

## ORIGINAL ARTICLE

# Concentration-dependent response of soil parameters and functions to trifluoroacetic acid

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## Abstract

Despite growing environmental concerns about perfluoroalkyl and polyfluoroalkyl substances (PFAS) worldwide, the ultra-short-chain PFAS, for example, trifluoroacetic acid (TFA), have been largely neglected in the context of soil pollution. Given the persistence and increasing occurrence of TFA in the soil environment, accumulated TFA could impact soil properties and functions. Therefore, we investigated the effects of a wide range of concentrations of TFA (0.001–100  $\mu\text{g g}^{-1}$ ) on chemical, physical and biological indicators of soil health, using a six-week microcosm experiment. Our results showed that TFA treatments decreased soil pH, sulphate content, soil respiration, litter decomposition and bacterial abundance, and increased phosphate content ( $p < 0.05$ ). These effects were observed for TFA concentrations  $\geq 1 \mu\text{g g}^{-1}$ . As expected, because of its strong acidity, TFA decreased soil pH, and this change likely contributed to effects on other soil parameters and functions, for example, reducing bacterial abundance and soluble nutrients. However, importantly, TFA clearly also affected soil parameters at concentrations at which soil pH was not changed. Soil aggregation, fungal abundance, and enzyme activities were not affected by TFA in this study. Considering the current reported environmental levels of TFA of  $< 2.4 \mu\text{g g}^{-1}$ , only litter decomposition would be affected under current field conditions. Our data also show that future accumulation of higher concentrations, or hotspots of TFA, will likely negatively affect soils with similar properties as our test soil. Thus, we demonstrated that microbial processes can be impacted by the accumulation of the ‘forever chemical’ TFA in the tested sandy, low organic matter soil.

## Highlights

- Trifluoroacetic acid (TFA) decreased soil pH, respiration, litter decomposition and bacterial abundance.
- TFA affected soil soluble nutrients.
- TFA affected litter decomposition at  $1 \mu\text{g g}^{-1}$  with no change in soil pH.

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- Effects of TFA on soil enzyme activity and aggregation and fungal abundance were negligible.

**KEYWORDS**

litter decomposition, perfluoroalkyl and polyfluoroalkyl substances (PFAS), soil pH, soil pollution, soil respiration, soil soluble nutrients, ultra-short-chain perfluoroalkyl acids

## 1 | INTRODUCTION

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) represent a class of thousands of synthetic chemicals of growing concern worldwide, basically defined as chemicals with at least one perfluoroalkyl moiety ( $-C_nF_{2n-}$ ; Buck et al., 2011; Kwiatkowski et al., 2020; OECD, 2018). Given the longevity of PFAS in natural environments, they are popularly known as ‘forever chemicals’ (Beans, 2021). To date, research has mostly focused on the long-chain PFAS ( $>C7$  for carboxylic acids and  $>C5$  for sulphonic acids), particularly perfluorooctanesulphonic acid (PFOS) and perfluorooctanoic acid (PFOA), due to their persistence, bioaccumulation, and toxicity, largely overlooking the ultra-short-chain PFAS ( $C2-C3$ ; Ateia et al., 2019; Björnsdotter et al., 2019).

The ultra-short-chain PFAS of greatest current interest is trifluoroacetic acid (TFA). This environmental contaminant has raised concerns since the 1990s, recently in particular because of advances in analytical techniques (Frank et al., 1995; Joudan et al., 2021). Trifluoroacetic acid can be produced by the atmospheric oxidative degradation of hydrofluorocarbons, hydrochlorofluorocarbons, and hydrofluoroolefins, but also by other sources, and enters terrestrial ecosystems via precipitation (Björnsdotter et al., 2019; Richey et al., 1997; Wu et al., 2014). In Germany, TFA concentration in precipitation from 2018 to 2019 was as high as  $38.0 \mu\text{g L}^{-1}$ , and the average TFA concentration increased by 3–4 times over the last decades (Freeling et al., 2020). The biotransformation of  $CF_3$ -containing precursors in landfill sites is also a possible pathway of TFA entering soil (Sun et al., 2020). Another important source of TFA in the soil environment is the direct release from fluorochemical manufacturing plants (Chen et al., 2018).

Recently, there has been discussion on whether TFA is an environmental issue (Joudan et al., 2021; Kwiatkowski et al., 2021; Singh & Papanastasiou, 2021). Although TFA does not bioaccumulate in the food chain due to its very low aliphaticity, its high environmental persistence has resulted in significant accumulation in the environment, and its mobility within the environment is regarded as a concern comparable to bioaccumulation (Hale et al., 2020; Kwiatkowski et al., 2021). Compared to long-chain PFAS, TFA is weakly retained

by the soil and prone to transport to groundwater (Richey et al., 1997). Despite this fact, TFA pollution has been widely detected in soils (Solomon et al., 2016), for example,  $0.42-2.4 \text{ ng g}^{-1}$  across Germany (Behringer et al., 2021), and  $92.7-188 \text{ ng g}^{-1}$  in Shanghai, China, dominating the tested PFAS (Li et al., 2010); TFA concentration in soil from a fluorochemical manufacturing park in China was even up to  $2400 \text{ ng g}^{-1}$ , 1–2 orders of magnitude higher than other single PFAS (Chen et al., 2018). Given significant environmental consequences, TFA and its salt produced from new refrigerants (hydrofluoroolefins) have been listed as a concern for global biodiversity conservation for 2022 (Sutherland et al., 2022).

