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Strong bioaccumulation of a wide variety of PFAS in a contaminated terrestrial and aquatic ecosystem

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ABSTRACT

The widespread use of Per- and Poly Fluorinated Substances (PFAS) in a multitude of industrial and consumer applications, together with their persistence and mobility, has led to global contamination of the abiotic and biotic environment. Nevertheless, important knowledge gaps remain concerning PFAS occurrence and bioaccumulation, with studies tending to focus either on aquatic or on terrestrial ecosystems, with a bias towards the aquatic environment. The aim of the present study was therefore to investigate the distribution and bioaccumulation of various PFAS in a contaminated terrestrial and aquatic ecosystem. Subsequently, it was examined if the calculated bioaccumulation factors are related to PFAS molecular descriptors. Abiotic and biotic samples were collected from the aquatic and terrestrial compartments of a PFAS contaminated ecosystem and screened for 44 compounds. PFAS were present in all environmental compartments with varying profiles and concentrations. Generally, higher concentrations were found in aquatic than in terrestrial biota as well as in animals compared to plants. Biota-to-soil and biota-to-sediment accumulation factors (BSAFs) demonstrated a strong bioaccumulation of PFAS, reaching 96,708 kg sediment/kg biota. Similarly, a high bioconcentration potential from water was observed, with bioconcentration factors (BCFs) reaching 55,597 L water/kg biota. The membrane-water partition coefficient (K_{mw}) explained PFAS bioaccumulation to some extent, but the still limited understanding of factors driving PFAS bioaccumulation calls for further mechanistic research. Nonetheless, it is concluded that many of the 44 analyzed PFAS strongly bioaccumulate in terrestrial and aquatic primary producers and animals, making these compounds of great environmental concern for the coming decades.

1. Introduction

Per- and Poly Fluorinated Substances (PFAS) are a widely used group of anthropogenic compounds, characterized by their high stability and great environmental persistence (Parsons et al., 2008). Their properties make PFAS suitable for a plethora of industrial and consumer applications, but also make them hazardous contaminants, due to their bioaccumulation potential and consequent adverse effects on environmental and human health (Fiedler et al., 2019; Guo et al., 2019). On top of that, many PFAS are highly mobile, leading to their widespread global presence in many environmental compartments and organisms (Ahmed et al., 2020; Houde et al., 2011; Kurwadkar et al., 2022; Murakami et al., 2011).

The ubiquitous environmental presence of PFAS, in combination with their concerning characteristics, have initiated extensive research on their occurrence, distribution and bioaccumulation. However, these efforts remained biased towards a limited spectrum of monitored PFAS in a limited number of organisms, albeit during the last decade this is steadily expanding (Gkika et al., 2023). Nonetheless, the currently available information on the environmental distribution and bioaccumulation of PFAS is not representative of the over 4,700 Chemical Abstracts Service (CAS)-registered PFAS that have been identified on the global market (OECD, 2018). Although especially long-chain PFAS are considered to bioaccumulate and transfer along the food chain, shorter

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PFAS are also commonly found in many organisms (Huang et al., 2022). Their higher mobility and increasing production volumes, following restriction of longer-chain homologues, may fuel their observed bioaccumulation. Hence, short-chain PFAS are of emerging concern (Brendel et al., 2018), and there is an urgent need to enlarge the spectrum of monitored PFAS.

PFAS bioaccumulation studies tend to focus either on aquatic or on terrestrial ecosystems, with a bias towards the aquatic environment (Byns et al., 2022; Gkika et al., 2023; Lewis et al., 2022; Miranda et al., 2021). This distinction does, however, not do justice to the life cycle of the many organisms that spend part of their lifetime in the aquatic and part of it in the terrestrial environment, such as many insects (Kraus et al., 2023). When the terrestrial environment is studied, this frequently remains limited to laboratory (Jarjour et al., 2021; Karnjanapiboonwong et al., 2018; Zhao et al., 2013) and crop studies (Ghisi et al., 2019), while field studies including different plant and invertebrate taxa still remain scarce (D'Hollander et al., 2014; Groffen et al., 2019; Heimstad et al., 2024; Rijnders et al., 2021). Yet, the few available terrestrial field studies revealed specific differences in the extent of PFAS bioaccumulation between species (Ecke et al., 2023; Hopkins et al., 2023; Groffen et al., 2023; Koch et al., 2020), highlighting that combined aquatic-terrestrial studies on a variety of organisms are essential to unravel the complex environmental distribution and bioaccumulation of PFAS.

The aim of this study was therefore to investigate the distribution and bioaccumulation of a wide variety of PFAS in a contaminated terrestrial and aquatic ecosystem. Subsequently, it was examined if there are relationships between the calculated bioaccumulation factors and PFAS molecular descriptors. To this end, abiotic and biotic samples were collected from terrestrial and aquatic environmental compartments located in close proximity to a fluorochemical industrial site. The concentrations of 44 PFAS were quantified in primary producers and animals and *in situ* bioaccumulation factors were calculated based on the concentrations in the environmental compartment that they inhabit (soil, sediment, water). Finally, potential relationships between these bioaccumulation factors and PFAS chain length, polar head, and membrane-water partition coefficient (K_{mw}) were investigated.

2. Materials and methods

2.1. Sampling site

Samples were collected from Lake Blokkersdijk (48 ha, average depth 0.7 m) (Louette et al., 2008) and the surrounding terrestrial ecosystem, in Antwerp, Belgium (51°13′56.8″N 4°20′52.1″E) (Fig. 1). The location was chosen because it is a contamination hotspot in close proximity to the 3 M factory. It is a typical shallow lake with a large littoral zone, representative of the north-western European plane. Lake Blokkersdijk was established as a nature reserve in 1978, with the north-western part bordering the 3 M factory premises, and functions as an important wintering and breeding location for several wetland bird species (Denys et al., 2014; Buytaert et al., 2023; Hoff et al., 2005). Blokkersdijk is a eutrophic, shallow, permanently mixed lake and besides rainwater, the upwelling groundwater supplies water to the lake (Denys et al., 2014). The littoral zone of the lake consisted of wide reed beds, while the surrounding terrestrial ecosystem consisted of bushes and grasslands. Since the water was very eutrophic and the terrestrial environment was ruderal, the present communities were relative species poor.

2.2. Sampling of environmental compartments and biota

The terrestrial and aquatic ecosystems were sampled at five sites each along the western bank of Lake Blokkersdijk, between 200 and 300 m apart (Fig. 1). Sampling included the environmental compartments soil, sediment, water and suspended particulate matter (SPM), and for the biota both primary producers (plants and algae) and animals. The level of identification was not the same for all sampled organisms and therefore for some organisms the taxa name was used instead of the species name. Terrestrial primary producers included four plant species (*Rubus plicatus, Urtica dioica, Crataegus monogyna* and *Alnus glutinosa*), and terrestrial animals consisted of five taxa (Oligochaeta/Lumbricidae, the diplopod (Julidae) *Schizophyllum sabulosum,* Isopoda/Oniscidea, and the snails (Gastropoda) *Arion rufus,* and *Cepaea* spp. (including both *Cepaea nemoralis* and *Cepaea hortensis*)). The aquatic organisms were categorized as either benthic or pelagic. Benthic primary producers included three sediment rooting macrophyte species (*Phragmites australis, Elodea canadensis,* and *Potamogeton crispus*) and benthic animals consisted of three taxa of Insecta *(Chironomus riparius, Cloeon dipterum,* and Trichoptera). Pelagic primary producers contained four groups (*Lemna minor*, *Chara vulgaris,* phytoplankton, and periphyton from reed stems), while pelagic animals were represented by the taxon Corixidae. Further details on the sampling can be found in Section S1 (Text S1; Table S1).

