Effects of perfluoroalkyl and polyfluoroalkyl substances (PFAS) on soil structure and function

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ABSTRACT

Soils are impacted globally by several anthropogenic factors, including chemical pollutants. Among those, perfluoroalkyl and polyfluoroalkyl substances (PFAS) are of concern due to their high environmental persistence, and as they might affect soil structure and function. However, data on impacts of PFAS on soil structure and microbially-driven processes are currently lacking. This study explores the effects of perfluorooctanesulfonic acid (PFOS), perfluoroctanoic acid (PFOA) and perfluorobutanesulfonic acid (PFBS) at environmental-relevant concentrations on soil health, using a 6-week microcosm experiment. PFAS (even at 0.5 ng g\textsuperscript{-1} for PFBS) significantly increased litter decomposition, associated with positive effects on β-glucosidase activities. This effect increased with PFAS concentrations. Soil pH was significantly increased, likely as a direct consequence of increased litter decomposition affected by PFAS. Soil respiration was significantly inhibited by PFAS in week 3, while this effect was more variable in week 6. Water-stable aggregates were negatively affected by PFOS, possibly related to microbial shifts. PFAS affected soil bacterial and fungal abundance, but not microbial and certain enzyme activities. Our work highlights the potential effects of PFAS on soil health, and we argue that this substance class could be a factor of environmental change of potentially broad relevance in terrestrial ecosystem functioning.

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1 Introduction

Human activity is progressively and fundamentally affecting the Earth's surface, including soils, which operate at the interface between biosphere, hydrosphere, atmosphere, and lithosphere, and are suffering from physical, chemical, and biological stressors related to anthropogenic activities (Morgado et al., 2018; Zhu and Penuelas, 2020). One significant category of these influences is chemical pollution, which is widely recognized as a global change factor (Zhu and Penuelas, 2020; Rillig et al., 2021). Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a highly diverse family of chemicals of concern (Lim, 2019). These compounds contain the perfluoroalkyl moiety \( \text{C}_n\text{F}_{2n+1}^- \) (Buck et al., 2011), and the relatively high-energy carbon-fluorine (C–F) bonds make them extremely resistant to breakdown, and subsequently persistent in the environment. Therefore, they are also called “forever chemicals” in public discourse (Beans, 2021). Certain PFAS, perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are on the list and on the waiting list of persistent organic pollutants (POPs) under the Stockholm Convention (UNEP, 2019), respectively.

Soil is a major sink for persistent organic chemicals in the environment. There are many pathways for PFAS entering the soil environment. Typically, fluoride factory emission, sludge application, the degradation of aqueous film-forming foam, and landfill leachates constitute direct sources, and atmospheric deposition and runoff constitute non-point sources (Cai et al., 2021; Ma et al., 2022). PFAS have been widely detected in soils with a broad range of concentrations. Generally, PFAS concentrations in non-hotspot soil are lower than 300 ng g\(^{-1}\), while in hotspots of PFAS-contaminated soil, they can be as high as several or even tens of \( \mu \text{g g}^{-1} \), mostly dominated by PFOA and PFOS (Brusseau et al., 2020). Jin et al. (2015) also reported that perfluorobutanesulfonic acid (PFBS) was a prominent type of PFAS in the soil around a fluoride-factory park.

Existing evidence has demonstrated that PFAS can exert some impacts on soil functions, in particular on soil enzymes, and microbial activities and communities, and these influences were closely related to properties of soil and PFAS (Cai et al., 2021). According to the number of carbon atoms, perfluoroalkyl carboxylic acids with 7 or more and perfluoroalkyl sulfonate with 6 or more carbons are categorized as long-chain PFAS, otherwise, short-chain PFAS (Buck et al., 2011). Short-chain PFBS might activate sucrose and urease activities, while long-chain PFOS might reduce these activities in soil (Qiao et al., 2018). Cai et al. (2019) showed that PFAS with sulfonic groups and longer chains had higher toxicity to soil microbial activities, and that soil with higher organic matter content and higher pH (neutral) exhibited lower impact by PFAS. Differences in sorption affinity of PFAS to soil are likely to regulate the impact of PFAS on soil microbes (Cai et al., 2021). Changes in structure and function of microbial communities by PFAS were shown by previous research (Qiao et al., 2018; Cai et al., 2020; Chen et al., 2020; Xu et al., 2021), and these shifts were suggested to affect soil processes and ecosystem functions. However, the implications on process rates in soil, for example, litter decomposition and soil aggregation, were not addressed.