Existing studies have mostly focused on TFA effects on terrestrial and aquatic organisms, and it appears that the test organisms were not sensitive to TFA and its salt (Solomon et al., 2016). TFA is considered to be low to moderately toxic to a range of organisms, but this has been only tested on a limited range of organisms (Boutonnet et al., 1999; Solomon et al., 2016). On the other hand, the environmental levels of TFA are continuously increasing. As reported by Zhai et al. (2015), the TFA concentration in water increased by 17 times from 2002 to 2012 in Beijing, China. Therefore, we need to reconsider the environmental effects of TFA in the context of increasing and widespread accumulation of PFAS.

In fact, the influence of TFA contamination on soil *per se* has been largely overlooked so far. Therefore, the present study investigated TFA effects on soil properties and functions, employing chemical, physical and biological indicators, including soil pH and soluble nutrients, soil aggregation, soil respiration, litter decomposition, enzyme activities, and microbial populations.

## 2 | MATERIALS AND METHODS

### 2.1 | Soil and trifluoroacetic acid

Trifluoroacetic acid ( $CF_3COOH$ , 99%, TFA) was purchased from Fluorochem Ltd. (Derbyshire, UK). It was diluted with deionised water to a stock solution with the concentration of  $10 \text{ g L}^{-1}$ . As a strong organic acid with a

$pK_a$  of 0.23, TFA is highly polar and thus has a low bioaccumulation potential (Bouttonnet et al., 1999).

A sandy loam soil sample (Albic Luvisol, 73.6% sand, 18.8% silt, and 7.6% clay) was collected at a grassland field site of Freie Universität Berlin, Germany (52°28'N, 13°18'E). The grassland is managed by mowing (twice a year), and the sampling depth was 30 cm. The percentage of total C and N was 1.87% and 0.12%, respectively, measured using a Euro EA analyser (HEKAtech GmbH, Wegberg, Germany; Rillig et al., 2010). Soil pH (1:5, H<sub>2</sub>O, wt/vol) was 6.8. Fresh soil was thoroughly mixed, sieved to <2 mm, and stored at 4°C until the experiment.

## 2.2 | Microcosm experiment

We first added the TFA solution of the respective concentration (see below) to 5 g sterilised (121°C, 20 min, twice) 'loading' soil in a 200-ml plastic bottle; this sterilised loading soil was used to avoid exaggerated effects on soil microbial communities and to allow more homogenous mixing (Rillig et al., 2019). We then mixed this loading soil with 35 g of live soil for 2 min to achieve TFA evenly distributed in the soil. The blank control was treated exactly the same way, adding the same volume of deionised water. This soil was then transferred into a 50-ml mini-bioreactor (Corning Inc., Corning, USA) with vented caps to allow air exchange, and we slowly added deionised water to reach 60% water holding capacity. We set the nominal concentration of TFA as 0.001, 0.01, 0.1, 1, 10, and 100  $\mu\text{g g}^{-1}$ . Although the highest TFA concentration observed in the soil is 2.4  $\mu\text{g g}^{-1}$  (Chen et al., 2018), owing to its longevity and continuing input in soil, two very high concentrations were included to consider future scenarios. The experimental tubes were placed randomly inside a dark incubator and incubated at 20°C for 6 weeks. We watered each tube weekly to maintain soil moisture, and also re-arranged tubes to minimise positional effects. Each TFA concentration treatment and blank control had 10 replicates, giving 70 tubes in total. After the incubation, fresh soil samples were collected and stored at 4°C (less than 2 weeks) and at -80°C prior to enzyme activity measurements and DNA extraction, respectively. The rest was air-dried for the measurement of soil soluble nutrients, pH, and water-stable aggregates.

## 2.3 | Measurement of soil properties and functions

### 2.3.1 | Litter decomposition

We used litter bags to investigate TFA effects on organic matter decomposition, the most common technique for

measuring litter decomposition in soil (Xie, 2020). Litter bags were manually made with nylon mesh (38  $\mu\text{m}$ ) in a size of 2.5 × 1.5 cm (Lehmann et al., 2020), and contained 300 mg of fine green tea (Meßmer Tee GmbH, Seevetal, Germany). These bags were microwaved for 1 min to minimise microbial contamination, and included in the microcosms at the start of the experiment. At harvest, litter bags were recovered, cleaned with deionised water, and dried at 60°C. Mass loss was calculated as an indication of decomposition (%).

### 2.3.2 | Soil respiration

We measured soil respiration in weeks 3 and 6, respectively. Airtight caps with rubber stoppers were used during this measurement. Tubes were flushed with CO<sub>2</sub>-free air for 5 min to eliminate background CO<sub>2</sub> and then incubated for 3 h (Rillig et al., 2019). Then, we sampled 1 ml of air in the headspace of each tube to measure CO<sub>2</sub> concentration using an infrared gas analyser (LI-6400XT, LI-COR Inc., Bad Homburg, Germany). Soil respiration was expressed as net CO<sub>2</sub> production ( $\mu\text{M M}^{-1} \text{h}^{-1}$  per microcosm).

### 2.3.3 | Soil enzyme activities

At harvest, we collected 5.0 g of fresh soil to measure four enzyme activities linked to C, N, and P cycling, that is,  $\beta$ -glucosidase and  $\beta$ -D-1,4-cellobiosidase (C-related),  $\beta$ -1,4-*N*-acetyl-glucosaminidase (N-related), and phosphatase (P-related). Enzyme activities were measured in 96-well plates with the use of artificial *p*-nitrophenyl linked substrates and quantified colorimetrically using a microplate reader (Bio-Rad Lab., Hercules, USA). Detailed procedures have been reported in previous studies (Jackson et al., 2013; Liang et al., 2021).

