists of higher C and lower O content than HA and FA fractions, and represents a more stabilized fraction of the soil organic carbon (Nissenbaum and Schallinger 1974).

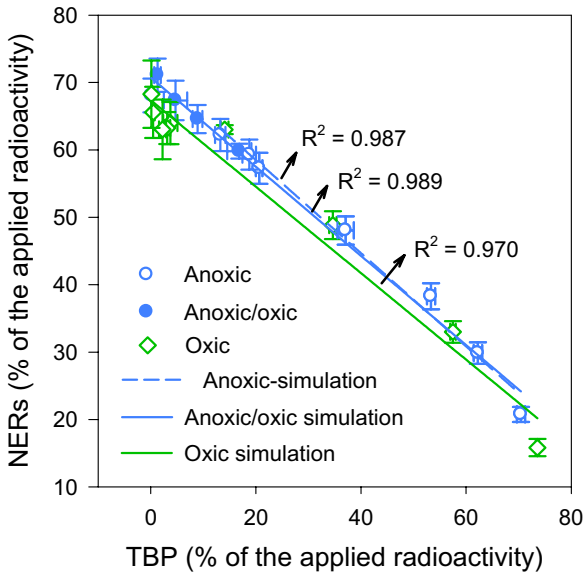

Fig. 5 Linear correlation between organic solvent-extractable TBP and the NERs formed in the active soil during incubation under continuously oxic, continuously anoxic, and anoxic–oxic alteration conditions. The results are presented as the average ± standard deviation ($n = 3$)

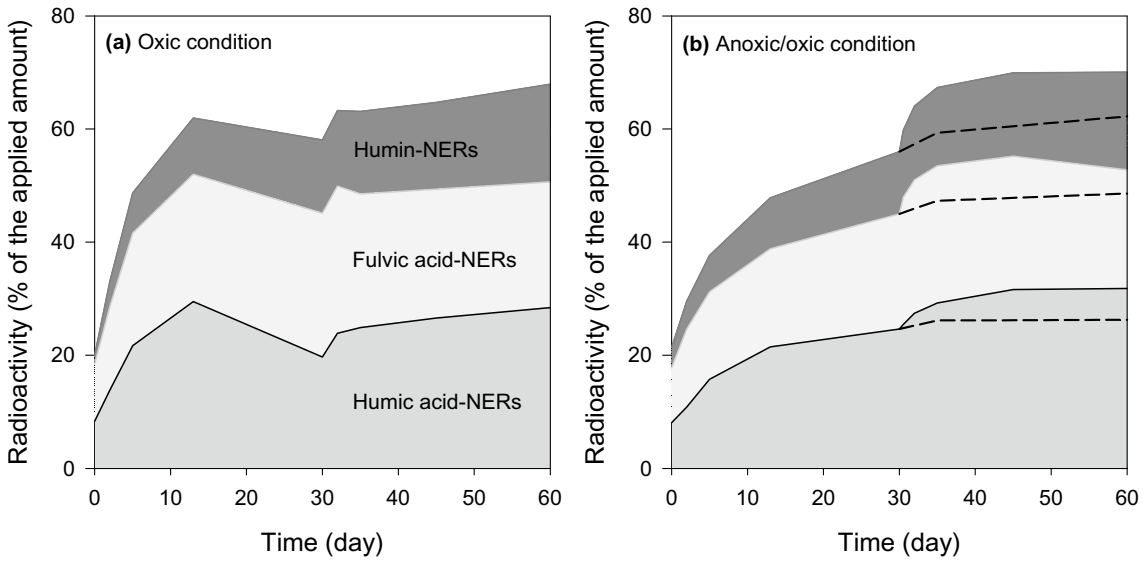

Fig. 6 Distribution of ^{14}C-TBP-derived NERs in three humic fractions during incubation in the active soil under **a** continuously oxic and **b** anoxic–oxic alteration of conditions. The results are presented as the average ± standard deviation ($n = 3$). The Dash lines represent for fractionation of NERs in the continuously anoxic condition during day 30–60

The stability of the predominant and less stable HA- and FA-bound NERs may still be of environmental concern, which needs to be further investigated.

In summary, this work is the first study revealing the fate and transformation of TBP in soil under continuously anoxic, continuously oxic, and especially the alteration of anoxic and oxic conditions. The results showed that TBP dissipated rapidly ($t_{1/2} = 5.2$–17 days) in soil and transformed into CO_2 (13–26%), metabolites (2–12%), and NERs (62–70%). NER formation was the major fate of TBP in the soil and was mainly bound to the HA and FA fractions. Dissipation and transformation of TBP was significantly faster under oxic conditions than under anoxic conditions. Redox alteration from anoxic to oxic state could boost TBP transformation. Our findings emphasize the need to consider the formation and release potential of NERs when assessing the environmental risks of TBP.

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