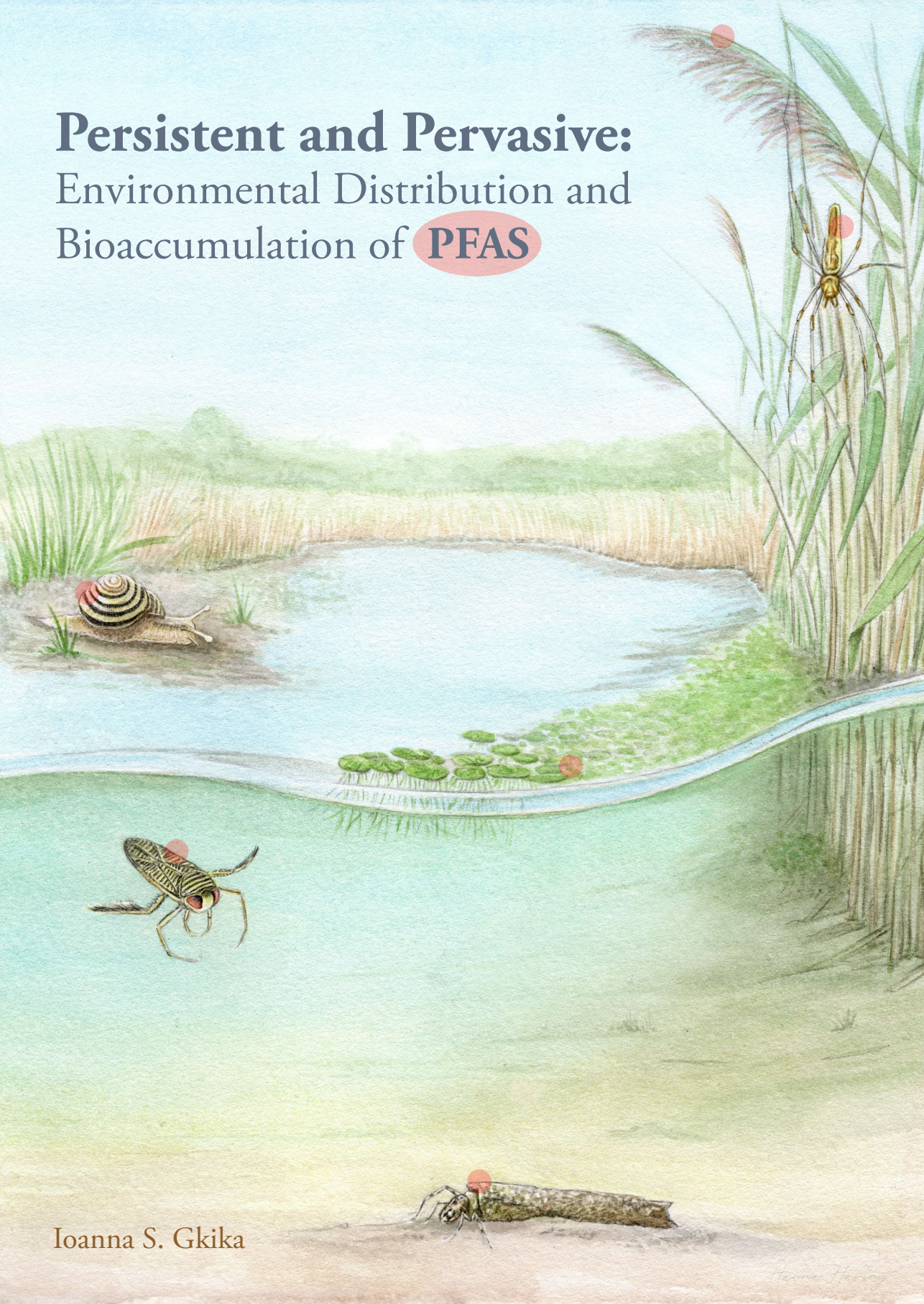
Persistent and Pervasive:
Environmental Distribution and Bioaccumulation of PFAS