Given the persistent nature of PFAS, it is important to explore if these chemicals can affect soil process rates and properties. Here, we investigate effects of three PFAS (i.e., PFOA, PFOS, and PFBS) on soil processes, including soil respiration, litter decomposition, soil aggregation, enzyme and microbial activities, as well as microbial population. We discuss the environmental implications of our results and suggest that PFAS be considered as a global change factor of importance in terrestrial ecosystems.

2 Materials and methods

2.1 Test soil and PFAS

The test soil was Albic Luvisol (IUSS Working Group WRB, 2015) collected at the agricultural field station of Freie Universität Berlin (52°28' N, 13°18’ E) in December 2020. The soil has a sandy loam texture (73.6% sand, 18.8% silt and 7.6% clay), with 1.87% total C, 0.12% total N and a soil pH (in water) of 5.9 (Rillig et al., 2010; Lehmann et al., 2020). Fresh soil samples were thoroughly mixed, passed through a 2-mm sieve, and then stored at 4°C.

Three PFAS, namely PFOS, PFOA and PFBS were selected in this study due to their wide occurrence in the soil environment (Ahmed et al., 2020). Values of their octanol-water partition coefficients (Log \( K_{ow} \)) are 5.26, 4.59 and 2.73, respectively (Milinovic et al., 2015), and their chemical structures and other physicochemical properties are listed in Supporting Information (SI) Table S1.

2.2 Experimental setup

PFAS standards were dissolved in sterilized deionized water to prepare the stock solution with the concentration of 100 mg L\(^{-1}\). A five-gram portion of previously sterilized soil samples (121°C, 20 min, twice) was supplemented with appropriate doses of PFAS in solution (we used this sterilized ‘loading soil’ to avoid any exaggerated effects on soil communities (Rillig et al., 2019)), and thoroughly mixed this soil with 25 g of soil by manual stirring for 2 min. A total of 30 g soil was placed in 50-mL mini-bioreactor tubes (Corning Inc., Corning, USA) with vented lids to establish experimental microcosms. Each tube was watered to 70% soil water holding capacity (WHC) with deionized water. The nominal concentrations of PFOS and PFOA in soil were 1, 10, 100 and 1000 ng g\(^{-1}\), and that of PFBS were 0.5, 5, 50 and 500 ng g\(^{-1}\), corresponding to their environmentally-relevant levels (Brusseau et al., 2020). The analytical method and actual concentrations of PFAS in soil are reported in SI Text S1, and Table S2, respectively. Tubes were
placed in a randomized fashion inside a dark temperature-controlled incubator at 20 °C for 6 weeks, and each tube was watered weekly to maintain soil moisture. This experiment ran with 10 replications of blank control (without any PFAS added, but handled exactly the same way) and 8 replications of each treatment, for a total of 106 microcosms.

2.3 Proxies for soil health

We measured well-established proxies for soil health, including soil respiration, litter decomposition, soil pH, enzyme activities, soil aggregates, and soil bacterial and fungal abundance. Soil respiration was measured with an infrared gas analyzer (LI-6400XT, LI-COR Inc., Bad Homburg, Germany), and litter decomposition was determined by the mass loss of tea bags (Lehmann et al., 2020). Soil pH was measured in deionized water with a 1:5 ratio by a pH meter (Hanna Instruments, Smithfield, USA). Soil enzymes activities were measured, including four enzymes concerning C (β-glucosidase and β-D-1,4-cellobiosidase), N (β-1,4-N-acetyl-glucosaminidase), and P (phosphatase) cycling, and fluorescein diacetate hydrolase (FDA) representing general soil microbial activity. Water-stable aggregates, as the basic unit of soil structure, were qualified using a wet-sieving apparatus (Eijkelkamp, Giesbeek, Netherlands) with the package “gghost” (Wickham, 2016). Soil DNA was extracted with DNeasy PowerSoil Pro Kit (QIAGEN GmbH, Germany) following the technical protocol, and we amplified using the universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') for soil bacteria, and primers FungiQuant-F (5'-GGRAAACTCACCAGGTCCAG-3') and FungiQuant-R (5'-GSWCTATCCACCACGAGCAGA-3') for soil fungi (Liu et al., 2012) with quantitative polymerase chain reactions (qPCR) in a CFX 96 Real-Time System (Bio-Rad Laboratory, Hercules, USA). For more information on measurement procedures, qPCR conditions and quality control, see SI Text S2.