### 2.3.4 | Soil aggregation

Water-stable aggregates (WSA) were quantified by the wet sieving method (Kemper & Rosenau, 1986). Briefly, 4 g air-dried soil samples were rewetted by capillarity on a 250- $\mu\text{m}$  sieve for 5 min, and wet-sieved for 3 min with a sieving machine (Eijkelkamp, Giesbeek, Netherland). The soil remaining on the sieve (fraction 1; consisting of stable soil aggregates and dry matter) was dried at 60°C and weighed, and then crushed in the wet sieve manually to obtain the coarse matter (fraction 2). The coarse-matter corrected fraction of WSA was calculated by weight as  $\text{WSA} (\%) = (\text{fraction 1} - \text{fraction 2}) / (4 - \text{fraction 2}) \times 100\%$ .

### 2.3.5 | Soil pH and soluble nutrients

A portion of 5.0 g air-dried soil was mixed with 25 ml deionised water, and shaken horizontally at 200 rpm for 30 min at room temperature. Soil pH was determined in the suspension with a pH meter (Hanna Instruments, Smithfield, USA) after allowing the soil to settle for 30 min.

Soil soluble nutrients ( $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{PO}_4^{3-}$ ) were extracted by shaking the soil solution (dry soil: water, 1:5) for 1 h and centrifuging at 3000 rpm for 10 min. The supernatant was passed through a 0.22- $\mu\text{m}$  filter and concentrations of nutrients were determined using a Dionex ICS-1000 ion chromatography instrument (Thermo Fisher Scientific, Waltham, USA).

### 2.3.6 | Soil DNA extraction and bacterial and fungal abundance qualification

Soil DNA was extracted using  $\sim 250$  mg soil using DNeasy PowerSoil Pro Kit (QIAGEN GmbH, Hilden, Germany) following the technical protocol and stored at  $-20^\circ\text{C}$ .

Bacterial and fungal abundances were quantified by quantitative PCR (qPCR) in a CFX 96 Real-Time System (Bio-Rad Lab., Hercules, USA) in 96-well plates. Soil DNA was amplified using the universal primers, 515F (5'-GTGCCAGCMGCCGCGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') for soil bacteria, and FungiQuant-F (5'-GGRAAACTCACCAGGTCCAG-3') and FungiQuant-R (5'-GSWCTATCCCCAKCACGA-3') for soil fungi (Liu et al., 2012) with the KAPA HiFi PCR kit (Roche, Basel, Switzerland) and EvaGreen Fluorescent DNA Stain (Jena Bioscience GmbH, Jena, Germany). The 20- $\mu\text{l}$  reaction per well was composed of  $1 \times$  KAPA HiFi buffer, 0.3 mM KAPA dNTP Mix, 0.75  $\mu\text{M}$  of each primer, 0.1 U KAPA HiFi Polymerase, 0.5  $\mu\text{M}$  EvaGreen, and 5  $\mu\text{l}$  DNA template. The thermocycler conditions for bacterial qPCR were: 3 min of initial denaturation at  $95^\circ\text{C}$ , followed by 30 cycles of 20 s of denaturation at  $95^\circ\text{C}$ , 30 s of annealing at  $50^\circ\text{C}$ , and 30 s of extension at  $68^\circ\text{C}$ , and ended with 5 min of final extension at  $68^\circ\text{C}$ ; for fungal qPCR: 3 min of initial denaturation at  $95^\circ\text{C}$ , followed by 30 cycles of 15 s of denaturation at  $98^\circ\text{C}$ , 45 s of annealing and extension at  $67^\circ\text{C}$ , and ended with 5 min of final extension at  $72^\circ\text{C}$ . A melting curve was subsequently performed to verify the specificity of amplicons. The qPCR standards were prepared from a DNA pool from the same soil samples amplified under the same conditions but without EvaGreen. The amplification products were confirmed in agarose gel for their specificity and size. After purification with magnetic beads (CleanNA, Waddinxveen, Netherlands), the DNA concentration of the standards was measured using a Qubit 3 Fluorometer (Fisher Scientific

GmbH, Germany), diluted from  $10^3$  to  $10^9$  copies and used in the standard curves for each qPCR plate. Standard curves had  $R^2$  values higher than 0.997, and amplification efficiencies were  $89.8\% \pm 0.6\%$  and  $91.6\% \pm 2.3\%$  for bacterial and fungal primers, respectively.