Ioanna S. Gkika

Persistent and Pervasive: Environmental Distribution and Bioaccumulation of PFAS

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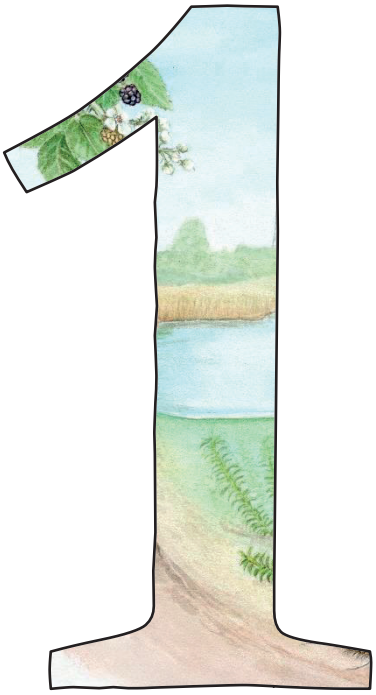
The system is rigged. They want us to believe that it will protect us, but that's a lie. We protect us. We do. Nobody else. Not the companies, not the scientists, not the governments. Us.

Robert Billot, Dark Waters

PFAS Soundtrack

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Chapter One

General Introduction

"*We are now in an era of our making and our own choosing. Earth is increasingly becoming what we choose to make of it. Choosing not to choose is a choice as well and business as usual is part of that.*" These were the words of the famous environmentalist Andrew C. Revkin in 2011, when he was interviewed about the Anthropocene. Some 50 years earlier, even before the concept of the Anthropocene was launched, Rachel Carson wrote a similar expression in her book Silent Spring (1962): According to her "[…] *Only within the moment of time represented by the present century has one species – man– acquired significant power to alter the nature of his world.*"

It is clear that we have manipulated and reshaped many aspects of the world we live in. Although this brought us prosperity by economic growth, welfare, convenience etc., we have also managed to push the planetary boundaries to their limits. Especially for novel chemical entities, released into the environment at an unprecedented rate, it has been argued that we are now already outside the safe operating space of our planet (Persson et al., 2022). Consequently, the Anthropocene differs from the preceding eras by the ubiquitous presence of human-made chemicals in the environment (Waters et al., 2016). The synthetic per- and polyfluoroalkyl substances (PFAS) have the dubious honor of contributing significantly to exceeding the planetary boundaries of chemical pollution (Brunn et al., 2023).

Per- and polyfluoroalkyl substances (PFAS)

It all started with the accidental discovery of the world's first fluoropolymer, later identified as polytetrafluoroethylene (PTFE), but most commonly known as Teflon, by Dr. Roy Plunkett in 1938. A few years later, industrial production of more PFAS, including perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), took off at a large scale serving various applications (Baran, 2001; Buck et al., 2011; Gaines, 2023). Already more than 4,700 Chemical Abstracts Service (CAS)-registered PFAS have been identified on the global market (OECD, 2018), while over 7 million unique per- and polyfluoroalkyl structures have been recorded in the PubChem database (Schymanski et al., 2023).

PFAS nomenclature, as well as PFAS definitions have been rather inconsistent throughout the years. In this thesis the most recent definition by the Organization for Economic Co-operation and Development (OECD, 2020) is adapted, defining PFAS as fluorinated substances that contain at least one fully fluorinated methyl (-CF_3) or methylene carbon atom (-CF_2-), without any H, Cl, Br, or I atom attached to it. PFAS can be classified as either polymers or non-polymers and only the latter are addressed in this thesis. The non-polymers are categorized either as polyfluoroalkyl substances, which are partly fluorinated, or as perfluoroalkyl substances, which are fully fluorinated. Each of these two categories consists of various subclasses, each containing a plethora of compounds, from which a limited number is examined in the present thesis (Figure 1).

