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Reference:

Groffen Thimo, Rijnders Jet, Verbrigghe Niel, Verbruggen Erik, Prinsen Els, Eens Marcel, Bervoets Lieven.- Influence of soil physicochemical properties on the depth profiles of perfluoroalkylated acids (PFAAs) in soil along a distance gradient from a fluorochemical plant and associations with soil microbial parameters

Chemosphere - ISSN 0045-6535 - 236(2019), UNSP 124407

Full text (Publisher's DOI): <https://doi.org/10.1016/J.CHEMOSPHERE.2019.124407>

To cite this reference: <https://hdl.handle.net/10067/1621850151162165141>

Accepted Manuscript

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PII: S0045-6535(19)31628-5

DOI: <https://doi.org/10.1016/j.chemosphere.2019.124407>

Article Number: 124407

Reference: CHEM 124407

To appear in: *ECSN*

Received Date: 24 May 2019

Revised Date: 17 July 2019

Accepted Date: 18 July 2019

Please cite this article as: Groffen, T., Rijnders, J., Verbrigghe, N., Verbruggen, E., Prinsen, E., Eens, M., Bervoets, L., Influence of soil physicochemical properties on the depth profiles of perfluoroalkylated acids (PFAAs) in soil along a distance gradient from a fluorochemical plant and associations with soil microbial parameters., *Chemosphere* (2019), doi: <https://doi.org/10.1016/j.chemosphere.2019.124407>.

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Influence of soil physicochemical properties on the depth profiles of perfluoroalkylated acids (PFAAs) in soil along a distance gradient from a fluorochemical plant and associations with soil microbial parameters.

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- Microbial activity
- Microbial biomass
- Soil Respiration

pH
Temperature
Organic carbon
Clay content

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Abstract

The widespread use of perfluoroalkylated acids (PFAAs) has led to a global presence in the environment, in which they accumulate and may cause detrimental effects. Although soils are known sinks for many persistent organic pollutants, still little is known on the behaviour of PFAAs in soils. Furthermore, studies that examine the relationships between PFAA concentrations and soil microbial parameters are scarce.

The 3M fluorochemical plant near Antwerp has been characterized as a PFAAs hotspot. In the present study, we examined the vertical distribution of 15 PFAAs and their associations with multiple physicochemical soil properties along a distance gradient from this hotspot. Additionally, we tested the relationships between PFAA concentrations in the top soil with soil respiration, microbial activity and microbial biomass.

Our results show that both perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) concentrations were elevated in the subsurface layer (up to 50 cm), after which concentrations decreased again, suggesting a downward migration of both analytes in the soil. This downward movement might pose a potential threat for the contamination of the groundwater and, consequently, organisms that rely on this water for consumption. The soil concentrations were influenced by multiple physicochemical properties of the soil, which suggests differences in bioavailability and sorption/desorption capacities between different soil types. We did not observe any influence of PFAA contamination in the top soil on microbial activity and biomass nor soil respiration.

Keywords

PFOS; PFOA; microbes; soil; vertical distribution; sorption

Capsule

47 The vertical distribution of PFAAs, which was influenced by multiple soil physicochemical properties but
48 had no influence on soil microbial parameters, suggests the downward movement of PFAAs in soil,
49 indicating a potential risk of groundwater contamination.

1. Introduction

Perfluoroalkylated substances (PFAAs) have been produced for almost seven decades, for use in a wide range of consumer products and industrial applications (Cousins et al., 2016). However, during the past decades, there has been a growing scientific attention and public concern towards these chemicals as a result of their toxicity in combination with their persistence and bioaccumulative potential (e.g. Conder et al., 2008; Giesy et al., 2010; Houtz et al., 2013). As a consequence, PFAAs have been detected globally in the environment, wildlife and even humans (e.g. Butt et al., 2010; Giesy and Kannan, 2001, 2002; Groffen et al., 2017, 2018, 2019a, 2019b, Houde et al., 2006; Lasters et al., 2019; Lopez-Antia et al., 2019; Miller et al., 2015).

Soils are known sinks for many persistent organic pollutants (POPs), including polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) (Cetin et al., 2017; Maqsood and Murugan, 2017; Mueller et al., 2006; Rankin et al., 2016; Xiao et al., 2016). However, the behaviour of PFAAs in the environment differs from other POPs due to their extreme surface-active properties (Goss and Bronner, 2006). PFAAs consist of a hydrophobic (oleophobic) perfluorinated carbon chain in combination with a hydrophilic functional group. This means that the behaviour of PFAAs, in contrast to other non-ionic POPs, is governed not only by hydrophobic interactions, but also by electrostatic interactions (Higgins and Luthy, 2007). Hence, the sorption of PFAAs cannot be predicted from a single soil property such as organic carbon (OC) content due to the complexity of the PFAAs chemistry and therefore there are still many uncertainties on how various physicochemical properties, such as pH, interact to determine the binding of PFAAs to soils (Li et al., 2018).

While it is not a sole linear predictor, organic carbon is one of the most important sorbents for PFAAs in soils (e.g. Milinovic et al., 2015). The sorption of PFAAs to soil organic matter (SOM) describes a nearly linear relation between sorption (described as the organic carbon-water partitioning coefficient (K_{oc}))

and the chain-length of the compounds (Labadie and Chevreuil, 2011), which is attributed to an increase in the hydrophobicity with each CF_2 moiety. Although the functional group of the PFAAs also affects the sorption (Ahrens et al., 2010; Campos Pereira et al., 2018; Higgins and Luthy, 2006), due to its electrostatic negativity (Du et al., 2014), chain-length is considered the dominating structural feature concerning the adsorption (Ahrens et al., 2010; Higgins and Luthy, 2006). Multiple studies have reported differences in PFAAs sorption between fractions of soil organic matter. For example, it has been reported that the humin fraction is the most important factor for the sorption of long-chained PFAAs (e.g. Campos Pereira et al., 2018; Zhang et al., 2015; Zhao et al., 2014), although the humic and fulvic acid fractions might also be important for the sorption of shorter-chained PFAAs (Campos Pereira et al., 2018).