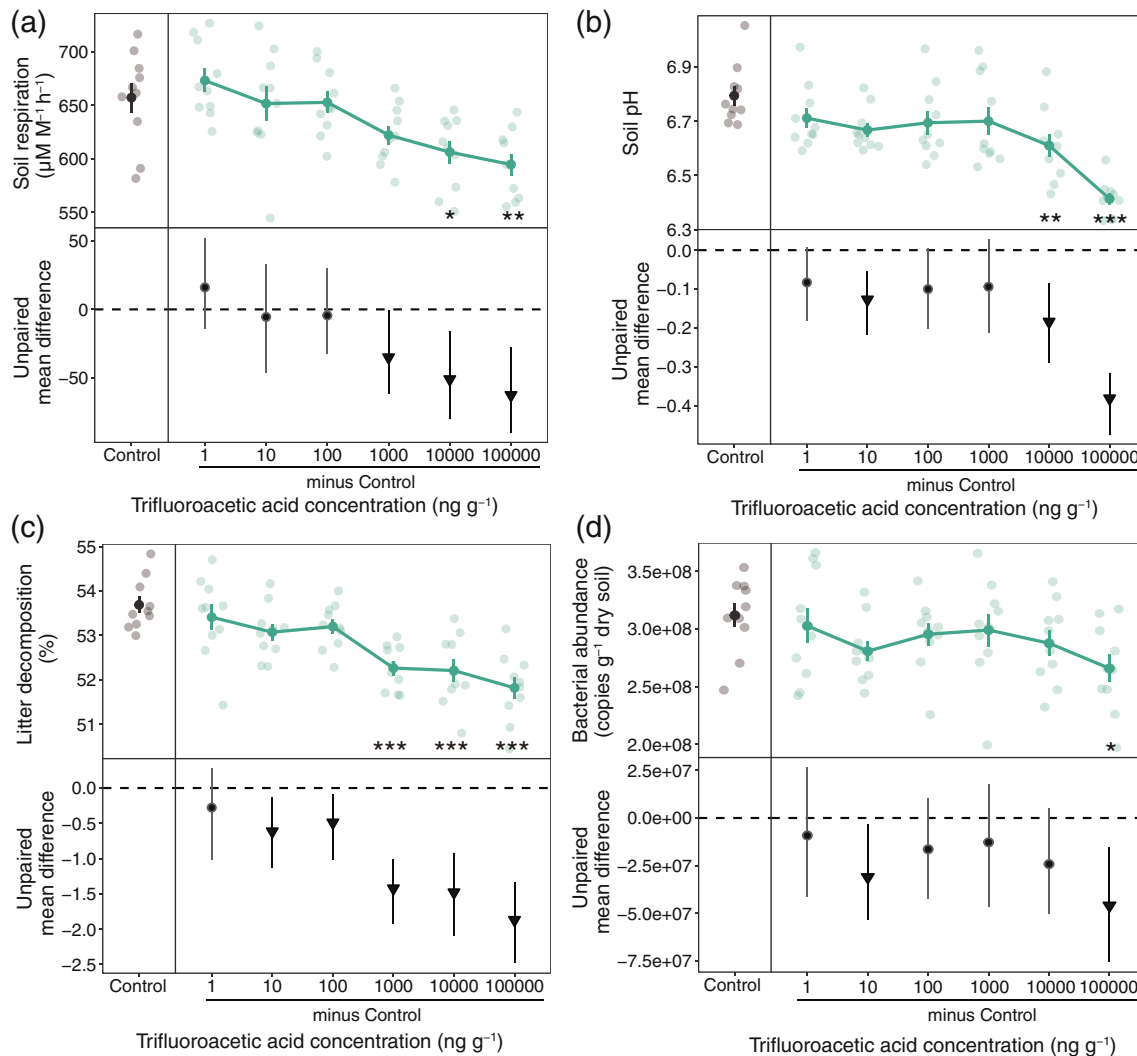
### 2.4 | Data analysis

We performed all statistical analyses and plotting in R v.4.0.6 (R Core Team, 2020). The effect of TFA treatments on soil properties and functions was tested using the R package 'dabestr' (Ho et al., 2019). We calculated the 95% confidence interval (CI) of unpaired mean differences of each TFA treatment minus control by a bootstrapping method. This approach brings the effect size and its precision to the forefront, and it can also avert the pitfalls of significance testing (Ho et al., 2019). As a supplement, we carried out a one-way ANOVA followed by Dunnett's test using the R package 'multcomp' to compare each treatment to the control (Hothorn et al., 2008), and report the adjusted  $p$  values by a single-step method. The 10% effective concentration ( $\text{EC}_{10}$ ) and median effective concentration ( $\text{EC}_{50}$ ) of certain soil responses were estimated using the function 'LL4' in the R package 'drc' (Ritz & Streibig, 2005). Spearman correlations among TFA concentrations, soil property, and function were estimated using the R package 'psych' (Revelle, 2010), and  $r$  and  $p$  values adjusted for false discovery rate (FDR) were presented using the R package 'corrplot' (Wei & Simko, 2017), and all plots were produced with the package 'ggplot2' (Wickham, 2016).

## 3 | RESULTS

### 3.1 | TFA treatments decreased soil respiration, pH, litter decomposition, and bacterial abundance

Soil respiration in week 3 was not affected by TFA treatments (Figure S1), while overall levels of respiration were lower in week 6, and a significant effect was observed at TFA concentrations higher than  $10 \mu\text{g g}^{-1}$  ( $p < 0.05$ , Figure 1a). At the highest concentration, TFA exerted the most negative effect ( $-62.4 \mu\text{M M}^{-1} \text{h}^{-1}$ , 95% CI [ $-89.8$  to  $-27.3 \mu\text{M M}^{-1} \text{h}^{-1}$ ]). TFA treatments tended to decrease soil pH, and this effect was significant at only high concentrations (i.e., 10 and  $100 \mu\text{g g}^{-1}$ ,  $p < 0.01$ , Figure 1b). Particularly, at the highest concentration, there was a sharp decrease and the effect size was  $-0.381$  (95% CI:  $-0.315$  to  $-0.462$ ). We observed that TFA treatments significantly decreased litter decomposition at already low concentrations of  $1 \mu\text{g g}^{-1}$  ( $p < 0.001$ , Figure 1c), where there were