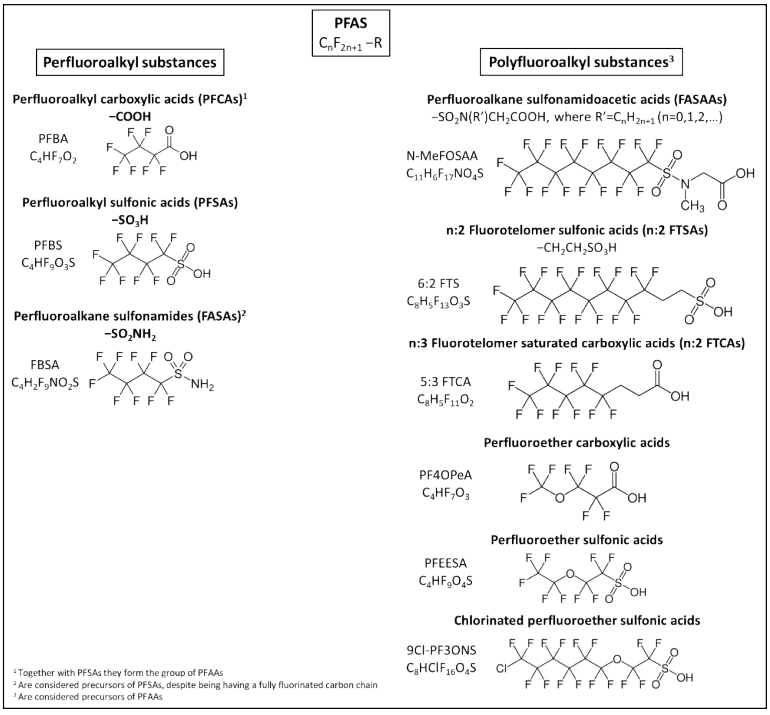


Figure 1: Examples of PFAS subclasses and structures examined in this thesis (adapted from Buck et al., 2011).

Despite the diversity of the structural spectrum of PFAS, a common feature is the presence of the C-F bond, which is considered to be one of the strongest single covalent bonds in organic chemistry (Buck et al., 2011; Smart, 1994). This bond gives PFAS most of their highly useful and enduring properties, including chemical inertness, thermal stability, low surface energy, UV and weather resistance. These properties make PFAS suitable for a wide range of applications like cosmetics, textiles, paints, food packaging, coatings, firefighting, medical devices and non-stick coatings (Baran, 2001; Buck et al., 2011; Gaines, 2023; Itumoh et al., 2024; Rekik et al., 2024). Yet, the C-F bonds also make PFAS very persistent, since a substantial amount of energy is required to reach mineralization of these compounds, if possible at all (Cheng et al., 2024). Another characteristic of many PFAS is their amphiphilic nature, due the concurrent presence of a hydrophobic fluorinated carbon chain and a hydrophilic polar head. This amphiphilic nature manifested in most PFAS is quite unique, as most synthetic chemicals tend to be either hydrophobic or hydrophilic. The amphiphilic nature makes PFAS very effective surfactants (Baran, 2001), further enlarging their application in industrial processes and in consumer products (Gaines, 2023) but also complicating their environmental fate.

PFAS in the environment

Although the widespread use of PFAS is acknowledged, defining the exact types of compounds, the amounts used as well as the quantities of PFAS that are eventually released or disposed into the environment remains a challenge (Gaines, 2023). The release of PFAS into the environment is encompassing and global, as they can enter the environment via many different routes and end up in different environmental compartments including water, sediment, soil and air. The main contributors to PFAS emissions into the environment include, but are not limited to industrial and manufacturing discharges, application of aqueous film forming foams (AFFF), wastewater treatment plants and landfill leachates (Lu et al., 2023; Sunderland et al., 2019; Trobisch et al., 2024). Furthermore, PFAS released from consumer products, constantly contribute to background levels via diffusion, although on a lower scale compared to emissions from point sources (Yang et al., 2025). Once released into