Besides SOM, the sorption of PFAAs is promoted by a decreased pH and increased cation concentration (e.g. Higgins and Luthy, 2006; Chen et al., 2009; Wang et al., 2015; Zhang et al., 2013). The effects of pH on the adsorption of PFAAs to soil are typically described as due to protonation or deprotonation of the organic acids (Higgins and Luthy, 2006). However, pH-dependent changes in the sorbent, such as surface charge of SOM, may also explain the pH effects (Higgins and Luthy, 2006). The decrease in adsorption with increasing pH is possibly caused by the decrease of electrostatic interactions, rather than protonation or deprotonation of the sorbate (Chen et al., 2009; Higgins and Luthy, 2006; Wang et al., 2012). However, the influence of pH changes in the presence of a sufficient amount of divalent cations (Chen et al., 2009; Du et al., 2014). Adsorbent surfaces develop more basic sites to bind these cations when the pH increases, resulting in increased sorption of PFAAs (Du et al., 2014; Wang et al., 2012).

The clay content, although poorly studied, might also play a role in the sorption of PFAAs as soils with smaller particles, such as clay, will have more functional groups (e.g. hydroxyl and carboxyl groups) and thus more binding sites to facilitate the sorption of contaminants (Qi et al., 2014).

The environmental threats posed by PFAAs have received an increasing attention in recent years. The composition and activity of the soil microbial community, important attributes of the soil ecosystems, are of great significance to the maintenance of soil fertility and can indicate changes in environmental quality of the soil (Qiao et al., 2018). Therefore, the assessment of the ecological toxicity of soil pollutants, such as PFAAs, is of great importance. However, studies that examine the effects of PFAAs on microbial communities in the environment are scarce. Pasquini et al. (2013) investigated the impact of PFOA and PFOS on a laboratory strain of *E. coli* and on activated sludge from an urban wastewater treatment plant. They observed a total absence of toxicity at high PFOA and PFOS concentrations (up to 10^2 µg/L). In addition, they observed that both PFOS and PFOA significantly induced an increase of bound extracellular polymeric substances (EPS), a polymeric network that enables microorganisms to live at high cell densities under adverse conditions and thus ensure their survival, adsorbing pollutants, nutrients and minerals (Finlayson et al., 1998; Flemming and Wingender, 2001), in the activated sludge at PFOS and PFOA concentrations ≥ 0.1 µg/L. This indicated that the biomass in the sludge had to cope with new conditions and that the microbial biomass was sensitive to these pollutants. Contradictory results were reported by Ochoa-Herrera et al. (2016), who observed no toxic effects of PFOS and short-chained PFAAs to the methanogenic activity of anaerobic wastewater sludge.

The main objective of this study was to assess the influence of multiple soil physicochemical properties (pH, temperature, total organic carbon (TOC) and clay content) on the vertical distribution of PFAAs in soils along a distance gradient from a fluorochemical plant. In addition, we examined the relationship between surface-layer PFAA pollution and soil respiration, microbial activity and biomass.

2. Materials and method

2.1 Study area and sample collection

Soil samples were collected during autumn 2018 at five sampling sites with increasing distance from a 3M fluorochemical plant in Antwerp. Based on prior monitoring studies (e.g. Dauwe et al., 2007; Groffen

et al., 2017, 2019a, 2019b, Lopez-Antia et al., 2019) we selected Vlietbos (1 km SE from 3M), Middenvijver-Rot (hereafter Rot; 2.3 km ESE from 3M) and Burchtse Weel (3 km SE from 3M) as sampling sites. As a reference site, Westmalle (25 km NE from 3M) was selected (Figure 1).

At each site, five soil samples were collected at varying depths (0 – 5 cm, 25 – 30 cm, 50 – 55 cm, 75 – 80 cm and 100 – 105 cm) using an Edelman auger and a stainless steel shovel. Due to a rocky sublayer, at Rot and Burchtse Weel only the top layer could be collected and at 3M only four layers could be sampled. Of each sample the temperature and pH were recorded using a portable multimeter (HI9125, Hanna Instruments) prior to storing them into 50 mL polypropylene (PP) tubes. Additionally, we filled two 10 L plastic buckets, using a stainless steel shovel, with top soil ($\pm$ 0 – 5 cm) from each site to use in further analyses on microbial biomass, activity and soil respiration. The 50 mL PP tubes were stored at -20 °C prior to PFAA analysis and determination of TOC and clay content. The buckets were stored, at a similar length of time, in a dark room at room temperature.

2.2 Chemical extraction and analysis

The used abbreviations are all according to Buck et al. (2011). Target analytes included four perfluorosulfonic acids (PFSAs) and eleven perfluorocarboxylic acids (PFCAs). The target analytes and the isotopically mass-labeled internal standards (ISTDs; Wellington Laboratories, Guelph, Canada) are displayed in Table 1. During the extraction HPLC grade acetonitrile (ACN; LiChrosolv, Merck Chemicals, Belgium), ammonium acetate (VWR International, Belgium), ammonium hydroxide (Filter Service N.V., Belgium) and Milli-Q water (MQ; 18.2 mΩ; TOC: 2.0 ppb; Merck Millipore, Belgium) were used.