2.4 Statistical analysis

All statistical analyses and data visualization were performed in R (R Core Team, 2020). The effects of PFAS treatment (four concentrations per PFAS type) on soil functions were tested with a two-step method. First, we calculated the 95% confidence interval (CI) of unpaired mean differences (treatment minus control) using the R package “dabestr” (Ho et al., 2019). This approach focuses on the effect size and its precision, and can avoid the pitfalls of significance testing. Secondly, One-way analysis of variance (ANOVA) followed by Dunnett’s test in the R package “multcomp” was implemented to compare each treatment with the control (Hothorn et al., 2008). Model residuals were checked for heteroscedasticity and normal distribution. Spearman correlations among actual concentrations of PFAS and soil structure and function were performed with the package “corrplot” (Wei and Simko, 2017). Adjusted p values by a single-step method are reported in the SI Table S3. All plots were generated with the package “ggplot2” (Wickham, 2016).

3 Results and discussion

3.1 PFAS increased litter decomposition and soil pH

Positive effects of three PFAS on litter decomposition were observed (Fig. 1A), and PFBS, particularly, at all tested concentrations significantly increased the decomposition rate (p < 0.05, Table S3). Regardless of tested type, increasing concentrations of PFAS significantly enhanced litter decomposition (R = 0.22, p = 0.023, Fig. 1B). In terms of PFAS type, PFOA and PFBS resulted in significantly positive effects on litter decomposition (p < 0.01, Table S3), and PFBS exerted the most remarkable positive effect (Fig. 1C). The treatment of all PFAS significantly increased soil bacterial abundance (F = 1.87, p = 0.049) and decreased fungal abundance (F = 2.74, p = 0.047) in terms of copy number per gram of dry soil, but single PFAS did not show any significant difference to the control (Figures S1 and S2, Table S3). Among three PFAS, it appears that PFBS also caused the most obvious effects on both bacterial and fungal abundance (Figures S1 and S2).

We also observed that soil pH was increased in PFAS treatments (Fig. 2A), and PFOA at 100 ng g⁻¹ and PFBS at all tested concentrations significantly increased soil pH values (p < 0.05, Table S3). Irrespective of concentrations, PFOA (p = 0.022) and PFBS (p < 0.001) significantly increased soil pH (Figure S3). There was moderately strong evidence that soil pH was positively correlated with PFAS concentrations regardless of tested type (R = 0.20, p = 0.045, Fig. 2B), while there was clear evidence that soil pH was positively affected by litter decomposition (R = 0.22, p < 0.001, Fig. 2C). Refer to Muff et al. (2022) for the interpretation of p values.

Microorganisms, including bacteria and fungi, play an essential role in the biological decomposition of organic matter in soil, a process in which bacteria generally predominate in neutral or alkaline soils, while fungi are more important in acidic soils (Rousk et al., 2010; Valentín et al., 2013). In this study, we found that in our moderately acidic soil with various PFAS treatments, there were no significant correlations between litter decomposition and bacterial abundance (R = 0.16, p = 0.11, Figure S4A) or fungal abundance (R = –0.11, p = 0.29, Figure S4B). Despite that, soil microbial community composition and structure can be altered by PFAS treatments (Qiao et al., 2018; Zhang et al., 2020; Xu et al., 2021), which might further affect organic matter decomposition.

Given the acidity of PFAS, it is surprising that PFAS treatments increased soil pH instead of decreasing it, particularly the strong acid PFBS (pKₐ = –3.31). As a matter
of fact, there are two possible explanations for this phenomenon. One is that we only applied a small amount of PFAS in soil, and it can be expected that PFAS would not change soil pH significantly considering the soil pH-buffering capacity. A recent study also showed that soil pH was not altered by PFOA and PFOS at the final concentration of 1500 ng L\(^{-1}\) in soil solution, an experiment where there was not exogenous organic matter addition (Xu et al., 2021). Another possibility is that the increased soil pH was likely associated with the increased litter decomposition rather
than with PFAS per se, as evidenced by the stronger correlations of soil pH with litter decomposition than with PFAS concentrations (Fig. 2). Previous studies have demonstrated that decomposition of plant residues (particularly leaves) can increase soil pH, through the release of alkalinity derived from decomposition of organic anions, and ammonification of N in residues (Sparling et al., 1999; Xu et al., 2006). Therefore, we believe that pH changes were an indirect consequence of PFAS treatment, but a direct one of increased litter decomposition, and also pH changes in this microcosm were not a driving factor of litter decomposition.

Response of soil bacteria to PFAS treatments seems varied in different studies. A recent study showed that PFOA and PFOS reduced soil bacterial gene abundance in an acidic soil over a 90-day incubation (Xu et al., 2021). We think that a possible reason is that we employed litter bags in our microcosms, and there might be interactive effects of PFAS and organic matter on soil microorganisms. This inference needs further confirmation in future research. Previous studies attributed the increased bacterial biomass in soil receiving organic pollutants to the potential consumption of chemicals as a carbon (Zhang et al., 2008). Given the fact that PFAS compounds can barely be consumed by soil microbiota naturally, the reason for increasing bacterial
abundance remains unclear. Future studies on shifts in soil microbial community composition responding to PFAS in soil with and without litter bags may give insights into the microbial processes occurring in this experimental system.