the environment, PFAS partition between the different environmental compartments (Alam et al., 2024) and consequently can be found in all types of environmental matrices. PFAS can be detected even in remote areas, where no PFAS point sources are present at close proximity (Alam et al., 2024; Björnsdotter et al., 2021), and especially some short-chain compounds are widespread in groundwater (Sadia et al., 2023). Their distribution and long-range transport potential depends on various factors, including the intrinsic compound properties like molecular weight, charge distribution, molar volume, solubility, as well as site- and media-specific characteristics like organic carbon content, pH, surface charge, redox conditions, and the presence of other contaminants (Alam et al., 2024). Understanding the interplay between these variables is essential in order to unravel and predict the environmental fate of PFAS, as these compounds can ultimately end up in living organisms.

PFAS bioaccumulation

The first peer-reviewed study that raised awareness of the bioaccumulation potential of PFAS, specifically PFOS, in wildlife was by Giesy & Kannan, (2001). Later and especially during the last decades, the topic of PFAS bioaccumulation has received increasing attention and it is now beyond doubt that many PFAS are highly bioaccumulative (Buytaert et al., 2025; Houde et al., 2011). Since PFAS are present in all environmental compartments, their uptake by organisms can also take place from different media and via different routes. For terrestrial plants, PFAS uptake via pore water by the roots comprises a major pathway (Gobelius et al., 2017). External adsorption and active uptake are two more PFAS uptake pathways relevant for both terrestrial and aquatic plants. Unlike most persistent organic chemicals, like polychlorinated biphenyls (PCBs), for which uptake is mostly dependent on passive diffusion (Burkhard, 2021; Burkhard & Votava, 2023a), PFAS uptake by plants seems to be concentration dependent, indicating that PFAS are also actively taken up (Ankley et al., 2021; Burkhard, 2021; Higgins et al., 2007; Ng & Hungerbühler, 2013; Ng & Hungerbühler, 2014). This combination of passive diffusion and active uptake often leads to high bioaccumulation factors, which cannot be solely explained

by diffusion driven partitioning as defined by the equilibrium partitioning (EqP) theory (Jonker & van der Heijden, 2007). Atmospheric deposition of particle-sorbed or free PFAS on leaves and subsequent absorption through leaf surfaces can also contribute to the PFAS load of terrestrial plants and emergent macrophytes (Li et al., 2022). For submerged macrophytes, their habitat and extent of contact with the sediment, water and air largely affect their PFAS uptake (Wang et al., 2019; Zhang et al., 2020a).

Animals share many of the PFAS uptake mechanisms of plants, like adsorption, active transport, exposure through the air and via the pore water, the latter being mainly important for sediment and soil inhabiting invertebrates (Yun et al., 2023). Next to that, many invertebrates are exposed to PFAS through dermal uptake and ingestion of particles or food to which PFAS are sorbed (Yun et al., 2023; Zhao et al., 2013). Apart from environmental exposure, dietary uptake can be especially relevant for animals (Hedgespeth et al., 2023). Dietary exposure can result in biomagnification across trophic levels, which has been reported for some PFAS in various food webs that included primary producers, invertebrates, birds, fishes and mammals (Dai et al., 2025; Hopkins et al., 2023; Huang et al., 2022b; Ibor et al., 2025). It is concluded that the exposure of organisms to PFAS via these diverse pathways translates into a complicated interplay of factors affecting PFAS bioaccumulation, which to a considerable extent remains to be elucidated.