The extraction procedure described and validated by Groffen et al. (2019c) was used. Soil samples were oven-dried at 60 °C prior to the analysis. To each sample ($\pm$ 0.3 g dw) 10 ng (80 μ L, 125 pg/ μ L) of each ISTD and 10 mL of ACN was added. After vortex-mixing, the samples were sonicated for 3 x 10 min (Branson 2510) and left overnight on a shaking plate (135 rpm, room temperature, GFL 3020, VWR

International, Leuven, Belgium). After centrifugation (4 °C, 2400 rpm, 10 min, Eppendorf centrifuge 5804R, rotor A-4-44), the supernatant was transferred into a new PP tube. For the extraction we used Chromabond HR-XAW Solid Phase Extraction (SPE) cartridges, which are weakly basic secondary and tertiary ammonium polymeric anion exchangers. The cartridges (3mL, adsorbent weight 200 mg) were preconditioned and equilibrated with 5 mL of ACN and 5 mL of MQ, respectively, before loading the sample onto the cartridges. Hereafter, the SPE cartridges were washed with 5 mL of 25 mM ammonium acetate in MQ and 2 mL of ACN and eluted with 2 x 1 mL of 2% ammonium hydroxide in ACN. The eluent was completely dried using a rotational-vacuum-concentrator at 37 °C (Martin Christ, RVC-2-25, Osterode am Harz, Germany) and reconstituted with 200 µL of 2% ammonium hydroxide in ACN. Finally, the samples were vortex-mixed for at least 1 minute and filtrated through an Ion Chromatography Acrodisc 13 mm Syringe filter with 0.2 µm Super (PES) membrane (VWR International, Leuven, Belgium) into a PP auto-injector vial prior to the UPLC analysis.

2.3 UPLC-TQD analysis

We used ultra-performance liquid chromatography coupled tandem ES(-) mass spectrometry (ACQUITY, TQD, Waters, Milford, MA, USA) to analyze the target analytes. Individual PFAAs were separated using an ACQUITY BEH C18 column (2.1 x 50 mm; 1.7 µm, Waters, USA) and an ACQUITY BEH C18 pre-column (2.1 x 30 mm; 1.7 µm, Waters, USA) was inserted between the solvent mixer and injector to retain PFAA contamination from the system. As mobile phase solvents we used A) 0.1% formic acid in water and B) 0.1% formic acid in ACN. The injection volume was 10 µL at a flow rate of 450 µL/min. The gradient started at 65% A, decreased to 0% A in 3.4 min and returned to 65% A at 4.7 min. To identify and quantify the target PFAAs, multiple reaction monitoring (MRM) of two diagnostic transitions per target analyte (Table 1) was used.

2.4 Total organic carbon (TOC) and clay content

The loss on ignition method, as described by Heiri et al. (2001), was used to determine the organic carbon content of the soil. Approximately 10 g of the soil was oven-dried at 60 °C. Empty aluminum-foil bags were folded and dried at 105 °C for at least 2h after which they were cooled down to room temperature in a desiccator and weighed. The bags were then filled with the dried soil, weighed and oven-dried in a muffle furnace at 105 °C for at least 24 h. Again, the samples were cooled down to room temperature in a desiccator, weighed and incinerated in the muffle furnace at 550 °C for at least 5 h. After cooling down in a desiccator, the weight loss was determined and the TOC was calculated using Formula 1.

$$\text{TOC (\%)} = ((\text{DW}_{105} - \text{DW}_{550}) / \text{DW}_{105} * 100) / 1.742 \quad (\text{Formula 1})$$

With DW the dry weight of the sample after heating at 105 °C or 550 °C.

A Malvern Mastersizer 2000 and Hydro 2000G were used to determine the clay content (particles with a size <2 µm) of the soil. The samples were pretreated with 40 mL 33% hydrogen peroxide (VWR Chemicals, Leuven, Belgium) and 9 mL 30% hydrochloric acid (VWR Chemicals, Leuven, Belgium) to destruct iron conglomerates and organic material in the samples. Additionally, the samples were boiled to speed up the destruction process and sieved over a 2.0 mm test sieve prior to the analysis.

2.5 Soil respiration, microbial biomass and microbial activity

Approximately 50 g of fresh soil was weighed, transferred into a glass jar (closed) and oven dried at 70 °C for 48 h to determine the dry mass. Hereafter the lid was replaced by a lid that was attached to an Environmental Gas Monitor (EGM-4). As CO₂ can be released from soil disturbance, the samples were rested for 5 min to return to their normal respiration rates. The CO₂ concentration (ppm) outside of the jars (reference concentration) was determined. The soil respiration was then determined by purging the reference air through the glass jar into the EGM at 350 mL/min (Pumpanen et al., 2004).

The soil microbial biomass was determined by fumigation of soil samples according to Brookes et al. (1985). Fumigation of the soil will kill and lyse microbial cells and thus a subtraction of the carbon content measured with fumigation from the total carbon content will give a measure for the microbial biomass in each sample. To determine the carbon content approximately 2 g of each sample was transported to marked scintillation vials. After adding 250 mL of a 0.5 M potassium sulfate (K_2SO_4), the vials were placed on a shaking plate for 1 h. After the soil settled, the supernatant was filtered using a Whatmann no. 41 filter. Per 10 soil samples, one control sample (20 mL K_2SO_4) was added. Vials were closed and stored at -20 °C until further analysis. Another sample of approximately 2g was fumigated in a desiccator. Approximately 50 mL of chloroform was added to a measuring cup and placed in the desiccator, after which the desiccator was closed and vacuumed for 3 x 10 min. Hereafter, samples were rested in the dark for 24 – 48 h under vacuum. After removal of the chloroform, the samples were transferred into scintillation vials and the previously described K_2SO_4 extraction was performed. Each sample (10 mL) was diluted with 10 mL MQ prior to analysis with a Total Organic Carbon Analyzer (TOC- $V_{CPH/CPN}$, Shimadzu Corporation).