**FIGURE 1** Effects of trifluoroacetic acid on soil respiration (a), soil pH (b), litter decomposition (c), and bacterial abundance (d). In the first row of each panel, raw data are presented as both scattered points and the corresponding mean and 95% confidence intervals (CIs;  $N = 10$ ). In the second row, estimation plots present the unpaired mean difference between each treatment and the shared control. Circles in grey and triangles (arrowhead down) in black represent neutral and negative effects, respectively. \*, \*\*, and \*\*\* represent the significance levels of 0.05, 0.01, and 0.001, respectively.

no effects on pH (Figure 1b), and the most negative effect was found at  $100 \mu\text{g g}^{-1}$  ( $-1.87\%$  [95% CI:  $-2.48\%$  to  $-1.34\%$ ]). There was a tendency for TFA treatments to decrease soil bacterial abundance over all tested concentrations, and this effect was significant at the highest concentration ( $100 \mu\text{g g}^{-1}$ ,  $p < 0.05$ , Figure 1d). The dose-response curves of those four parameters are presented in Figure S2, and corresponding  $EC_{10}$  and  $EC_{50}$  values are shown in Table S1.

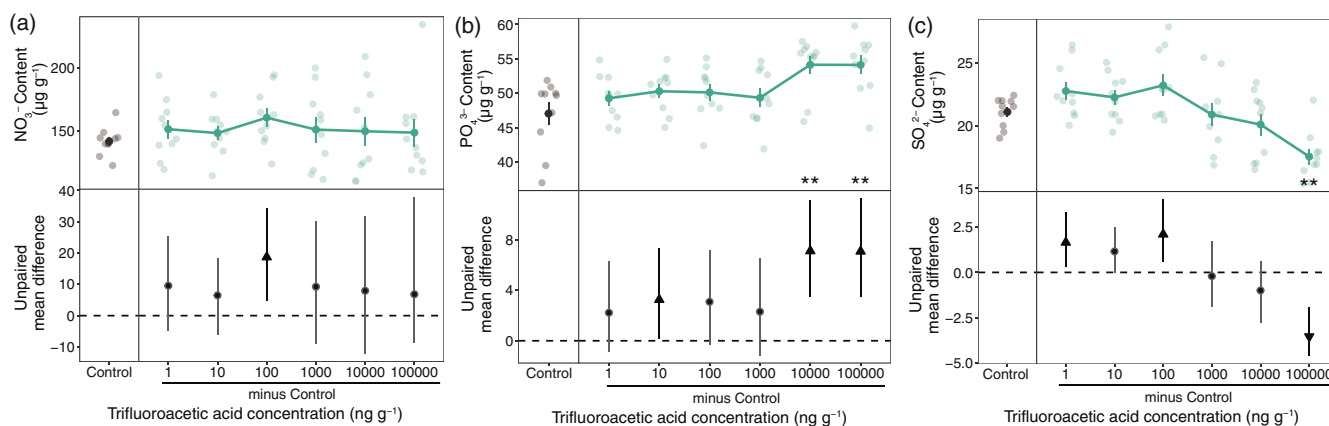
### 3.2 | Effects of TFA on soil soluble nutrients

TFA treatments did not significantly affect soil extractable  $\text{NO}_3^-$  content (Figure 2a), but significantly changed

$\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  content at high concentrations (Figure 2b,c). At 10 and  $100 \mu\text{g g}^{-1}$ , TFA significantly increased  $\text{PO}_4^{3-}$  content in soil ( $p < 0.01$ ), with the effect size of  $7.10 \mu\text{g g}^{-1}$  (95% CI: 3.44–11.09) and  $7.07$  (95% CI: 3.48–11.26), respectively. The significant difference in  $\text{SO}_4^{2-}$  content was observed only at  $100 \mu\text{g g}^{-1}$  of TFA ( $p < 0.01$ ), with the effect size of  $-3.55 \mu\text{g g}^{-1}$  (95% CI:  $-4.64$  to  $-1.94$ ).

### 3.3 | Limited effects on soil enzymes, aggregation and fungal abundance

Activities of four enzymes, that is,  $\beta$ -glucosidase and  $\beta$ -D-1,4-cellobiosidase,  $\beta$ -1,4-*N*-acetyl-glucosaminidase, and phosphatase, were not significantly affected by TFA



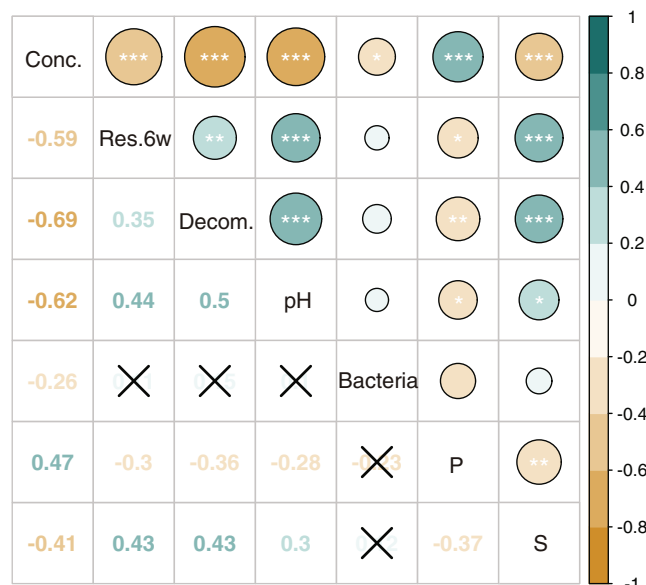
**FIGURE 2** Effects of trifluoroacetic acid on soil soluble nutrients, nitrate (a), phosphate (b), and sulfate (c). In the first row of each panel, raw data are presented as both scattered points and the corresponding mean and 95% confidence intervals (CIs;  $N = 10$ ). In the second row, estimation plots present the unpaired mean difference between each treatment and the shared control. Circles in grey and triangles (arrowhead up and down) in black represent neutral, and positive and negative effects, respectively. \*\* represents the significance level of 0.01.

treatments (Figure S3), nor were water-stable aggregates (Figure S4A), not even at the highest TFA concentration ( $100 \mu\text{g g}^{-1}$ ). In addition, soil fungal populations were not impacted by TFA treatments (Figure S4B).