Environmental factors and molecular properties drive PFAS bioaccumulation

Several environmental factors can influence PFAS bioaccumulation. Organic matter content, the presence of (dissolved) cations and soil and sediment composition (e.g. clay content) and properties (e.g. pH) are some of the factors that can alter PFAS bioavailability and consequently their uptake by organisms (Lasier et al., 2011; Wang et al., 2022a; Wen et al., 2016; Xia et al., 2015a; 2015b). Apart from environmental factors, intrinsic PFAS properties and characteristics also determine their bioaccumulation potential (Ahrens & Bundschuh, 2014; Yun et al., 2023; Zhang et al., 2020b). Long-chain PFAS are considered to bioaccumulate and biomagnify more



across food webs, however, shorter PFAS are increasingly detected in many organisms (Ateia et al., 2019; Brendel et al., 2018; Huang et al., 2022b). This could relate to their higher mobility, accompanied by increasing production volumes, following the restriction of longer-chain homologues. Inconsistent relationships between PFAS bioaccumulation and chain length have been reported (Hopkins et al., 2023; Lesmeister et al., 2021), which could relate to the importance of electrostatic interactions affecting PFAS uptake (Ding & Peijnenburg, 2013). This may especially be the case for short-chain PFAS, since hydrophobic interactions become less critical with decreasing chain length (Ehsan et al., 2024; Guelfo & Higgins, 2013; Sadia et al., 2024). Moreover, the unique amphiphilic characteristics of PFAS can result in uptake kinetics that cause exceedance of equilibrium partitioning-driven steady state concentrations in organisms within timeframes that are relevant to the life span of these organisms (Jonker & van der Heijden, 2007). This highlights that traditional lipophilicity driven molecular descriptors alone cannot accurately predict PFAS bioaccumulation.

Attempting to characterize the environmental distribution and bioaccumulation for the entire chemical space of PFAS is not realistic and therefore research on suitable descriptors that can predict their environmental fate has been initiated. The octanol-water partition coefficient (K_{ow}) is a common proxy for the bioaccumulation potential of organic contaminants. The K_{ow} is defined as the ratio between the concentration of a chemical in octanol, representing the lipid phase, and its concentration in water, representing the aqueous environment, with higher values indicating higher lipophilicity. However, determining the K_{ow} for surfactants and ionic chemicals like PFAS is associated with high uncertainty, stemming from their amphiphilic nature and ionic interactions (Droge, 2019; Hodges et al., 2019). In an attempt to more accurately predict PFAS bioaccumulation, the membrane-water partition coefficient (K_{mw}) holds potential to replace or compliment the K_{ow}. Phospholipid binding of PFAS has previously been reported (Chen et al., 2025; Qin et al., 2023; Xie, et al., 2010a; Xie, et al., 2010b; Zhao et al., 2023), and significantly affects, and in some cases can even be used to predict the tissue distribution of some

PFAS in organisms (Armitage et al., 2012; Ng & Hungerbühler, 2014; Shi et al., 2018). So far, only few K_{mw} values for PFAS have been published, although this research field is steadily expanding (Chen et al., 2025; Droge, 2019). Next to phospholipids, proteins comprise an equally, if not even more important determinant of PFAS bioaccumulation and distribution in biota (Chen et al., 2025; Starnes et al., 2025; Xiong & Li, 2024). Although some PFAS probably have a higher affinity for proteins compared to membrane lipids (Qin et al., 2023), a recent study showed that the PFAS binding affinity for both types of biomolecules was concentration dependent (Chen et al., 2025). At high PFAS concentrations non-specific binding was observed, while at lower, environmentally relevant exposure concentrations, protein binding seems to largely control the bioaccumulation of PFAS (Chen et al., 2025).

Ecological traits also influence PFAS bioaccumulation

Several ecological factors also affect PFAS bioaccumulation and biomagnification. Habitat plays a crucial role, since it defines through which environmental compartments organisms are exposed and to what extent (Gkika et al., 2025; Groffen et al., 2023; Pi et al., 2017; Yun et al., 2023). Different organisms have different diets and foraging modes, which further define PFAS exposure (Chumchal et al., 2022; Lewis et al., 2023; Penland et al., 2020; Prosser et al., 2016; Xiong & Li, 2024). PFAS bioaccumulation and internal distribution in organisms also depends on their tissue composition, especially their protein and phospholipid content, since PFAS preferentially bind to these biomolecules (Fremlin et al., 2023; Qin et al., 2023; Smeltz et al., 2023). It is concluded that even for a single species and a single PFAS there may not be a universal bioaccumulation factor (Liu et al., 2011), the more so since additional (external) factors also play a role in the exposure and uptake of PFAS by organisms, as discussed above.