The soil microbial activity was assessed using the bait-lamina tests. This is an integrative method that consists of perforated PVC-strips (16 holes) filled with a standard substrate mixture and allows us to compare the feeding activity of soil organisms in soils from different sites, containing different PFAA concentrations. The disappearance of the bait material is directly associated with the activity of soil microorganisms living on the substrate (Bart et al., 2018; Hamel et al., 2007; Kratz, 1998). In each 10 mL bucket, five bait-lamina test strips (Terra Protecta GmbH, Berlin, Germany) were placed (in total 10 strips per site) and checked regularly to determine the feeding activity. In case the strip was fully consumed, a new strip was placed directly next to it. After four weeks, the number of consumed holes were counted and used as a measure of microbial activity (Kratz, 1998).

2.6 Quality assurance

Per batch of 20 samples, one procedural blank (10 mL of ACN) was analyzed as quality control for the PFAAs analysis. The concentrations in the blanks were all < limit of quantification (LOQ). The method recoveries for the target analytes ranged between 3% and 100% in the soil samples. Despite the low recoveries in some samples (Table S1), the previously described method was shown to be highly accurate even at low recoveries of 1% (Groffen et al., 2019c). Therefore, we argue that the low detection frequencies for some analytes are not the result of the low recovery in these samples. Individual LOQs were determined based on a signal-to-noise (S/N) ratio of 10 and are displayed in Table 2, for the analytes with a detection frequency of at least 50% in at least one soil layer at a site (i.e. PFBA, PFOA, PFUnDA and PFOS).

2.7 Statistical analyses

Statistical analyses were performed using R Studio. We used the Shapiro-Wilk test to examine the validity of the models' assumptions and data were log-transformed when needed to fulfil the normality assumptions. The level of significance for all tests was set at $p \leq 0.05$. Concentrations that were below the LOQ were given a concentration of the LOQ/2 (Bervoets et al., 2004; Groffen et al., 2017; Lasters et al., 2019). Whenever the quantified concentrations of an analyte were below the LOQ in more than 50% of the samples at a certain layer or at a location, these data were excluded from the analyses.

Differences in concentrations between soil layers and between locations were examined using ANOVAs (in cases where comparisons could be made between multiple sites or layers) or t-tests (in cases where a comparison could only be made between two sites or layers). When significant differences were obtained, Tukey's post-hoc tests were used to compare mean PFAA concentrations among layers and sites. We used multiple linear regression to assess the relationships between soil physicochemical properties and PFAA concentrations in the soil and to assess relationships between PFAA concentrations and microbial parameters. We selected the best fitting model based on the Akaike Information Criterion (AIC). These models were only used as explanatory models and not predictive models.

3. Results

3.1 PFAA concentrations and vertical distribution

Only PFBA, PFOA, PFUnDA and PFOS were detected in more than 50% of the samples in at least one soil layer from at least one sampling site. Table 2 shows the concentrations and detection frequencies of these analytes. PFBA was detected in the top soil (0 – 5 cm) at Vlietbos, Rot and Burchtse Weel with mean concentrations of 0.82, 2.78 and 1.11 ng/g dw respectively. In addition, PFBA was detected in the 25 – 30 cm layer at Vlietbos (0.38 ng/g dw). PFOA was detected in all layers at 3M, with concentrations up to 3.19 ng/g dw. At Vlietbos, PFOA was detected up to 80 cm deep at concentrations ranging from <LOQ to 1.52 ng/g dw. Mean PFOA concentrations in the top layer at Rot and Burchtse Weel were 1.53 and 0.99 ng/g dw, respectively. At Westmalle, PFOA was detected at low detection frequencies in all layers (<LOQ – 2.41). PFUnDA was only detected in the top layer at Vlietbos at a mean concentration of 0.52 ng/g dw. PFOS concentrations ranged up to 202 ng/g dw at 3M, 45 ng/g dw at Vlietbos, 21 ng/g dw at Rot and 4.46 ng/g dw at Burchtse Weel and were detected at all studied layers. At Westmalle, on the contrary, PFOS was only detected in 40% of the samples from the top layer and ranged from <LOQ up to 0.88 ng/g dw. As the detection frequencies at Westmalle, with exception of PFOA in the deepest layer, were <50% we will exclude this location from further analysis when looking at individual locations.

The vertical distribution of PFOA and PFOS could only be determined at 3M and Vlietbos as only top-soil was collected at Rot and Burchtse Weel (see M&M). Figure 2 illustrates the vertical distribution in the soils from these locations. Significant differences in PFOA concentrations were observed between the soil layers at 3M ($p < 0.001$, $F_{3,16} = 15.2$). The concentrations in the third layer (50 – 55 cm) were significantly higher than those in the other layers (all $p < 0.020$). At Vlietbos, the PFOA concentrations did not differ between layers ($p = 0.229$, $F_{4,18} = 1.6$). Concentrations of PFOS at 3M also differed significantly between layers ($p < 0.001$, $F_{3,16} = 14.3$), with PFOS concentrations being significantly lower in the top soil compared to the second (25 – 30 cm) and third (50 – 55 cm) layer (both $p < 0.001$).