## 4 | DISCUSSION

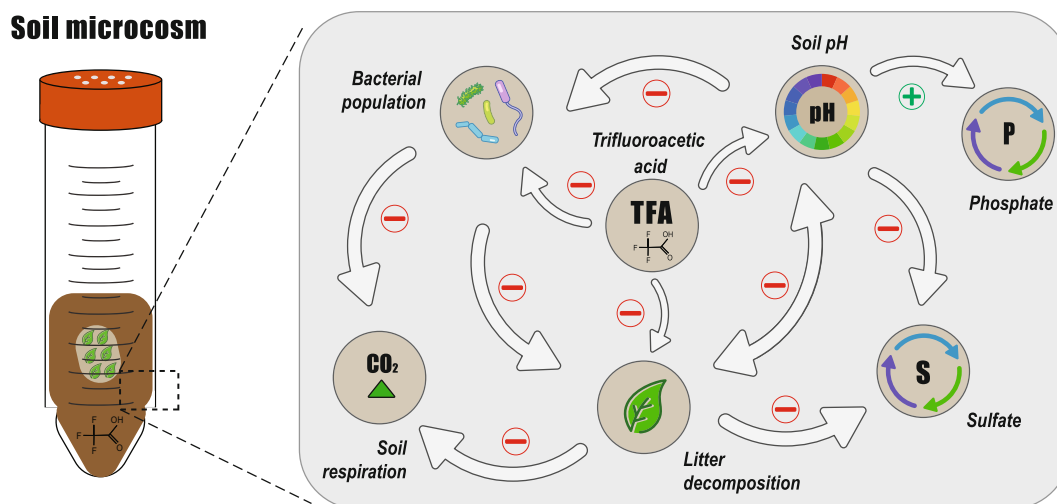
### 4.1 | Significant concentration-dependent response to TFA treatments

All notable responses had significant correlations with TFA concentrations (Figure 3), that is, soil respiration in week 6 ( $r = -0.59$ ,  $p < 0.001$ ), litter decomposition ( $r = -0.69$ ,  $p < 0.001$ ), soil pH ( $r = -0.62$ ,  $p < 0.001$ ), and bacterial abundance ( $r = -0.26$ ,  $p < 0.05$ ), phosphate ( $r = 0.47$ ,  $p < 0.01$ ), and sulphate ( $r = -0.41$ ,  $p < 0.001$ ), showing an apparent dose-dependent response to TFA treatments. In other words, within a wide range of TFA concentrations, where there was a significant effect on soil property and function, and there was an apparent concentration-dependent response to TFA treatments (Figure 3). In fact, such a dose-response relationship is important for evaluating the (eco)toxicity of chemicals on organisms, and whether such a relationship is valid might also depend on the test concentrations. In contrast to this result here, we did not find a clear concentration-dependent effect of long- and short-chain PFAS on soil function in a previous study (Xu et al., 2022), which probably partially resulted from the use of a different concentration range ( $1$ – $1000 \text{ ng g}^{-1}$ ) to detect such a dose-response, compared to the concentrations ( $1$ – $1,000,000 \text{ ng g}^{-1}$ ) used in the present



**FIGURE 3** Spearman correlation among trifluoroacetic acid concentrations and proxies for soil property and function. Significance levels of 0.05, 0.01, and 0.001 are presented as \*, \*\*, and \*\*\*, respectively. Conc., trifluoroacetic acid; res.6w, soil respiration at the 6th week; Decom., litter decomposition; pH, soil pH; bacteria, soil bacterial abundance; P, phosphate; S, sulphate. Adjusted  $p$  values and correlations of all variables are presented in Table S2 and Figure S5, respectively.

experiment. Previous studies also reported an obvious dose-dependent response of earthworms and soil microbial activity to various PFAS within the concentration of up to  $1$ – $100 \mu\text{g g}^{-1}$ , and  $100$ – $1600 \mu\text{g g}^{-1}$ , respectively (Cai et al., 2019; Zareitalabad et al., 2013).



**FIGURE 4** Schematic illustration of trifluoroacetic acid (TFA) effects on soil health in the test soil microcosm. TFA treatments were likely to directly change soil pH, litter decomposition, and soil bacterial abundance, while soil soluble nutrient contents and respiration were indirectly affected. The plus sign in green and minus sign in red represent the positive and negative effects, respectively.