Despite the adverse effects of environmental contaminants like PFAS on organisms, the latter are not just passive receivers of pollution, since they also actively shape their environment and can affect the environmental fate of chemicals. Burrowing



and feeding by animals rework soils and sediments, altering their composition, structure and texture (Aller, 1994; Kristensen et al., 2012; van der Meer et al., 2022). Plants are also able to alter the hydrological conditions, and microbial communities present in soils and sediments (Berhanu et al., 2023; Gamalero & Glick, 2024). Animal activity and plant roots drive dynamic changes between oxidizing and reducing conditions resulting in oscillating redox conditions, increased oxygen penetration and altered pH (Aller, 1994; Aller & Cochran, 2019; Kristensen et al., 2012). These processes can affect the speciation, residence depth, resuspension, bioavailability, biodegradation and transformation potential of contaminants (van der Meer et al., 2022), highlighting that organisms can play a crucial role in the environmental fate and organism exposure to PFAS. Apart from some studies showing that plants can affect PFAS environmental fate (Costello & Lee, 2024; Evangelou & Robinson, 2022; Scearce et al., 2023), limited research has been performed on how sediment dwelling organisms can affect PFAS bioavailability and distribution (Gkika et al., 2024; Wu et al., 2021).

Environmental risk assessment of PFAS

Traditionally, environmental risk assessment of chemicals is performed on a compound-by-compound basis (Drakvik et al., 2020). For PFAS, this started with environmental risk assessments of PFOS and PFOA, which were the first extensively studied compounds. Still, at this stage only for a handful of PFAS environmental quality limits have been derived and most are not legally binding (O'Sullivan Bakshi et al., 2025; Rüdel et al., 2022). Yet, PFAS are rarely found as single entities, since they are primarily produced and released as mixtures, which apart from legacy PFAS like PFOS and PFOA also include emerging PFAS, for which we lack vital occurrence data and effect concentrations (Dhore & Murthy, 2021; MacMillan et al., 2025). When trying to address PFAS mixtures, there is, however, not a single, generalized approach that can accommodate the diversity of these compounds, and therefore, risk assessment strategies vary based on the specific compounds, scenario, regulatory context and protection goals (Adewuyi & Li, 2024; Cousins et al., 2020a). Moreover, there are no standardized methods for

PFAS risk assessment, and approaches again vary based on region, specific compounds and assessment objective (Scaggiante et al., 2025). As risk is defined as the ratio between exposure and hazard, information on both these aspects is required. Yet, our insights into exposure and hazard are limited to only a small fraction of the PFAS present in the environment, highlighting that there are still major data gaps preventing a comprehensive environmental risk assessment for this diverse group of compounds (Ankley et al., 2021b).

Development of PFAS bans over time

After almost 40 years of intensive use, the United States Environmental Protection Agency (US EPA) first got alerted by 3M (3M, 1998) about the potential health hazards of PFAS. This marked the onset of awareness, but did not lead to immediate legally binding regulatory actions. A few years later, around 2000, the US EPA launched voluntary programs that encouraged manufacturers to phase out certain PFAS, but these were not legally binding (Brennan et al., 2021). During the same time, various US states implemented legal PFAS restrictions (Brennan et al., 2021). In Europe, the first PFAS-specific regulation was adopted under the European Union's persistent organic pollutants (POPs) framework (Directive 2006/122/EC) and in 2009 PFOS was listed in Annex B (restriction) of the Stockholm Convention, which is enforced globally to all member countries. Some eight years later in 2017, PFOA and its related compounds were restricted under the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation and listed in Annex A (elimination) of the Stockholm Convention (Commission Regulation 2017/1000). In the period 2021-2024, PFHxS and its related compounds followed (Delegated regulation 2023/1608), as well as the C9-C14 PFCA subgroup (Regulation 2021/1297) and PFHxA (Regulation 2024/2462).