Similarly, the concentrations in the deepest layer (75 – 80 cm) were lower than those in the second ($p = 0.005$) and third ($p = 0.020$) layers. At Vlietbos, PFOS concentrations differed between layers ($p = 0.005$, $F_{4,18} = 5.3$), as concentrations in the top layer were significantly higher ($p = 0.011$) than in the deepest layer (100 – 105 cm), but no other significant differences were observed. PFBA concentrations at Vlietbos did not differ between the first two layers ($p = 0.319$, $F_{4,18} = 1.4$).

PFBA concentrations in the surface soil differed between locations ($p < 0.001$, $F_{2,12} = 15.0$) were significantly higher at Rot compared to Vlietbos ($p < 0.001$) and Burchtse Weel ($p = 0.003$). PFOA concentrations in the top layer did not differ significantly between sites ($p = 0.084$, $F_{3,16} = 2.7$). The second ($p = 0.558$) and fourth ($p = 0.157$) layers at 3M and Vlietbos did not differ either. However, soil at 3M showed significantly higher PFOA concentrations in the third layer than Vlietbos ($p < 0.001$). PFOS concentrations in the surface layer did differ between sites ($p < 0.001$, $F_{3,16} = 13.2$) and were significantly higher at Rot compared to 3M ($p = 0.001$) and Burchtse Weel ($p < 0.001$). Additionally, the PFOS concentrations in the surface soil at Burchtse Weel were significantly lower than those at 3M ($p = 0.014$) and Vlietbos ($p < 0.001$). PFOS concentrations in the second ($p = 0.008$) and third ($p = 0.001$) layer were higher at 3M than at Vlietbos ($p = 0.008$). Concentrations in the fourth layer were not different between sites ($p = 0.548$).

3.2 Relationships with physicochemical soil properties

The average physicochemical properties of the soil are displayed in Table 3 for each location and each soil layer. When all locations were combined, PFBA concentrations in the soil were significantly positively related to TOC ($p < 0.001$, $R^2 = 0.27$) and there was a negative trend with clay content ($p = 0.052$, $R^2 = 0.05$). PFOA concentrations were, on the other hand, positively related to the soil temperature ($p = 0.042$, $R^2 = 0.07$) and there was a positive trend with pH ($p = 0.059$, $R^2 = 0.06$). Similarly, PFOS was only positively related to the soil pH ($p = 0.001$, $R^2 = 0.15$) and there was a positive

trend with temperature ($p = 0.075$, $R^2 = 0.04$). When distinguishing the individual locations, at 3M the PFOA concentrations were not related to any soil property (all $p > 0.100$), whereas PFOS concentrations were negatively related to the temperature ($p = 0.011$, $R^2 = 0.23$) and positively to the TOC ($p < 0.001$, $R^2 = 0.48$). At Vlietbos, the PFBA concentrations were positively related to the TOC ($p < 0.001$, $R^2 = 0.72$), the PFOA concentrations were positively related to the clay content ($p = 0.040$, $R^2 = 0.24$) and PFOS concentrations were not related to any soil property (all $p > 0.440$).

3.3 Associations with microbial parameters

All data on soil respiration, microbial biomass and microbial activity (determined with the bait-lamina) are displayed in Table 4. No significant associations between concentrations of PFBA, PFOA and PFOS were observed with soil respiration, microbial activity and microbial biomass (all $p > 0.100$). Neither were any of these microbial parameters associated with soil physicochemical properties (all $p > 0.230$).

4. Discussion

4.1 Soil concentrations

Our results showed no clear gradient of decreasing PFAA concentrations with increasing distance from the fluorochemical plant. This was contradictory to a previous study, performed by Groffen et al. (2019a) on soil and isopods in the same study area. This gradient has also been reported by other studies on PFAA concentrations in wildlife at this site (e.g. Groffen et al., 2017, 2019b; Lopez-Antia et al., 2019).

In order to compare the PFAA concentrations in the soil with literature, a few examples of PFAA concentrations in the surface soils around fluorochemical plants are shown in Table 5. In 2016, many PFAAs were detected in the surface soils at 3M, Vlietbos, Rot and Burchtse Weel, often at higher concentrations than those in the present study (Groffen et al., 2019a). Surprisingly, the PFBA and PFOS concentrations at Rot were higher in the present study, which was likely the result of differences in

physicochemical soil properties and thus sorption. The TOC was much lower in the study by Groffen et al. (2019a) and TOC is known to play an important role in the sorption of PFAAs (e.g. Milinovic et al., 2015). Groffen et al. (2019a) also observed a higher contribution of PFBA to the total PFAA concentrations in songbird eggs with increasing distance from the fluorochemical plant and suggested that this was likely the result of different pollution pathways or different pollution sources. It is possible that concentrations close to the plant are mainly influenced by the direct industrial pollution, whereas further away the atmospheric degradation of volatile precursor compounds could play a role. The differences between both studies could also be explained by differences in sampling strategy. In the present study, the soil samples were collected in a small area, all relatively close to each other, whereas Groffen et al. (2019a) collected soil samples in the vicinity of nestboxes that were placed across the entire study sites. In addition, we collected soil samples approximately 50 – 100 m south-west from the 3M plant, whereas samples in 2016 were collected in the east of the plant (but still at the plant site). The dominant wind direction in Belgium is from the south-west (Royal Meteorological Institute Belgium (KMI), 2018), indicating that aerial deposition of PFAAs should mainly affect areas north to east from the fluorochemical plant. This hypothesis could also explain the higher PFOS concentrations observed by D'Hollander et al. (2014) at Blokkersdijk, approximately 0.5 – 1.5 km east from 3M (69 ng/g dw). PFBA concentrations at a fluorochemical industrial park in China (0.6 ng/g dw, Lu et al., 2018) were similar to the concentrations we observed at Vlietbos, but lower to those at Rot and Burchtse Weel. Both PFOA (50.1 ng/g dw) and PFOS (2583 ng/g dw) concentrations at a fluorochemical manufacturing facility in Wuhan, China were much higher than those reported in the present study (Wang et al., 2010). However, concentrations of PFOA (0.79 ng/g dw) and PFOS (7.06 ng/g dw) near a fluorochemical manufacturing facility in Hubei Province, China (Wang et al., 2010), were similar to the ones detected at 3M. Nevertheless, it must be noted that, in most of the studies reported in Table 5, it is often unclear which

soil layer (i.e. how deep) was collected and defined as top/surface layer. Therefore, it is possible that these comparisons were based on different soil layers.