2.3. PFAS extraction and analysis

All environmental and biota samples were analyzed for 44 PFAS

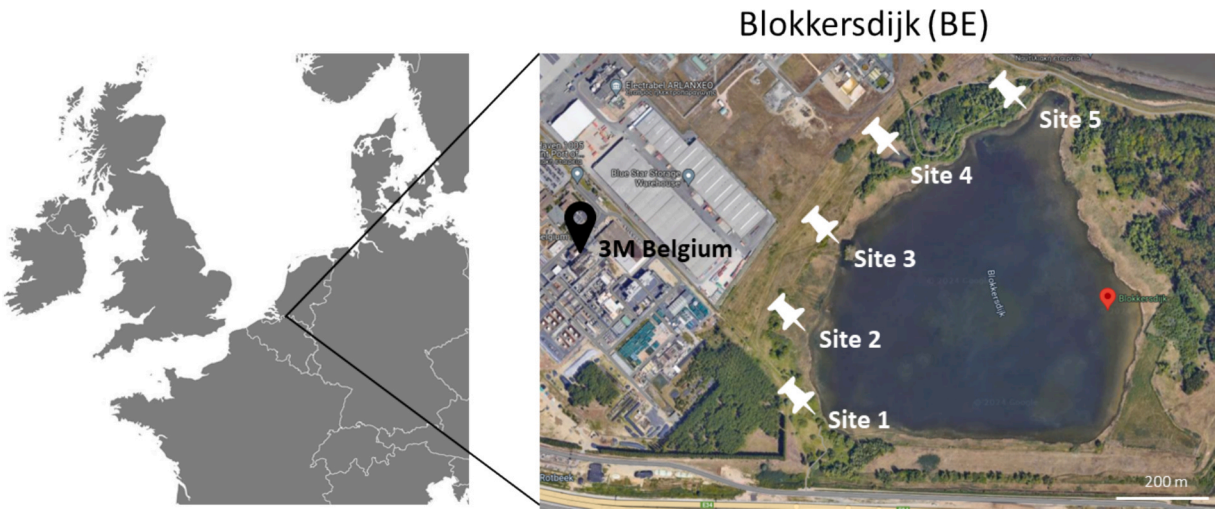

Fig. 1. The location of Lake Blokkersdijk in Belgium and the five sampling sites (1–5) (Site 1; 51°13′46.2″N 4°20′29.6″E. Site 2; 51°13′56.0″N 4°20′28.9″E, Site 3; 51°14′05.4″N 4°20′40.1″E, Site 4; 51°14′09.3″N 4°20′43.0″E, Site 5; 51°14′09.9″N 4°20′51.8″E).

covering a wide range of structures, including six isomer pairs (listed in Section S2, Table S2). Individual branched isomers were not differentiated in the analysis and the term "branched isomers" refers therefore to the sum of all quantified branched isomers per compound. PFAS were divided into five subclasses: short and long Perfluorosulfonic acids (PFSAs), short and long Perfluorocarboxylic acids (PFCAs) and the category "other PFAS and precursors". The protocols used for PFAS extraction, analysis and quality assurance/quality control (QA/QC) assessment have been described previously (Gkika et al., 2024; Sadia et al., 2020; 2023). Briefly, for the water samples a weak anion exchange solid phase extraction was applied. For the soil, sediment and biota samples, solid–liquid extraction was performed followed by a weak anion exchange solid phase extraction and a clean-up step. A detailed description of the PFAS extraction for all matrices, the quantification method, and the quality assurance/quality control criteria can be found in Section S2 (Texts S2 and S3; Tables S3-S5). Due to low PFAS recovery in Oniscidea, results from these samples were excluded from further analysis (Text S3).

2.4. Environmental distribution and bioaccumulation of PFAS

The environmental distribution of the 44 PFAS was characterized by their concentrations in the abiotic compartments and the sampled terrestrial and aquatic organisms. In water and sediment, one compound (7:3 FTCA) and in benthic and pelagic biota two compounds (PFBS and 7:3 FTCA) did not fulfill the QA/QC criteria. Therefore, the distribution of 43 and 42 PFAS was characterized in these samples, respectively (Text S3). Concentrations in organisms from the five sampling sites were first averaged per species for the whole study area. For species that were not present at all sampling sites, only the sites where the species were present were taken into account when calculating the average for the whole study area. Next, these concentrations were used to calculate the average for the whole study area for each of the following categories: terrestrial primary producers, terrestrial animals, benthic primary producers, benthic animals, pelagic primary producers and pelagic animals. These average concentrations were then summed per PFAS subclass to determine the PFAS profiles in each of these six categories on a weight basis (ng/g dry weight (dw) or ng/L).

For the PFAS bioaccumulation calculations, the same categorization of primary producers and animals into terrestrial, benthic or pelagic was maintained. Bioaccumulation in terrestrial organisms was related to the PFAS concentrations in the soil, in benthic organisms to those in the sediment and in pelagic organisms to those in the water. First, the bioaccumulation factors for each species were calculated per site, by dividing the PFAS concentration in the organism from that specific site by the PFAS concentration in the respective matrix (soil, sediment or water) from the same site. Then, the five site-specific bioaccumulation factors per species were averaged for the whole study area (Equations ((1A)) and (1B)). This number was in turn used to calculate the average bioaccumulation factor for the whole study area for each of the six categories mentioned above.

For the terrestrial and benthic primary producers and animals, the enrichment of PFAS from the soil or sediment was quantified by calculating the average biota-to-soil or biota-to-sediment accumulation factor (BSAF), according to Equations (1A) and (1B) (Van Gestel et al., 2019). For the pelagic primary producers and animals, the enrichment of PFAS from the water was assessed by calculating the bioconcentration factor (BCF), using Equations (2A) and (2B) (Van Gestel et al., 2019).

$$\mathrm{BSAF_{taxon}} = \mathrm{AVG}^{5}_{\mathrm{site=1}} \frac{[\mathrm{PFAS}]_{\mathrm{taxon}}}{[\mathrm{PFAS}]_{\mathrm{soil\ or\ sediment}}} \left[\frac{\mathrm{kg_{soil\ or\ sediment}\,dw}}{\mathrm{kg_{org}\,dw}}\right] \quad (1A)$$

$$\mathrm{AVG\,BSAF_{organism\ category}} = \frac{\sum_{n=1}^{n=x}\mathrm{BSAF_{taxon}}}{\#\,\mathrm{taxa}} \left[\frac{\mathrm{kg_{soil\ or\ sediment}\,dw}}{\mathrm{kg_{org}\,dw}}\right] \quad (1B)$$

$$\mathrm{BCF_{taxon}} = \mathrm{AVG}^{5}_{\mathrm{site=1}} \frac{[\mathrm{PFAS}]_{\mathrm{taxon}}}{[\mathrm{PFAS}]_{\mathrm{water}}} \left[\frac{\mathrm{L_{water}}}{\mathrm{kg_{org}\,dw}}\right] \quad (2A)$$

$$\mathrm{AVG\,BCF_{organism\ category}} = \frac{\sum_{n=1}^{n=x}\mathrm{BCF_{taxon}}}{\#\,\mathrm{taxa}} \left[\frac{\mathrm{L_{water}}}{\mathrm{kg_{org}\,dw}}\right] \quad (2B)$$