#### 4.2 | Relationships among soil pH, bacterial abundance, soil respiration, and litter decomposition affected by TFA treatments

Given the nature of microbial drive in our soil microcosms, we considered soil bacterial abundance as the main driver of soil process and function. However, it was refuted by insignificant correlations of bacterial numbers with other proxies (Figure 3). Instead of the changes in the total population of soil bacteria, the shift in bacterial community composition and structure might play a more important role in influencing the measured soil processes and properties. Unfortunately, the effects of TFA on soil microbial community currently remain unknown, while as a member of the PFAS family, its potential to change microbial communities can be expected, as evidenced by other effects produced by PFAS (Qiao et al., 2018; Xu et al., 2021). In terms of the tested soil parameters and functions, TFA treatments were likely to directly change soil pH, litter decomposition, and soil bacterial abundance, while soil soluble nutrient contents and respiration were indirectly affected (Figure 4).

As a strong organic acid, TFA significantly decreased pH in soil solution at 10 and 100  $\mu\text{g g}^{-1}$ , but not at lower TFA concentrations, for which we have already observed biological effects (i.e., litter decomposition). The complex composition of the soil matrix makes it resistant to changes in pH of soil solution through cation exchange and protonation on clay and organic colloids (Weil & Brady, 2017). However, this buffering effect is limited. Soil acidity is a state variable affecting a wide range of soil chemical and biological properties, as well as soil

microbial communities and activities (Weil & Brady, 2017). Rousk, Bååth, et al. (2010) and Rousk, Brookes, and Bååth (2010) reported that soil pH was significantly positively correlated with bacterial gene copy number in arable soils, and lower-pH soil contained lower bacterial abundance. This supported the point that the strong acidity of TFA likely contributed to the decreased bacterial population at the highest concentration of TFA. Over the test concentrations, however, there was not a significant correlation between pH values and bacterial population ( $p > 0.05$ ), while bacterial abundance was significantly associated with TFA concentrations ( $r = -0.26$ ,  $p < 0.05$ , Figure 3), which means that soil bacterial abundance was more likely driven by TFA treatments (trifluoroacetic anions), rather than indirectly by soil pH changes ( $\text{H}^+$  ions).

Soil respiration is one measure of microbial activity and organic matter decomposition. In our test soil systems, the inhibited soil respiration indicated the microbial respiration in the litter bag and in the soil was decreased by TFA treatments (Figure 4), and the significant correlation of soil respiration with litter decomposition ( $r = 0.35$ ,  $p < 0.01$ ), but not with bacterial abundance ( $p > 0.05$ , Figure 3) suggested that the decreased litter decomposition might account for the major changes in soil respiration.

Litter decomposition is governed by soil microbiota in this soil microcosm, including bacteria and fungi, in which usually bacteria play a vital role in neutral or alkaline soils, while fungi do in acid soils (Rousk, Brookes, & Bååth, 2010). Correspondingly, we observed a decrease in bacterial populations in the test pH-neutral soil, which partly explained the decline in decomposition rate, since

there was little evidence that litter decomposition was associated with soil bacterial abundance ( $p > 0.05$ , Figure 3). Remarkably, there was clear evidence that litter decomposition was correlated to soil pH ( $r = 0.50$ ,  $p < 0.001$ ). However, effects might be caused by TFA or by litter decomposition. On the one hand, litter decomposition is usually investigated as a response to environmental factors, and soil pH can affect litter decomposition. Previous studies have shown that a lowered soil pH affected by acid rain apparently decreased litter decomposition (Liu et al., 2017; Lv et al., 2014). On the other hand, litter decomposition also can change soil pH. It was reported that the decomposition of plant residues increased soil pH values, as a consequence of the release of alkalinity and ammonification during decomposition (Sparling et al., 1999; Xu et al., 2006). Therefore, soil pH changes could be a consequence of decreased litter decomposition, that is, the decreased decomposition (e.g., blank control) possibly leading to the higher soil pH values compared with the promoted decomposition (e.g.,  $100 \mu\text{g g}^{-1}$ ).

The ultra-short-chain PFAS, TFA, appeared to induce changes in soil properties and functions that differed from the effects of long- and short-chain PFAS. Our recent research has shown that PFOS, PFOA, and perfluorobutanesulphonic acid (PFBS) significantly elevated litter decomposition and soil pH in a similar experimental system (Xu et al., 2022). It is interesting to note the contrasting results among these PFAS, and it also might imply that PFAS containing various chain-length are likely to cause a contrasting impact on soil health, an assertion that needs validating in future studies. In fact, PFBS ( $\text{p}K_a = -3.31$ ) has stronger acidity than TFA ( $\text{p}K_a = 0.23$ ), while significantly increasing soil pH. Although we cannot explain the mechanism underpinning this difference here, this observation could give some evidence that the pronounced effect might not be attributed to the acidity of TFA.

#### 4.3 | Effects of TFA on soluble nutrients associated with changes in soil pH and decomposition

Microbially-driven decomposition in the test soil microcosm was the main driver of nutrient transformation and cycling. During the decomposition of organic matter, organic nutrients would be transformed into inorganic forms that are available to organisms. The reduced content of  $\text{SO}_4^{2-}$  resulting from TFA treatments is likely due to the decreased litter decomposition and subsequent release of sulphate (Figure 4), because of the significant positive correlation between litter decomposition and  $\text{SO}_4^{2-}$  content ( $r = 0.43$ ,  $p < 0.001$ , Figure 3). Meanwhile,

the availability of sulphate might also be affected by soil pH, as indicated by the significant correlation between soil pH and sulphate ( $p < 0.01$ , Figure 3).