Elevated concentrations of PFOS and PFOA were mainly observed in subsurface layer, up to approximately 50 cm depth. Hereafter, the concentrations decreased again, suggesting a downward movement of both compounds. This downward movement might result in a contamination of the groundwater and thus pose a potential source of exposure for organisms and communities that rely on this water. The downward movement of PFOS and PFOA has also been reported by Xiao et al. (2015) in soils at a U.S. metropolitan area with both PFOS and PFOA concentrations generally increasing with depth. Similarly, concentrations of ammonium perfluorooctanoate (APFO), the salt of PFOA, were highest in the surface soil and decreased with depth in soils on an alluvial floodplain of the Ohio River (Davis et al., 2007). Sepulvado et al. (2011) also reported the downward migration of PFOA and PFOS in biosolid-amended soils to depths of 120 cm.

4.2 Associations with soil physicochemical properties

As was already described before, TOC plays a key role in the sorption of PFAAs to soils (Campos Pereira et al., 2018; Miao et al., 2017; Milinovic et al., 2015). Therefore, the positive relationship between TOC and PFOS concentrations at 3M was expected. Although chain-length is considered a dominant factor concerning the adsorption to soils (Ahrens et al., 2010; Higgins and Luthy, 2006), we also observed a positive relationship between TOC and PFBA concentrations when all locations were combined and at Vlietbos separately, which might be caused by larger humic and fulvic acid fractions, which are considered important for the sorption of short-chained PFAAs, such as PFBA (Campos Pereira et al., 2018).

Soils with a higher clay content will have more functional groups to facilitate the sorption of PFAAs (Qi et al., 2014). Although clay content itself is not a measure of the surface charge of the soil, it is considered

an indication of potential binding sites for electrostatic interactions (Li et al., 2018). Therefore, it was expected that clay content would be positively associated with PFAA concentrations, as was the case for PFOA concentrations at Vlietbos. By contrast, we observed a negative trend between PFBA and clay content when all locations were combined, which could potentially be explained by the difference in water solubility between long-chained and short-chained compounds. For example, Ahrens et al. (2010) reported a variation in PFBA concentrations in water at varying depths, indicating that concentrations can vary depending upon sampling depth of the water column. As short-chained compounds are less hydrophobic, they are more water soluble (e.g. Deng et al., 2012) and as a result it is less likely that they will bind to solid matrices such as soil.

The positive relationship between pH and PFOS concentrations, as well as the positive trend between pH and PFOA concentrations were unexpected as PFAAs are weak acidic chemicals and the proportion of anionic molecule increases with increasing pH, resulting in a decreased sorption to soils (Li et al., 2018). In addition, the pH can affect surface properties of the sorbent as an increased pH results in a less positive surface charge on mineral particles (Johnson et al., 2007). The increased PFOA and PFOS concentrations at a higher pH could be the result of the development of more basic surface sites, consisting of carboxyl, alcoholic, phenolic and quinone groups (Schwarzenbach et al., 2003). These surface sites may increase the sorption of calcium (II) ions onto the soil, which further enhances the sorption of PFOS through electrostatic effects and Ca-bridging mechanisms (You et al., 2010).

Jia et al. (2010) investigated the effect of temperature on the sorption of PFOS on humic acid, an important part of TOC, and found that the sorption capacity was doubled when the temperature increased from 5 to 35°C. This could potentially also explain the positive trend between PFOS concentrations and soil temperature. However, it was expected that the concentrations would decrease with increasing temperature, as we observed for PFOS at 3M, as adsorption is often an exothermic reaction (Zhou et al., 2010).

Despite the significant correlations between PFAA concentrations and soil physicochemical properties, these correlations were often weak. This is likely the result of a high similarity in soil properties at the study sites. Therefore, future studies should include sites that contain distinct differences in physicochemical properties to further improve our understanding of these associations.

4.3 Associations with soil microbial parameters

PFAAs have a dual effect on stimulating the growth of some soil bacteria, while inhibiting the growth of others and the extents of this effect varies among analytes (Qiao et al., 2018). Although we did not observe any associations between PFAA concentrations and soil microbial parameters, it is likely that other factors, which we did not measure, affected these results. For example, seasonal variations in microbial respiration and activity have been reported in turfgrass systems, where a lower microbial biomass and activity in September were associated with lower soil available nitrogen (Yao et al., 2011). To minimize the influence of environmental conditions, we recommend investigating the associations between microbial parameters and PFAA concentrations under controlled conditions. Studies that investigate the effects of PFAAs on soil microbial communities are scarce and often only investigate species abundance, richness and diversity (e.g. Bao et al., 2018; Li et al., 2017). The highest bacterial abundance was found in the top soil and was potentially influenced by PFHxS, at a site with a long exposure to severe PFAA pollution (Li et al., 2017). The links between PFHxS concentrations and bacterial abundance were not observed by Bao et al. (2018), who reported that soil TOC might be a key determinant of bacterial abundance. Similarly, Li et al. (2017) reported that archaeal abundance could be affected by PFHxS, whereas Bao et al. (2018) did not observe such associations. Ochoa-Herrera et al. (2016) also observed no toxic effects of PFOS and short-chained PFAAs on the methanogenic activity (performed exclusively by archaea) of anaerobic wastewater sludge.