In Equations (1A) and (2A), $[\mathrm{PFAS}]_{\mathrm{taxon}}$ is the PFAS concentration in each organism at each of the five sites, $[\mathrm{PFAS}]_{\mathrm{soil\ or\ sediment}}$ and $[\mathrm{PFAS}]_{\mathrm{water}}$ are the PFAS concentrations in the soil, sediment or water at the same site. In Equations (1B) and (2B) the $\sum_{n=1}^{n=x}\mathrm{BSAF_{taxon}}$ and $\sum_{n=1}^{n=x}\mathrm{BCF_{taxon}}$ are the sums of BSAFs and BCFs, respectively of all taxa that belonged to the same organism category ($x = 1 - 5$) and # taxa is the number of taxa in this organism category. Units are given in between squared brackets. More details on the calculation of the bioaccumulation factors for each organism category can be found in Section S3 (Text S4-S5, Equations (S4-S9)).

2.5. Relationships between bioaccumulation and PFAS molecular descriptors

To investigate potential relationships between bioaccumulation and PFAS molecular descriptors, the BSAFs/BCFs for each of the six organism categories (terrestrial primary producers; benthic primary producers; pelagic primary producers; terrestrial animals; benthic animals; pelagic animals) were plotted against the number of fluorinated carbons for PFAS with different polar heads, like PFSAs and PFCAs. In addition, the calculated BSAFs/BCFs were plotted against previously published K_{mw}, available for a smaller set of PFAS (Droge, 2019). K_{mw} is important for describing the bioaccumulation potential of (charged) compounds (Bittermann et al., 2016) and is defined as the ratio between the concentration of the chemical in a membrane and the concentration of the chemical dissolved in water. To evaluate the statistical significance of the observed patterns, a Pearson correlation test was performed for the average BSAF/BCF value per taxon and for the average BSAF/BCF value of each of the six organism groups, by checking if the Pearson's correlation coefficients were greater than the critical values from the Pearson table. Linear regression trendlines were drawn when correlations were statistically significant ($p < 0.05$).

3. Results

3.1. PFAS concentrations in the abiotic environment

All environmental compartments contained numerous PFAS, with varying profiles and individual PFAS concentrations (Section S4, Table S6). In the soil, 80 % (35/44) of the targeted PFAS were quantified and PFAS profiles were dominated by long-chain PFSAs (62 %), followed by the category "other PFAS and precursors" (25 %), while few PFCAs and short-chain compounds were present (Fig. 2A-2B). Average total ∑PFAS concentration in the soil was 183 ng/g dw. L-PFOS and FOSA exhibited the highest concentrations (75 and 41 ng/g dw, respectively), accounting for more than 64 % of the total PFAS concentration in the soil.

In the sediment, 73 % (32/44) of the targeted PFAS were quantified and the concentrations were almost 10 times lower than in the soil, dominated by long-chain PFSAs and precursors with almost equal contributions (45 % and 42 %, respectively) (Fig. 2A-2B). Average total ∑PFAS concentration in the sediment reached 20 ng/g dw, and like in the soil, FOSA and PFOS exhibited the highest concentrations (6.5 and 5.4 ng/g dw, respectively), accounting for 60 % of the total PFAS concentration.

In the water, a higher number of PFAS was quantified compared to soil and sediment; 36 out of 43, which corresponds to 84 % of the targeted compounds. In contrast to soil and sediment, short-chain PFCAs

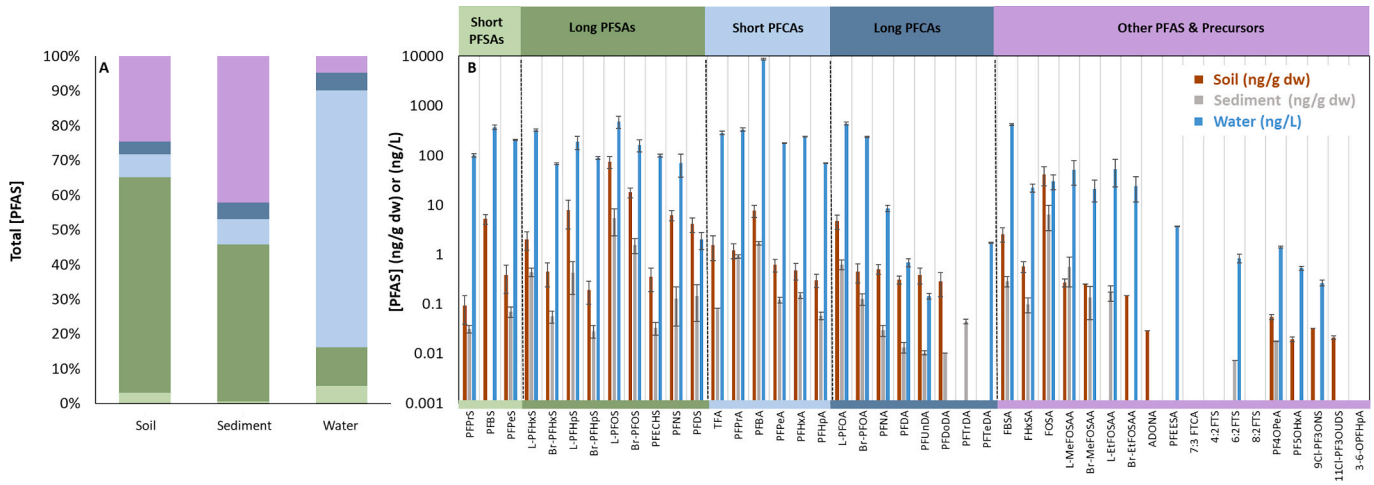

Fig. 2. Relative PFAS profiles per subclass (weight basis) and average (n = 5 sites) individual PFAS concentrations in soil, sediment and water sampled from Lake Blokkersdijk, reported in ng/g_{soil} dw, ng/$g_{sediment}$ dw and ng/L_{water}, respectively. In panel A, the different colors correspond to the five different PFAS subclasses given at the top of panel B. In panel B, compounds are plotted with increasing number of fluorinated carbons within subclasses, and error bars represent the Standard Error of the Mean (SEM) of the concentrations measured at the five sampling sites within the area. For visualization purposes, the Y axes with PFAS concentrations in panel B are shown on a log scale. The horizontal bars indicate the different subclasses, which are separated by vertical, dashed black lines. All raw data behind the plots are included in Table S6.

accounted for the majority (74 %) of the PFAS concentrations in water, followed by long-chain PFSAs (12 %) and an approximately equal contribution of the other three categories (5 %) (Fig. 2A). Average ∑PFAS concentrations reached 13,305 ng/L and PFBA showed by far the highest concentration (8,732 ng/L), accounting for more than 65 % of the total PFAS concentration measured in the water, followed by L-PFOS, L-PFOA and FBSA with comparable concentrations of 481, 438 and 418 ng/L, respectively (Fig. 2B).