Litter decomposition and the ensuing nutrient release, governing carbon and nutrient cycling, is a key process in terrestrial ecosystems (Berg and McClaugherty, 2014). Our results showed that PFAS had a positive effect on litter decomposition, and particularly the PFBS treatment, even at 0.5 ng g\(^{-1}\) in soil, resulting in a significant enhancement of litter decomposition and consequently of soil pH.

### 3.2 Soil respiration is inhibited by PFAS

We observed that the tested PFAS produced significantly negative effects on soil respiration in week 3 (Figs. 3A and S5), while more variable effects were present in week 6 (Figure S6). In week 3, PFOA and PFBS at all tested concentrations exerted negative effects on soil respiration, while effects of PFOS were dependent on its concentration (\(F = 5.46, p < 0.001\)). Irrespective of concentration, our tested PFAS had negative effects on soil respiration in week 3 (Figure S6C). However, this negative effect was significant only at its highest concentration (500 ng g\(^{-1}\), \(p = 0.019\), Table S3).

Waning effects of organic compounds on soil respiration during the incubation period have been previously reported (Xiong et al., 2014; Zhang et al., 2019). For example, the fungicides tebuconazole and carbendazim significantly suppressed soil respiration during the first 30 days, while this effect was no longer present on the 90th day (Wang et al., 2016). Therefore, PFAS might act as other exogenous organic chemicals, inducing the time-dependent response of soil microorganisms (Zhang et al., 2019), that is, an inhibitory effect during the first stage, and gradual recovery from this inhibition during the incubation. Moreover, various PFAS indeed induced responses with different degrees, which might be associated with physicochemical properties of PFAS, and possible reasons are discussed in Section 3.5.

As for the correlation between litter decomposition and soil respiration, it is not necessarily positive, at least in this soil microcosm system. There are three possible explanations. First of all, it should be noted that the measured soil respiration did not strictly correspond to litter decomposition. Litter mass loss is the result of a cumulative process over the entire duration of the experiment, while respiration measurements were episodic point-measurements particularly at week 3. Secondly, the measured soil respiration consisted of microbial respiration in both litter bags and soil. A previous study has shown that respiration derived from litter only contributed less than 50% of total respiration (Xiao et al., 2014). This means that even though microbes in litter bags produced more CO\(_2\) through facilitating decomposition, it did not necessarily imply that the total CO\(_2\) would be increased since CO\(_2\) in soils probably was largely decreased by PFAS treatments. Thirdly, when soil microorganisms utilize organic matter, they might convert a higher proportion into microbial biomass rather than respiring it off (i.e., higher carbon-use efficiency) (Sokol et al., 2022). A shift in microbial communities by PFAS treatments might increase the relative abundance of decomposers with higher carbon-use efficiency. In this case, this effect might be related to the decomposition stage, since the effect on soil respiration was attenuated from the third to sixth week. In fact, our previous studies have also demonstrated that the insecticide, neonicotinoid, increased litter decomposition, but decreased soil respiration in the same microcosm (Rillig et al., 2019).

### 3.3 Long-chain PFOS suppressed water-stable aggregates

Although effects of PFOS treatments on soil aggregate stability at the single concentration were insignificant (Fig. 3B, Table S3), PFOS treatment significantly decreased water-stable aggregates irrespective of concentration (\(p = 0.044\), Figure S7C).

Fungi are likely to play more important roles in the formation of macroaggregate (250–2000 μm), while bacteria contribute more to microaggregate stability (53–250 μm) (Lynch and Bragg, 1985). In our measurements, we tested the soil aggregates larger than 250 μm. Although soil aggregates were not significantly correlated to fungal abundance (Figure S8), there might be shifts in fungal community composition and structure, which possibly affected the stability of soil aggregates. Unfortunately, the impact of PFAS on soil fungi community is largely ignored.

### 3.4 Limited effects on soil enzyme and microbial activities

Four enzymes were not significantly affected by individual PFAS treatments (Figures S9–S12), nor the general microbial activities (Figure S13, Table S3), but regardless of concentration, PFBS significantly increased β-glucosidase activity (\(p = 0.029\), Figure S11C).