5. Conclusion

Our results show that both PFOA and PFOS concentrations were elevated in the subsurface layer (up to 50 cm), after which they decreased again, suggesting a downward migration of both analytes in the soil. This downward movement might pose a potential threat for the contamination of the groundwater and, consequently, people and organisms who rely on this water. The soil concentrations were influenced by multiple physicochemical properties of the soil, including TOC, pH, clay content and soil temperature, which suggests differences in bioavailability and sorption/desorption capacities between different soil types. Although expected, we did not observe any influence of PFAA contamination in the top soil on microbial activity and biomass nor soil respiration.

6. Acknowledgements

This work was supported by the Research Foundation Flanders (FWO; research project G038615N to LB and ME). The authors would like to thank T. Willems, who performed the UPLC analysis, and T. van der Spiet for his help with the particle size analysis.

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Figures and Tables

Figure 1. Overview of the study areas of this research. 1 = Westmalle (± 25 km from 3M), 2 = Burchtse Weel (± 3 km from 3M), 3 = Vlietbos (± 1 km from 3M), 4 = Middenvijver-Rot (± 2 km from 3M) and 5 = 3M. Produced with Google Maps.

Figure 2. Vertical distribution of PFOA and PFOS (ng/g dw $\pm$ st. error) in soils from 3M (triangles), Vlietbos (circles), Rot (squares) and Burchtse Weel (diamonds). The soil layers used in the analysis were; 0 – 5, 25 – 30, 50 – 55, 75 – 80 and 100 – 105 cm.

Table 1. Target analytes, isotopically mass-labelled internal standards (ISTDs) used for quantification and MRM transitions. Table adapted from Groffen et al. (2019a).

Compound	Internal standard (ISTD) used for quantification	Precursor ion (m/z)	Product ion (m/z)	
			Diagnostic product ion 1	Diagnostic product ion 2
PFBS	$^{18}\text{O}_2$ -PFHxS	299	80	99
PFHxS	$^{18}\text{O}_2$ -PFHxS	399	80	99
PFOS	[1,2,3,4- $^{13}\text{C}_4$]PFOS	499	80	99
PFDS	[1,2,3,4- $^{13}\text{C}_4$]PFOS	599	80	99
PFBA	$^{13}\text{C}_4$ -PFBA	213	169	169
PFPeA	$^{13}\text{C}_4$ -PFBA	263	219	219
PFHxA	[1,2- $^{13}\text{C}_2$]PFHxA	313	269	119
PFHpA	[1,2- $^{13}\text{C}_2$]PFHxA	363	319	169
PFOA	[1,2,3,4- $^{13}\text{C}_4$]PFOA	413	369	169
PFNA	[1,2,3,4,5- $^{13}\text{C}_5$]PFNA	463	419	169
PFDA	[1,2- $^{13}\text{C}_2$]PFDA	513	469	219
PFUnDA	[1,2- $^{13}\text{C}_2$]PFUnDA	563	519	169
PFDoDA	[1,2- $^{13}\text{C}_2$]PFDoDA	613	569	319
PFTTrDA	[1,2- $^{13}\text{C}_2$]PFDoDA	663	619	319
PFTeDA	[1,2- $^{13}\text{C}_2$]PFDoDA	713	669	169

Table 2. Individual limits of quantification (LOQs; ng/g dw, determined as 10x the S/N ratio), median and mean concentrations (ng/g dw), range (ng/g dw) and detection frequencies (Freq. (%)) of PFAAs in soils collected at varying depths at five sampling sites: a perfluorochemical plant (3M) and at four other sites with increasing distance from 3M (i.e. Vlietbos 1km, Rot 2.3 km, Burchtse Weel 3 km and Westmalle 25 km).

Location	Layer (cm)		PFBA	PFOA	PFUnDA	PFOS
3M	0 – 5 (N = 5)	LOQ	0.3	0.73	0.27	0.67
		Mean	<LOQ	0.89	<LOQ	6.77
		Median		0.87		7.05
		Range		<LOQ – 1.34		3.93 – 8.46
	25 – 30 (N = 5)	Freq.	0	80	0	100
		Mean	<LOQ	1.00	<LOQ	102
		Median		0.93		70
		Range		<LOQ – 1.46		30 – 202
	50 – 55 (N = 5)	Freq.	0	80	0	100
		Mean	<LOQ	2.61	<LOQ	63
		Median		2.58		56
		Range		2.04 – 3.19		46 – 83
	75 – 80 (N = 5)	Freq.	0	100	0	100
		Mean	<LOQ	1.30	<LOQ	19
		Median		1.43		18
		Range		<LOQ – 1.88		1.85 – 39
Vlietbos	0 – 5	Freq.	0	80	0	100
		Mean	0.82	0.94	0.52	8.91