SPM was examined as a compartment that connects the water phase with the sediment, in an attempt to link the exposure of the organisms through these two compartments (Section S5, Text S6, Table S7, Fig. S1). In SPM a similar number of PFAS was quantified as in the sediment (30 out of 44), however, composed of slightly different compounds. Profiles resembled to some extent those in the sediment, with long-chained PFSAs (32 %), precursors (28 %) and short-chained PFSAs (25 %) having comparable contributions. Average ∑PFAS concentration was much higher compared to soil and sediment, reaching 7,937 ng/g dw, with PFBS (1,944) and L-PFOS (1,840) showing by far the highest concentrations, together accounting for almost 50 % of the total PFAS concentration measured in SPM.

3.2. PFAS concentrations in biota

In terrestrial primary producers, half (22/44) of the targeted PFAS were quantified and profiles were dominated by short-chain PFCAs (93 %) (Fig. 3A). The average ∑PFAS concentration reached 615 ng/g dw with the ultrashort-chain TFA having the highest concentration (382 ng/g dw), followed by PFBA (178 ng/g dw) and PFBS (16 ng/g dw) (Fig. 3B). More compounds could be quantified in pelagic (35/42; 83 %) and in benthic primary producers (29/42; 69 %) compared to terrestrial primary producers, although these numbers differed between the individual taxa or groups (Section S6, Text S7). PFAS profiles in benthic and pelagic primary producers were comparable to each other, but quite distinct from those in terrestrial primary producers, with long PFSAs dominating in benthic primary producers (53 %), followed by short-chain PFCAs (32 %), while in pelagic primary producers these two subclasses had comparable contributions of 39 and 40 %, respectively. The category "other PFAS and precursors" was also comparable between benthic and pelagic primary producers, accounting for 12 and 10 % respectively (Fig. 3A). Average total ∑PFAS concentrations were similar in benthic and pelagic primary producers (3,207 and 3,522 ng/g dw, respectively) (Fig. 3B) and were near one order of magnitude higher than in terrestrial primary producers (615 ng/g dw). In both pelagic and benthic primary producers, L-PFOS, PFBA and TFA had the highest concentrations. In benthic primary producers, L-PFOS, PFBA and TFA reached 1,378, 457 and 448 ng/g dw, respectively, while in pelagic primary producers they reached 947, 568 and 561 ng/g dw, respectively. All PFAS concentrations in the different primary producers can be found in Section S6 (Tables S8-S10).

In terrestrial animals, 82 % (36/44) of the targeted PFAS were quantified, with an equal contribution of long-chain PFSAs and short-chain PFCAs (44 %), followed by the category "other PFAS and precursors" (8 %) (Fig. 3C). Average total ∑PFAS concentration reached 6,153 ng/g dw with TFA having the highest concentration (2,442 ng/g dw), followed by L-PFOS (1,707 ng/g dw) and L-PFHpS (348 ng/g dw) (Fig. 3D). A similar number of PFAS was quantified in benthic and pelagic animals (32 and 33, respectively), with profiles similar to each other, but distinctly different from those in terrestrial animals, dominated by long-chain PFSAs (75 % in benthic; 80 % in pelagic) followed by the category "other PFAS and precursors" (18 % in benthic; 15 % in pelagic) (Fig. 3C). Compared to terrestrial animals, total ∑PFAS concentrations were generally higher with up to 13,059 ng/g dw for benthic and 5,351 ng/g dw for pelagic animals (Fig. 3D). In both benthic and pelagic animals, L- and Br-PFOS had the highest concentrations. In benthic animals L- and Br-PFOS reached 3,736 and 2,891 ng/g dw, respectively, followed by PFNS (2,061 ng/g dw), while in the pelagic animals they reached 2,776 and 739 ng/g dw, respectively, followed by FOSA (478 ng/g dw). Detailed information on the concentrations of all PFAS measured in the various terrestrial, benthic and pelagic animals can be found in Section S7 (Text S8, Tables S11-S13).

3.3. PFAS bioaccumulation

BSAFs for terrestrial primary producers and animals are expressed in kg soil dw/kg primary producer or animal dw. The aquatic organisms were categorized as either benthic or pelagic and BSAFs and BCFs were expressed in kg sediment dw/kg primary producer or animal dw and L water/kg primary producer or animal dw, respectively.

BSAFs for terrestrial primary producers could be calculated for 19 PFAS and ranged from 0.13 to 392 (Fig. 4), with the highest average BSAF values being observed for four short-chain compounds, TFA (392), PFBA (41), PFPrS (18) and PFPrA (12). The specific PFAS for which