The increased content of extractable  $\text{PO}_4^{3-}$  might not be associated with the decomposition process, but was likely due to the change in soil acidity. Adsorption and desorption processes are the most important mechanism controlling the immobilisation and release of phosphate, which are highly dependent on soil pH (Gustafsson et al., 2012). Soil pH was decreased to around 6.5 in the 10 and  $100 \mu\text{g g}^{-1}$  TFA treatments (Figure 1b), and it is known that P has maximum availability near pH 6.5 (Penn & Camberato, 2019; Weil & Brady, 2017). The increased acidity in the test soil probably enhanced the desorption and solubility in the soil solution (Figure 4). Therefore, the increased  $\text{PO}_4^{3-}$  content is likely ascribed to the lowered soil pH by TFA treatment at high concentrations. This is also supported by the result that the significant effects on both soil pH and  $\text{PO}_4^{3-}$  content by TFA treatments were only observed at 10 and  $100 \mu\text{g g}^{-1}$ , and the two variables were significantly correlated ( $r = -0.28$ ,  $p < 0.01$ , Figure 3).

#### 4.4 | Limited effects on soil enzymes, aggregation and fungal abundance

Although TFA treatments significantly decreased litter decomposition, the C-related enzymes (i.e.,  $\beta$ -glucosidase and  $\beta$ -D-1,4-cellobiosidase) were only marginally affected. Soil enzyme activities were not correlated with microbial community composition structure and function (Wei et al., 2020), even though different microorganisms have varying environmental responses, enzyme capability, and metabolic features (Berg & McClaugherty, 2014). The relationship between litter decay, respiration, inorganic nutrients and enzyme activities can be highly dynamic, meaning that these soil processes would not simply and directly be reflected by enzyme activities (Moorhead et al., 2013). Impacts of PFAS on soil fungi populations and communities have been insufficiently investigated so far. Although we find that fungal abundance was not influenced by ultra-short PFAS, namely TFA, it is still possible that fungal community composition was affected, which might become important, particularly in acid soils where fungi often dominate organic matter decomposition and nutrient cycling (Rousk, Bååth, et al., 2010; Rousk, Brookes, & Bååth, 2010).

#### 4.5 | Limitations and future perspectives

Our study has captured the effects of TFA on soil that include any potential effects mediated by this substance



being an acid; this is important because any such effects would also unfold in the environment. However, as our experiment did not control for pH effects, the effects derived from acidity and trifluoroacetic anions cannot be clearly distinguished, particularly at high levels of TFA. Importantly, the effects of trifluoroacetate that are not mediated by pH were also found in this study. For example, at  $1 \mu\text{g g}^{-1}$ , TFA treatment did not significantly change soil pH, while it clearly decreased litter decomposition, which showed that trifluoroacetic anions impacted soil functions. Additionally, the stronger correlations of TFA concentrations with soil respiration, litter decomposition and bacterial abundance than those of soil pH suggested more direct effects on soil parameters and functions from TFA rather than from soil pH changes.

Having shown here effects of TFA on soil processes and functions, future research is now needed to address the following issues. Firstly, since TFA is a strong acid, additional controls including acid control (e.g., HCl) and trifluoroacetic anion control (e.g., potassium trifluoroacetate) are needed to disentangle the effect of changes in pH or the associated polyfluoroalkyl anions. This is also applicable to other acidic substances in the PFAS family (e.g., PFBS). Next, only one sandy loam soil with low C content (1.87%) was used in this study, but responses likely vary with different soil properties. For example, soils with higher organic matter, Al and Fe content showed strong sorption for TFA (Richey et al., 1997), and thus bioavailability in those soils might be different, leading potentially to a changed response in soil processes. Therefore, future priorities should include testing a range of soil types to obtain a more comprehensive picture of TFA effects on soil health in a range of terrestrial ecosystems.

## 5 | CONCLUSIONS

In our six-week microcosm experiment, we found that TFA significantly affected chemical and biological proxies of soil health and function, including soil pH and soluble nutrients, soil respiration, litter decomposition and bacterial abundance. These changes were dependent on TFA concentrations and partially associated with pH changes, attributed to the acidity of TFA. Trifluoroacetic acid affected litter decomposition at a concentration for which soil pH was not changed, which indicated that trifluoroacetic anions were likely to impact soil function in the absence of any pH changes. Soil physical structure, enzyme activities and fungal abundance were not significantly affected by TFA within the test concentrations. It should be noted that the observed effect concentration of TFA might be 1–2 orders of magnitude higher than the

currently reported environmental levels, but such concentrations could be possible in future scenarios of continued accumulation of these chemicals or after accidental spills of TFA. Considering current reported environmental levels, the decomposition of organic matter was significantly suppressed at currently environmentally-relevant concentrations of TFA (i.e.,  $1 \mu\text{g g}^{-1}$ ). These results indicate that soil-borne microbial processes can be affected by the accumulation of the ‘forever chemical’ TFA, at least in the tested soil.

## AUTHOR CONTRIBUTIONS

**Baile Xu:** Conceptualization (equal); data curation (equal); funding acquisition (equal); investigation (equal); methodology (equal); writing – original draft (lead). **Rabea Alizray:** Data curation (equal); investigation (equal); writing – review and editing (equal). **Daniel R. Lammel:** Investigation (supporting); visualization (supporting); writing – review and editing (equal). **Sebastian Riedel:** Conceptualization (equal); resources (equal); writing – review and editing (equal). **Matthias C. Rillig:** Conceptualization (equal); funding acquisition (equal); resources (equal); writing – review and editing (equal).

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## CONFLICT OF INTEREST

Authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

All data used for analyses and plotting are available online and can be accessed at <https://doi.org/10.6084/m9.figshare.19102586>.

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## SUPPORTING INFORMATION

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