	(N = 5)	Median	1.00	1.04	0.59	9.47
		Range	<LOQ – 1.25	<LOQ – 1.20	<LOQ – 0.69	7.48 – 10
		Freq.	80	80	80	100
	25 – 30 (N = 5)	Mean	0.38	0.94	<LOQ	4.32
		Median	0.38	0.88		4.42
		Range	<LOQ – 0.50	0.85 – 1.13		2.88 – 5.80
		Freq.	80	100	0	100
	50 – 55 (N = 5)	Mean	<LOQ	<LOQ	<LOQ	1.58
		Median		<LOQ		1.84
		Range		<LOQ – 1.40		<LOQ – 2.27
		Freq.	0	40	0	80
	75 – 80 (N = 5)	Mean	<LOQ	0.77	<LOQ	11
		Median		0.77		2.27
		Range		<LOQ – 1.52		1.85 – 45
		Freq.	0	60	0	100
	100 – 105 (N = 3)	Mean	<LOQ	<LOQ	<LOQ	1.21
		Median				1.14
		Range				<LOQ – 2.15
		Freq.	0	0	0	67
Rot	0 – 5 (N = 5)	Mean	2.78	1.53	<LOQ	14
		Median	2.97	1.50		13
		Range	2.06 – 3.67	1.11 – 2.27		9.50 – 21
		Freq.	100	100	0	100
Burchtse Weel	0 – 5 (N = 5)	Mean	1.11	0.99	<LOQ	3.95
		Median	1.11	0.94		3.89
		Range	<LOQ – 2.01	<LOQ – 1.55		3.65 – 4.46
		Freq.	80	80	0	100
Westmalle	0 – 5 (N = 5)	Mean	<LOQ	<LOQ	<LOQ	<LOQ
		Median		<LOQ		<LOQ
		Range		<LOQ – 0.95		<LOQ – 0.88
		Freq.	0	40	0	40
	25 – 30 (N = 5)	Mean	<LOQ	<LOQ	<LOQ	<LOQ
		Median		<LOQ		
		Range		<LOQ – 1.15		
		Freq.	0	20	0	0
	50 – 55 (N = 4)	Mean	<LOQ	<LOQ	<LOQ	<LOQ
		Median		<LOQ		
		Range		<LOQ – 1.72		
		Freq.	0	25	0	0
	75 – 80 (N = 5)	Mean	<LOQ	<LOQ	<LOQ	<LOQ
		Median		<LOQ		
		Range		<LOQ – 0.90		

		Freq.	0	40	0	0
	100 – 105 (N = 3)	Mean	<LOQ	1.04	<LOQ	<LOQ
		Median		1.02		
		Range		<LOQ – 2.41		
		Freq.	0	60	0	0

Table 3. Average physicochemical soil properties in each layer at the sampling sites.

Location	Depth (cm)	pH	Temperature (°C)	TOC (%)	Clay content (%)
3M	0 – 5	6.7	21.3	1.47	0.07
	25 – 30	7.0	18.7	2.89	0.06
	50 – 55	6.9	18.2	0.75	0.05
	75 – 80	6.9	18.7	0.95	0.11
Vlietbos	0 – 5	6.8	17.3	6.05	0.42
	25 – 30	6.8	16.3	0.73	0.05
	50 – 55	6.8	16.0	0.93	0.12
	75 – 80	6.8	15.9	0.93	0.02
	100 – 105	6.7	15.9	0.40	0.0
Rot	0 – 5	7.0	17.5	5.44	0.64
Burchtse Weel	0 – 5	6.9	18.8	3.86	0.41
Westmalle	0 – 5	6.7	13.8	3.99	1.77
	25 – 30	6.6	14.9	3.24	1.25
	50 – 55	6.6	15.0	2.10	2.38
	75 – 80	6.7	14.9	1.83	3.21
	100 – 105	6.7	14.7	1.07	0.87

Table 4. Microbial activity (average number of holes of the bait-lamina sticks consumed after 4 weeks), soil respiration and microbial biomass in the top soil at each location.

Location	Microbial activity (# holes consumed after 4 w)	Soil respiration (µg/h*g dw)	Microbial biomass (µg C/ g soil)
3M	2.0	-0.83	-3.23
Vlietbos	3.2	13.76	3.86
Rot	7.5	46.34	1.19
Burchtse Weel	0.6	0.143	4.01
Westmalle	7.4	11.93	4.54

Table 5. Mean PFAA concentrations (ng/g) in soils published in literature.

Location	Year	PFBA	PFOA	PFUnDA	PFOS	Reference
3M fluorochemical plant, Belgium	2016	1.92	24.0	8.89	1700	Groffen et al., 2019a
	2018	<LOQ	0.89	<LOQ	6.77	The present study

Vlietbos, Belgium	2016	1.33	2.05	<LOQ	22	Groffen et al., 2019a
	2018	0.82	0.94	0.52	8.91	The present study
Rot, Belgium	2016	<LOQ	2.71	<LOQ	3.26	Groffen et al., 2019a
	2018	2.78	1.53	<LOQ	14	The present study
Burchtse Weel, Belgium	2016	0.77	203	<LOQ	7.82	Groffen et al., 2019a
	2018	1.11	0.99	<LOQ	3.95	The present study
Blokkersdijk, Belgium	2006				69	D'Hollander et al., 2014
Daikon Co, Lit, Fluorochemical Industrial Park, China	2015	0.6	62.5	0.2	64.6	Lu et al., 2018
Fluorochemical manufacturing facility in Wuhan, China	2009		50.1		2583	Wang et al., 2010
Fluorochemical manufacturing facility in Hubei Province China	2009		0.79		7.06	

616

Highlights

- Soil concentrations did not decrease with distance from a fluorochemical plant
- The soil concentrations were often lower than other studies near PFAA industry
- Concentrations of PFOA and PFOS showed a downward migration in the soil
- Soil PFAA concentrations were associated with physicochemical properties
- No associations between PFAA concentrations and microbial parameters were observed