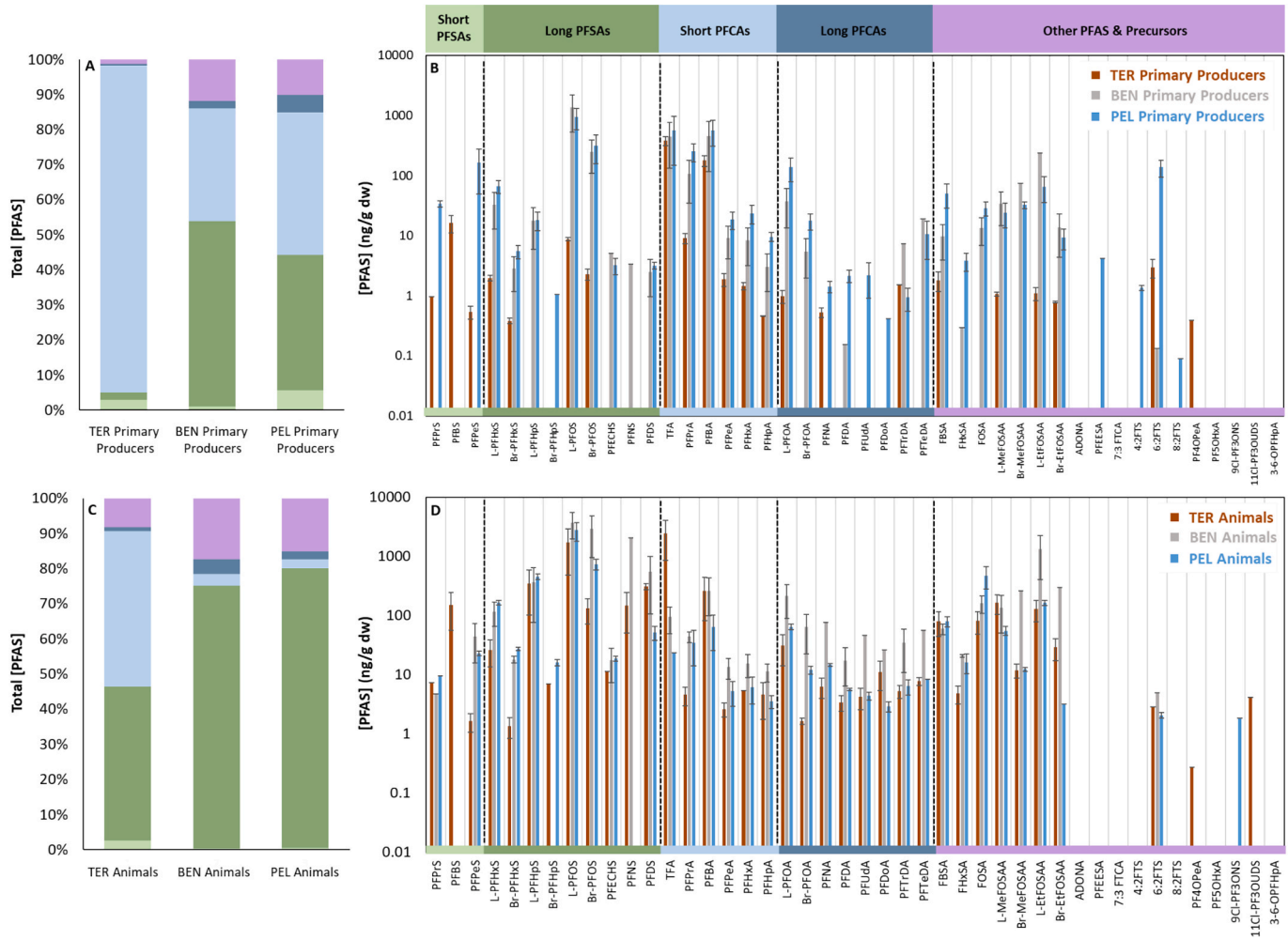

Fig. 3. Relative PFAS profiles per subclass (weight basis) and average (n = 5 sites) individual PFAS concentrations (ng/g_{org} dw) in six biota categories: terrestrial (TER), benthic (BEN) and pelagic (PEL) primary producers (A, B) and animals (C, D) sampled from Lake Blokkersdijk. In panels A and C, the different colors correspond to the different PFAS subclasses given at the top of the figure. In panels B and D, error bars represent the Standard Error of the Mean (SEM) of the concentrations in the different primary producer (B) or animal (D) taxa, except for pelagic animals where the error bars correspond to the SEM of the concentrations at the five sampling sites within the lake, since only one pelagic animal species (Corixidae) was analysed. For visualization purposes, the Y axes with PFAS concentrations are shown on a log-scale. On the X axis, compounds are plotted with increasing number of fluorinated carbons. The horizontal bars indicate the different subclasses, which are separated by vertical dashed lines. All raw data behind the plots, as well as PFAS concentrations in all individual taxa are included in Tables S8-S13.

BSAFs could be calculated as well as the calculated BSAFs varied between the different species, with *A. glutinosa* and *U. dioica* exhibiting high values, reaching up to 589 (TFA; *A. glutinosa*). For the benthic primary producers, BSAFs were calculated for 24 PFAS and ranged from 5.5 to 11,719, with substantially higher values compared to terrestrial primary producers (Fig. 4). More specifically, the highest average BSAF values were observed for TFA (11,719), L-EtFOSAA (1,927) and L-PFOS (903), but the PFAS with the highest BSAF differed between the different species and included short- and long-chain compounds from different subclasses. The highest species-specific average BSAF value for benthic primary producers was found for *P. crispus*, reaching up to 23,398 (TFA). For the pelagic primary producers, BCFs were calculated for 31 compounds, ranging from 10 to 227,104 (Fig. 4). High BCFs were encountered in all taxa and mainly for long-chain PFAS. The highest average BCF values were found for the long-chain compounds 6:2 FTS (227,104), PFUnDA (19,308) and PFTeDA (4,406). When comparing with the PBT/vPvB assessment criteria set by ECHA (2023), 10 compounds had a 3,000 < BCF < 5,000 and five had a BCF > 5,000 in at least one taxon. The compound with the highest BCF differed between taxa and the highest BCF of all pelagic primary producers was 6:2 FTS in phytoplankton (377,325). All PFAS bioaccumulation factors for the individual terrestrial, benthic and pelagic primary producers can be found in Section S8 (Table S14-S16).

BSAFs for terrestrial animals were calculated for 32 PFAS and ranged from 1.4 to 12,157, with TFA (12,157), L-MeFOSAA (475) and 11Cl-PF3OUDS (237) exhibiting the highest average values (Fig. 4). Short-chain compounds and precursors showed the highest BSAFs in most individual terrestrial animal taxa, with the highest average BSAF found for TFA in Lumbricidae (34,256). This species along with the diplopod *S. sabulosum* and the gastropod *Arion rufus* showed the highest BSAFs overall, while the Gastropoda *Cepaea* spp. showed the lowest BSAFs overall. BSAFs for benthic animals were calculated for 29 PFAS, with the highest average BSAFs found for the long-chain PFNS (96,708), PFDS (26,407) and L-EtFOSAA (25,523) (Fig. 4). Long-chain compounds and sulfonamide-based precursors tended to have the highest BSAF values in the individual taxa. *C. dipterum* exhibited the highest values compared to other benthic animals, with 96,708 and 79,104 for PFNS and PFDS, respectively. Overall, benthic animals showed higher BSAFs, accompanied by increased between-species variation, compared to terrestrial animals (Fig. 4). BCFs for pelagic animals were calculated for 31 PFAS,