The regulation of single compounds has frequently led to regrettable substitutions (Maertens et al., 2021). This was also the case for PFAS, where long-chain compounds were substituted by shorter chain homologues or other less stable PFAS, which were expected to be less persistent and bioaccumulative. These



alternatives, however, tend to be more mobile and many can (bio)transform into persistent PFAS (Ateia et al., 2019; Cheng et al., 2025), further perplexing the chemical space of PFAS present in the environment and further challenging the performance of a comprehensive risk assessment of PFAS as a group. This, in combination with an increasing body of knowledge and concerns about PFAS environmental and human health implications, has triggered a restriction proposal, aiming at a near-total phaseout of all PFAS. This process started in 2019 with a collaboration between the competent authorities of Denmark, Germany, the Netherlands, Norway, and Sweden, under the scientific coordination of the European Chemicals Agency (ECHA, 2025). Upon acceptance, the restriction will eventually be implemented through the REACH regulatory framework. A comprehensive environmental risk assessment for PFAS may aid the scientific underpinning of such restriction proposals, but currently still lacks the required information on PFAS distribution and bioaccumulation.

Aim, objectives and outline of the thesis

PFAS are a very large and diverse group of compounds possessing unique properties, which are therefore widely used and subsequently emitted into the environment. Despite the growing body of scientific publications on PFAS environmental occurrence and bioaccumulation, severe knowledge gaps still remain. The aim of this thesis was therefore to assess the environmental distribution, bioaccumulation and trophic transfer of PFAS in aquatic and terrestrial ecosystems. To meet this aim, a stepwise approach was followed. In order to appropriately steer the experimental efforts, **objective 1.** was to translate the general knowledge gaps concerning PFAS environmental risk assessment into more explicitly defined research priorities. This was done in **Chapter 2**, where current developments in PFAS environmental research were reviewed and knowledge gaps and subsequent research needs towards a more comprehensive environmental risk assessment of PFAS were defined. To this end, the open literature and databases on the environmental occurrence, hazards, and risks of PFAS were consulted. Based on the

identified research priorities the subsequent experimental objectives of the present thesis were defined:

2. To expand the spectrum of PFAS screened for in environmental and biotic matrices.
3. To expand the spectrum of organisms and taxa in which PFAS concentrations and bioaccumulation are measured.
4. To assess and compare the trophic transfer of a wide variety of PFAS in a terrestrial and an aquatic food web.
5. To investigate how the presence of benthic invertebrates and their bioturbation activity affect PFAS bioaccumulation.
6. To examine if PFAS molecular descriptors can describe their environmental distribution concerning **a)** bioaccumulation in primary producers and invertebrates, **b)** trophic transfer at the base of the food web, and **c)** redistribution in sediment-plant systems.

Chapter 3 aimed to investigate the distribution and bioaccumulation of a wide variety of PFAS in a contaminated terrestrial and aquatic ecosystem and to examine potential 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 near a fluorochemical industrial site. The concentrations of various PFAS from different subclasses 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). Potential relationships between these bioaccumulation factors and PFAS chain length, polar head, and membrane-water partition coefficient (K_{mw}) were investigated.

Chapter 4 aimed to unravel the PFAS transfer through the terrestrial and aquatic food web in a contaminated ecosystem, ending up in predatory spiders. First the structure of the integrated terrestrial-aquatic food web was unraveled using stable isotopes assessing the trophic position of each organism. Sampled organisms were



grouped according to their trophic position into primary producers, consumers (including herbivores and detritivores), and spiders as top predators. The concentrations and profiles of 44 PFAS were determined in all individual organisms. PFAS trophic transfer, expressed as either trophic magnification or trophic dilution factors, was examined by first excluding and then including the spiders as part of the food web.