Measuring enzyme activities provides evidence on how soil biochemical processes might be affected. β-glucosidase is responsible for catalyzing the hydrolysis of cellobiose (a product of cellulose breakdown) to glucose (German et al., 2011). We observed a positive trend on β-glucosidase by PFAS, particularly PFBS, which probably contributed to the litter decomposition by PFAS. The only significant effect on β-glucosidase corresponded with the most marked effect on litter decomposition by PFBS. Additionally, there was a significant correlation between decomposition rate and β-glucosidase activity (\(R = 0.29, p = 0.002\), Fig. 4).

Previous studies reported that soil dehydrogenase (proxy for total microbial activity), urease and sucrase activities were only insignificantly impacted by PFOA and PFOS with concentrations lower than 10 μg g\(^{-1}\) (He et al., 2016; Qiao
et al., 2018). Cai et al. (2019) also reported that microbial activity was barely affected by PFAS at 100 μg g⁻¹ in selected soils. Changes in enzyme activities are highly dynamic processes (Qiao et al., 2018; Zhao et al., 2021), and thus insignificant effects observed at harvest do not necessarily indicate that there were no remarkable changes during the incubation. In addition, there was no significant relationship between microbial activities (FDA) with other parameters (Figure S14). Overall, general microbial activities were affected only to a very limited degree.

3.5 Different effect sizes caused by three PFAS

The three PFAS examined here appeared to exert similar impact on soil microbes and functions, but with different effect sizes, which is likely related to their bioavailability and
bioaccumulation (Cai et al., 2021). Of the three PFAS, PFBS even at lower concentrations seemed to have the most remarkable impact on soil respiration, litter decomposition and soil bacterial abundance, while PFOS had a larger effect size on water-stable aggregates.

Sorption affinities of PFAS followed the order PFOS > PFOA > PFBS on soils with various soil textures and organic carbon contents, showing the same order of their hydrophobicity (Milinovic et al., 2015). With a low sorption affinity to soil particles, PFBS likely had an increased likelihood to interact with soil microbes, subsequently causing an impact. However, it is not a simple effect of hydrophobicity, because, for example, the higher hydrophobicity might result in higher bioaccumulation and hence exert higher toxicity in soil microorganisms (Qiao et al., 2018; Cai et al., 2019). This might explain the more apparent effect by PFOS on some processes via soil microbes.

3.6 Environmental implications and future perspectives

Within environmentally relevant concentrations, three PFAS, especially the short-chain PFBS, had a positive effect on litter decomposition in the tested soil. This effect indicates that the PFAS present in soils now might already affect ecosystem processes. The elevated decomposition might increase the release of carbon as CH$_4$ and dissolved organic carbon, affecting carbon sinks in soil (Dieleman et al., 2016). The precise mechanisms underpinning litter decomposition effects caused by PFAS need to be explored in further studies, with changes in microbial community composition likely playing a main role. On the basis of varied soil responses to PFAS treatments in this and other studies, further investigation on the potential interactive effects between exogenous organic matter and PFAS is warranted. In addition, we employed commercial green tea as a model litter, and different litter chemistry compositions may have contrasting responses to PFAS and also soil pH and nutrient cycling (Sparling et al., 1999; Tang and Yu, 1999). Effects are also likely influenced by soil and PFAS properties (Cai et al., 2019).

Soil aggregation is an essential feature of soil structure, principally driven by soil biota and their interactions (Lehmann et al., 2017). Our finding that certain PFAS negatively affected water-stable aggregates could indicate far-reaching consequences for soil health, given the many influences of soil structure on virtually all soil processes. Thus, future studies might explore these effects on the soil aggregation process in greater depth, including the formation, size distribution of soil aggregates and their intrinsic connections with soil biota.

4 Conclusions

Our study comprehensively analyzed PFAS impacts on soil structure and microbiologically-driven processes, which were largely neglected previously. The present results highlight the potential of PFAS to induce changes in soil properties and functions and we hope that our result inspires further studies that consider the impact of PFAS on soil ecosystem functions. We introduce the possibility of PFAS as persistent chemicals being a potential environmental change factor. The effects of various PFAS on soil functions should now be addressed in the context of global patterns of contamination.

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Conflicts of interest

We declare that there is no conflict of interest.

Availability of data and material

All data used for analyses and plotting are available online and can be accessed at https://doi.org/10.6084/m9.figshare.19772860.v1.

Electronic supplementary material

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Author contributions
B.X. and M.C.R. conceived the idea and designed experiments; B.X. conducted experiment and drafted the manuscript; G.Y. assisted in measurements of soil function; A.L. assisted in data analysis and presentation; S.R. assisted in the determination of PFAS concentrations; All authors contributed to the final version of this manuscript.

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