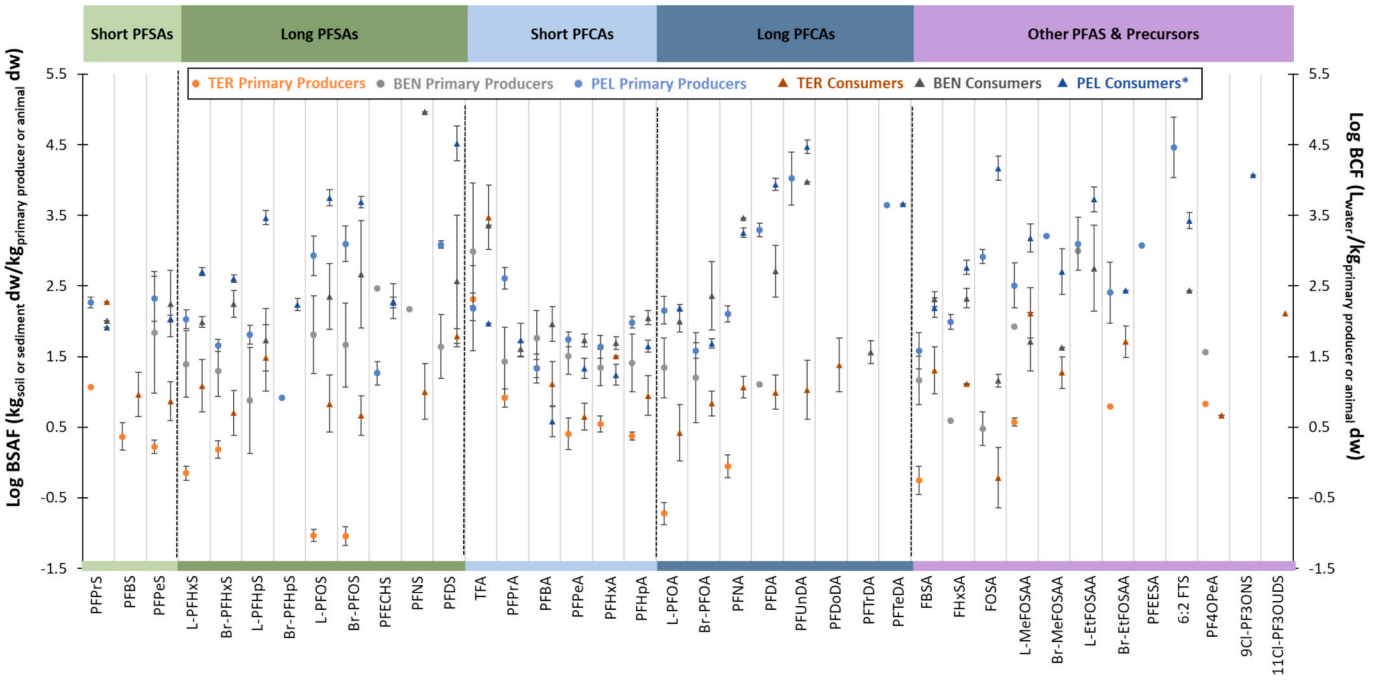

Fig. 4. Logarithmically transformed biota-to-soil and biota-to-sediment bioaccumulation factors (BSAFs) for the uptake of PFAS from soil and sediment into terrestrial (TER) and benthic (BEN) primary producers and animals (left Y axis), respectively, reported in $kg_{soil\ or\ sediment}$ dw/kg_{org} dw, and bioconcentration factors (BCFs) for the uptake of PFAS from water into the pelagic (PEL) primary producers and animals (right Y axis), reported in L_{water}/kg_{org} dw. The error bars represent the Standard Error of the Mean (SEM) of the BSAFs and BCFs in the different terrestrial, benthic and pelagic primary producers and animals sampled from Lake Blokkersdijk, (*except for the pelagic animals, where the error bars correspond to the SEM of the BCFs for the five sampling sites within the lake, since only one taxon (Corixidae) was found). On the X axis, compounds are plotted with increasing number of fluorinated carbons. The horizontal bars indicate the different subclasses, which are separated by vertical, dashed black lines. (n = 1–5; Tables S14-S19).

yet based on only one taxon (Corixidae) (Fig. 4). Higher BCFs were found for long-chain compounds from various subclasses with PFDS (55,597), PFUnDA (33,615) and FOSA (19,328) exhibiting the highest values and eight PFAS having a BCF > 5,000. All PFAS bioaccumulation factors for the individual terrestrial, benthic and pelagic animal species can be found in Section S9 (Tables S17-S19).

3.4. Relationships between bioaccumulation and PFAS molecular descriptors

Despite some group-specific patterns, no general, consistent pattern between bioaccumulation and PFAS chain length or polar head was observed across the different organism groups (Section S10; Text S9, Fig. S2). Although K_{mw} values were available for only a limited number of compounds (Section S11, Table S20), employing K_{mw} revealed that bioaccumulation in terrestrial primary producers significantly decreased with increasing K_{mw}, while it increased for pelagic primary producers, although this trend was only significant for *Phragmites australis* (Fig. 5). Linear regression analysis revealed that in many cases, the differences in the K_{mw} could explain most of the variation observed in the bioaccumulation factors for primary producers, with R^2 values ≥ 0.7 except for three cases in the benthic and one case in the pelagic group (Section S11, Tables S20 and S21). No patterns could be distinguished for animals (results not shown).

4. Discussion

To the best of our knowledge, this is the first field study to analyze a wide variety of targeted PFAS in a diverse group of primary producers and animal taxa in both the terrestrial and aquatic compartments of a contaminated ecosystem. Hence, the present study added the much-needed knowledge on the distribution and bioaccumulation of a wide variety of PFAS in various understudied primary producer and animal taxa. Our findings highlight that a wide variety of PFAS is indeed omnipresent in primary producers and animals, with ∑PFAS in some cases even reaching very high concentrations in the mg/kg dw range, with 42 out of the 44 targeted compounds quantified in at least one environmental compartment. ∑PFAS concentrations were higher in benthic and pelagic primary producers compared to terrestrial and had comparable values. In animals, ∑PFAS concentrations were higher in aquatic (benthic and pelagic) than in terrestrial species. Animals had a higher PFAS load compared to primary producers and accordingly, for most PFAS bioaccumulation factors were higher for animals compared to primary producers. Most importantly, PFAS concentrations were consistently far higher in all organisms compared to the abiotic environmental compartments that they inhabit. This highlights that many PFAS strongly bioaccumulate and that PFAS environmental contamination may be severely misjudged and underrated if only the abiotic environment is considered.

4.1. A wide variety of PFAS is present in the abiotic environment

With up to 36 of the 44 targeted PFAS quantified, the present study highlights that many understudied PFAS are present in the environment in significant concentrations. The detected compounds included legacy PFAS, sometimes in high concentrations, like PFOS, as well as emerging PFAS that serve as their alternatives. Ether-based compounds were present in water and soil, while sulfonamide-based alternatives were found in all environmental compartments. These precursors can be transformed into terminal PFAS like carboxylic or sulfonic acids (Liu and Avendaño 2013) and have therefore likely contributed to the PFSA subclass load, including PFOS. (Ultra)short-chain PFCAs and PFSAs were also present in all environmental compartments. This aligns with the current shift of manufacturing processes towards shorter chain compounds (Lee and Mabury 2014) and the use of larger quantities to obtain similar product performance, as the technical performance of

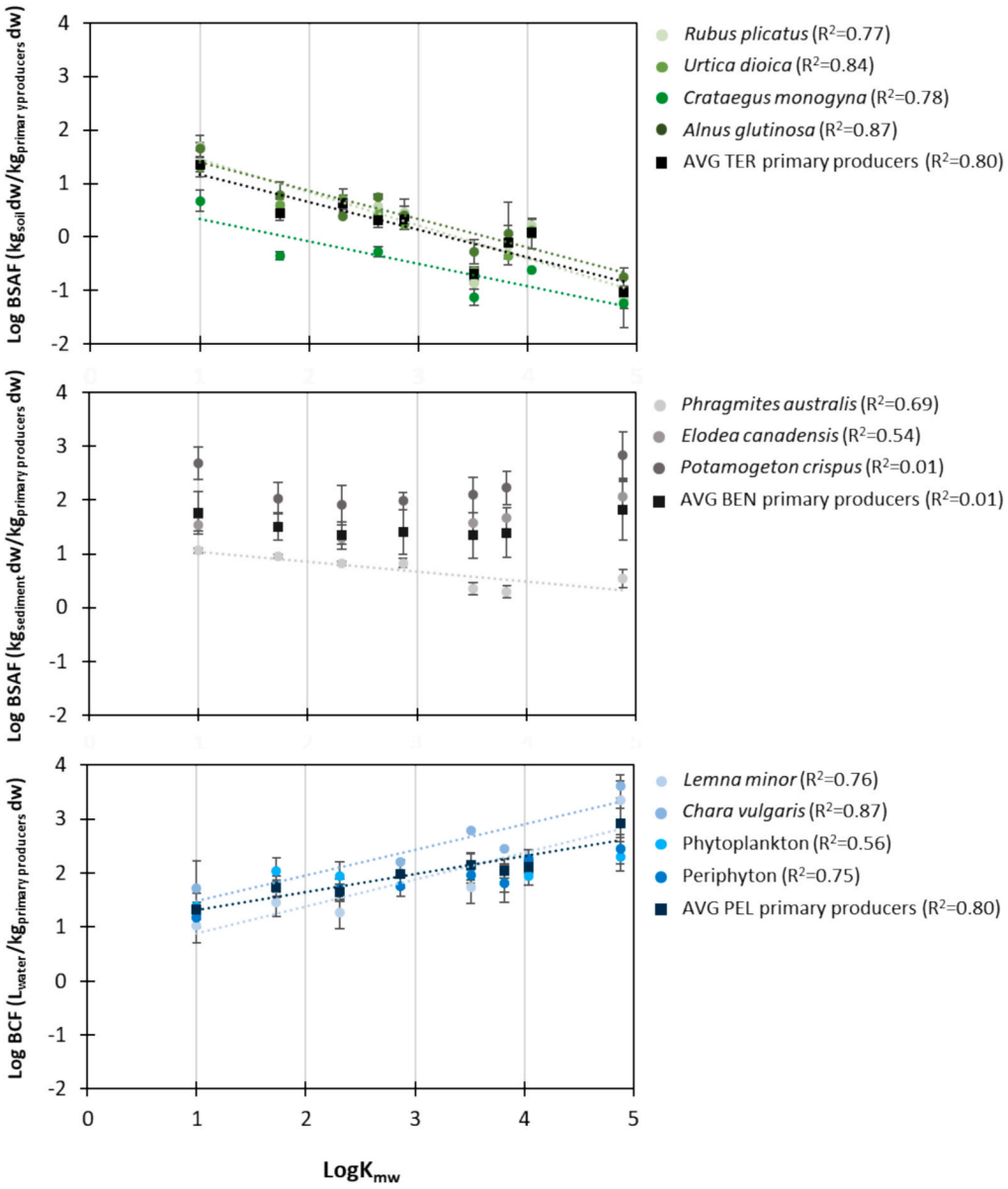

Fig. 5. Correlation between average bioaccumulation factors in primary producers and membrane-to-water partition coefficients (K_{mw}) of PFAS. For the uptake of PFAS from soil and sediment into terrestrial (A) and benthic (B) primary producers, the logarithmically transformed biota-to-soil or biota-to-sediment bioaccumulation factors (BSAFs; $kg_{soil\ or\ sediment}$ dw/kg_{org} dw) are plotted, while for the uptake of PFAS from water into the pelagic primary producers (C) the bioconcentration factors (BCFs; L_{water}/kg_{org} dw) are plotted, all against the logarithmically transformed K_{mw} reported by Droge (2019) (Section S11, Table S20). The error bars represent the Standard Error of the Mean (SEM) of the BSAFs and BCFs per taxon at the five sampling sites from Lake Blokkersdijk (n = 1–5), while for the organism categories the error bars represent the SEM of the BSAFs or BCFs of the taxa within each category. Regression lines were drawn only if the correlation was significant (Section S11, Table S21).

short-chain PFAS is lower than that of the long ones (Ateia et al., 2019; Lindstrom et al., 2011). The dominance of the short-chain PFCAs in water could also relate to their higher water solubility (S_w) (Table S6). Although S_w data were not available for all PFAS reported in this study and the different Sw predictions for a single compound sometimes differed (Sosnowska et al., 2023), generally PFAS with high solubilities were more dominant in the water compared to soil and sediment. Despite being phased out since the year 2000, L-PFOS was sometimes the most prominent compound in the environmental compartments, indicating that the Lake Blokkersdijk environment still suffers from historical pollution, nowadays accompanied by a wide variety of emerging PFAS that further perplexes the environmental PFAS mixture and raises the question how many more PFAS would be detected if we would further expand the target list (Bugsel et al., 2023).

Different studies reported a large variation in PFAS profiles and concentrations in surface water, covering several orders of magnitude (Abunada et al., 2020; Brusseau et al., 2020; Ehsan et al., 2024; Gebbink et al., 2017; Gerardu et al., 2023; Jonker 2024; Megson et al., 2024; Mussabek et al., 2019; Pan et al., 2018; Pulster et al., 2022). This renders comparisons difficult and also indicates that pollution is to a large extent related to (historical) emission patterns driven by local sources. Moreover, different PFAS are targeted by the different studies, hampering the evaluation and comparison of the severity of the different PFAS pollution hotspots. Yet, the concentrations reported in the present study systematically exceed various proposed or binding thresholds (SCHEER, 2022; Smit and Verbruggen 2022; Wintersen et al., 2019) for almost all PFAS in water and soil, by up to almost 70,000 times in the case of PFOS in water, when comparing with the RIVM risk limits (Smit and

Verbruggen 2022). Although the main focus of these guidance values is to protect human health (ITRC, 2023), the currently observed strong and frequent exceedances emphasize the severity of the present PFAS contamination of the Lake Blokkersdijk ecosystem.

4.2. Strong bioaccumulation of a wide variety of PFAS in primary producers

In terrestrial primary producers, short-chain PFAS were by far the most dominant and had among the highest BSAFs, which aligns with earlier findings (Bao et al., 2020; Groffen et al., 2023; Liu et al., 2023; Zhang et al., 2020; 2021) and with the hypothesis that shorter PFAS desorb more easily from soil particles and reach the plant shoots via the vascular tissue (Lan et al., 2018; Zhang et al., 2019). In our case and similar to a recent study (Groffen et al., 2023), PFAS concentrations in primary producers were not related to soil concentrations, and profiles were more similar to that of the water, showing the aqueous nature of the soil-root-shoot PFAS transport. In line with Felizeter et al. (2021) we thus conclude that uptake of the more mobile, pore water-dissolved PFAS dominates their accumulation in terrestrial plant shoots.

Accumulated PFAS profiles in benthic and pelagic primary producers were quite distinct from those in terrestrial primary producers, but with a still significant contribution of short-chain PFCAs. Moreover, PFAS concentrations and bioaccumulation factors were overall higher for benthic than for terrestrial primary producers, in spite of the higher PFAS concentrations in the soil compared to the sediment. These findings suggest that in addition to root-shoot transport the aqueous phase may also play an important role in the PFAS uptake by benthic macrophytes. This may have been further facilitated by the resuspension of sediment-associated PFAS in the currently studied shallow turbid lake, susceptible to wind disturbance. The contribution of the water phase to the PFAS exposure of benthic organisms is further supported by the SPM having a much higher PFAS load than the sediment. This indicates that some water-dissolved PFAS sorb to the SPM, which will eventually sink to the bottom to become the sediment, to which benthic biota are exposed. SPM plays a key role in the environmental fate of pollutants, including PFAS, and the extent of contamination may be undervalued if only water-dissolved and sediment-bound contaminants are considered (Liu et al., 2019). Earlier studies have also found significantly higher concentrations in SPM compared to sediment (Borthakur et al., 2021; Liu et al., 2019), highlighting the role of aquatic particles like SPM, as important transport carriers and as determinants of PFAS distribution (Jeon et al., 2011; Lee et al., 2020). Consequently, PFAS profiles were comparable between benthic and pelagic primary producers and more similar to the profiles in the sediment, with higher contribution of long-chain PFAS compared to the water. Yet, the aquatic PFAS footprint was also visible in the considerable contribution of short-chain PFCAs in the PFAS profiles and in the high bioaccumulation factors observed for both benthic and pelagic plants for many short-chain PFAS. This was especially the case for the pelagic primary producers, where the short-chain PFCAs made up the first most important group. With BSAFs for sediment rooting macrophytes exceeding in some cases 1,000 and BCFs for phytoplankton exceeding 100,000, it is concluded that many PFAS are extremely bioaccumulative in benthic and pelagic primary producers.

4.3. Strong bioaccumulation of a wide variety of PFAS in animals

The PFAS concentrations and bioaccumulation factors in animals were generally higher than those in primary producers for all three compartments. Studies concurrently investigating the bioaccumulation in various primary producer and animal species are scarce, which, combined with the different species being analysed, further complicates direct comparisons of the bioaccumulation factors between primary producers and animals. Moreover, all results here are reported on a total dry body weight basis, since the collected material was not sufficient to perform an analysis on the protein and lipid content of the organisms. Considering that PFAS have an affinity for proteins and to some extent phospholipids (Zhao et al., 2023) and that their composition probably differs between primary producers and animals, comparisons between these two groups of organisms should be interpreted with some caution.

In terrestrial animals, the most abundant subclasses were short-chain PFCAs and long-chain PFSAs, with very little contribution of short-chain PFSAs. PFAS profiles in terrestrial animals correlated to some extent to those in the water, where short-chain PFCAs were by far the most dominant, resembling what was observed for the primary producers, but also to the profiles in the soil, where long-chain PFSAs were dominant. This, however, did not translate into significantly higher bioaccumulation factors for the PFSA subclass over the other subclasses. This finding suggests that for some terrestrial animals, uptake of PFAS from the pore water (desorbed from soil particles), as well as soil particle ingestion could both be important exposure pathways.

Similar to what was observed for the plants, concentrations and bioaccumulation factors in benthic animals exceeded those in terrestrial animals. However, the PFAS profiles across the benthic and pelagic animal groups were almost identical and clearly dominated by long-chain PFSAs. Nonetheless, the most represented subclass of short-chain PFCAs and long-chain PFSAs did not always have higher BSAFs in terrestrial animals compared to other PFAS, while long-chain PFSAs had among the highest BSAFs/BCFs in benthic and pelagic animals.

In accordance with earlier findings the present study also reinforces that PFAS exposure and bioaccumulation may vary across animal species, individual foraging modes as well as habitat (Greger and Landberg 2024; Griffin et al., 2023; Mei et al., 2021; Prosser et al., 2016; Wen et al., 2013; Zhang et al., 2021). This may explain the differences between the bioaccumulation factors from various studies (Arnot and Gobas 2006; Burkhard and Votava 2023; Lewis et al., 2022), sometimes even reaching orders of magnitude. This is also the main reason why we refrained from specifically comparing our BSAFs/BCFs with those from other studies. Consequently, even for the same species and same compound there may not be a universal value for bioaccumulation factors (Liu et al., 2011), the more so since additional (external) factors also play a role in the exposure and uptake of PFAS. Nonetheless, with concentrations in animals being higher than in plants and with bioaccumulation factors approaching 100,000, it is concluded that many PFAS are extremely bioaccumulative in terrestrial and aquatic animals.

4.4. Bioaccumulation in relation to molecular descriptors

Our findings show that PFAS bioaccumulation does not clearly correlate with traditional molecular descriptors, such as chain length and polar head. Inconsistent relationships between PFAS bioaccumulation and chain length have previously been reported (Hopkins et al., 2023; Lesmeister et al., 2021) and may relate to the unique amphiphilic characteristics of these substances, resulting in uptake kinetics that might not allow equilibrium partitioning-driven steady state concentrations in (all) organisms (Jonker and van der Heijden 2007). With most PFAS being present in their anionic form in natural environments (Ding and Peijnenburg 2013), electrostatic interactions play a major role in their sorption and uptake. However, beyond a certain chain length, hydrophobic interactions may become more critical (Ehsan et al., 2024; Guelfo and Higgins 2013; Sadia et al., 2024). In addition, PFAS have different affinities for organic carbon, lipids and proteins and their uptake seems to be concentration dependent, indicating that apart from potential passive diffusion, a process not limited by concentration, active transportation processes are also involved (Ankley et al., 2021; Burkhard 2021; Higgins et al., 2007; Ng and Hungerbühler 2013; 2014). This distinguishes PFAS from many persistent organic chemicals, like PCBs, for which uptake is mostly dependent on passive diffusion (Burkhard 2021; Burkhard and Votava 2023), following the equilibrium partitioning (EqP) theory. Consequently, the BSAFs for macrophytes and benthic invertebrates observed for PFAS in the present study are much higher than the ones reported for PCBs

(Magnusson et al., 2006; Richard et al., 1997; Vanier et al., 2001). Although in those studies results were normalized to the lipid content of the organisms and the organic carbon content of the sediments, the orders of magnitude differences observed for PFAS in our study show that the conventional EqP theory alone fails to predict the strong PFAS bioaccumulation (Higgins et al., 2007; Ng and Hungerbühler 2013; 2014), which calls for novel molecular descriptors, better capable of explaining PFAS bioaccumulation.

Although lipophilicity does not exclusively drive PFAS bioaccumulation, some studies suggest that phospholipid partitioning could play a significant role in the tissue distribution of PFAS (Armitage et al., 2012; Ng and Hungerbühler 2014; Shi et al., 2018). Typically, the octanol–water partition coefficient (K_{ow}) is used as a proxy for bioaccumulation. However, determining K_{ow} for surfactants and ionic chemicals (like PFAS) is associated with high uncertainty, emanating from their amphiphilic nature and ionic interactions (Droge 2019; Hodges et al. 2019). To more accurately explain and predict PFAS bioaccumulation, the K_{mw} has been proposed as a promising descriptor (Droge 2019; Fitzgerald et al., 2018), since phospholipid binding of PFAS has previously been reported (Chen et al., 2025; Qin et al., 2023; Xie et al. 2010b; Xie et al. 2010a; Zhao et al., 2023). Plotting the calculated bioaccumulation factors against the K_{mw} data published by Droge (2019) indeed revealed a positive correlation for some pelagic primary producers, while the opposite trend with a decreasing bioaccumulation with increasing K_{mw} was observed for terrestrial primary producers. This aligns with our hypothesis that dissolved (more mobile) PFAS dominate bioaccumulation in terrestrial plant shoots, while partitioning seems to dominate bioaccumulation for pelagic plants. Nevertheless, since K_{mw} values were only available for a handful of PFAS, unraveling relationships with bioaccumulation factors remains challenging and highlights the need for further insight into the mechanisms of PFAS partitioning and bioaccumulation.

Next to phospholipids, proteins comprise an additional determinant of PFAS bioaccumulation and distribution in biota (Chen et al., 2025; Xiong and Li 2024). Although PFAS are thought to have higher affinity for proteins compared to membrane lipids (Qin et al., 2023), a recent study showed that PFAS' binding affinity for both of these biomolecules was concentration-dependent, and at high PFAS concentrations non-specific binding was observed (Chen et al., 2025). Further studies with human serum albumin have demonstrated the high affinity of PFAS for proteins (Qin et al., 2023; Smeltz et al. 2023). It would therefore be beneficial to develop a protein-related partition coefficient as a descriptor of the bioaccumulation of PFAS, complementary to K_{mw}.

5. Conclusions

The present study revealed that a wide variety of PFAS is omnipresent in the abiotic and biotic compartments of the examined contaminated terrestrial and aquatic ecosystem. The use of the alternative molecular descriptor K_{mw} did prove useful in explaining PFAS bioaccumulation to some extent, but the still limited understanding of the factors driving PFAS bioaccumulation calls for further mechanistic research. Nonetheless, it is concluded that many PFAS strongly bioaccumulate in terrestrial and aquatic primary producers and animals. The very persistent nature of most PFAS together with their high bioaccumulation factors, makes these compounds of great environmental concern for the long-term future.

CRediT authorship contribution statement

Ioanna S. Gkika: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **J. Arie Vonk:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Thomas L. ter Laak:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Cornelis A.M. van Gestel:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Jildou Dijkstra:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Thimo Groffen:** Writing – review & editing, Resources, Investigation. **Lieven Bervoets:** Writing – review & editing, Resources. **Michiel H.S. Kraak:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2025.109629.

Data availability

All data related to this work are included in the Supplementary Information file.

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