Chapter 5 aimed to investigate the effects of the presence of a benthic invertebrate and its bioturbation activity on the bioaccumulation of various PFAS from field-collected sediments by a rooting macrophyte and the invertebrates themselves. To this end, the concentrations and profiles of different PFAS subclasses in sediments collected from a reference and a contaminated site were determined. The bioaccumulation of these PFAS by a rooting macrophyte (*Myriophyllum spicatum*) and a benthic invertebrate (*Lumbriculus variegatus*) was calculated and the effect of the presence and bioturbation by the benthic invertebrate on PFAS bioaccumulation and redistribution in the sediment-plant system was assessed.

In the synthesis, **Chapter 6,** the findings of the present thesis are integrated and discussed, meeting the objectives phrased above. The synthesis ends with the final conclusions.



Chapter Two

Research Priorities for the Environmental Risk Assessment of Per- and Polyfluorinated Substances (PFAS)

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Ioanna S. Gkika, Ge Xie, Cornelis A.M. van Gestel, Thomas L. Ter Laak, J. Arie Vonk, Annemarie P. van Wezel, Michiel H.S. Kraak

Abstract

Per- and polyfluorinated substances (PFAS) are a group of thousands ubiquitously applied persistent industrial chemicals. The field of PFAS environmental research is developing rapidly, but suffers from substantial biases towards specific compounds, environmental compartments, and organisms. The aim of this perspective was therefore to highlight current developments, and to identify knowledge gaps and subsequent research needs that would contribute to a comprehensive environmental risk assessment for PFAS. To this end, we consulted the open literature and databases, revealing that knowledge of the environmental fate of PFAS is based on the analysis of less than 1% of the compounds categorized as PFAS. Moreover, soils and suspended particulate matter (SPM) remain largely understudied. PFAS bioavailability, bioaccumulation and food web transfer studies also focus on a very limited number of compounds and are biased towards aquatic biota. Fish are predominantly studied, while aquatic invertebrates and macrophytes are included less frequently. The available ecotoxicity data revealed that only a few PFAS have been well-studied for their environmental hazards, and that PFAS ecotoxicity data are strongly biased towards aquatic organisms as well. This emphasizes the need to catch up with ecotoxicity studies in the terrestrial environment. Considering the persistency and bioaccumulation of PFAS, more chronic, multigeneration and community ecotoxicity studies are needed. Finally, we identified the urgent need to unravel the relationship between sorption, bioaccumulation and ecotoxicity on the one hand and molecular descriptors of PFAS chemical structures and physicochemical properties on the other, to enable prediction of exposure, bioaccumulation and toxicity.

2.1 Introduction

Per- and polyfluorinated substances (PFAS) are a group of ubiquitously applied persistent industrial chemicals, which, depending on the definition, consist of over 6000 (Glüge et al., 2020) or even over 1 million compounds (Sha et al., 2019; Wang et al., 2021). The main feature of PFAS is the C-F bond, which is one of the strongest atomic bonds, and the cause of their great persistence in the environment. Consequently, PFAS are hardly degradable, and therefore often labelled as 'forever chemicals' (Miner et al., 2021). Persistency can be an advantageous feature, as it makes chemicals suitable for application in a wide variety of industrial products. PFAS are therefore economically highly relevant, driving an increasing number of societal applications (De Boer & Stapleton, 2019). However, persistency is also a chemical feature of environmental concern (Cousins et al., 2019b). When associated with a high lipophilicity, it gives rise to bioaccumulation, food chain transfer and adverse human and environmental health effects (Fiedler et al., 2019; Guo et al., 2019). Because of their persistency, the high production volumes and broad spectrum of applications, PFAS are ubiquitously present in various abiotic (Ahrens et al., 2010; Campo et al., 2016; Carrasquillo et al., 2008; Esparza et al., 2011; Knight et al., 2021; Kwon et al., 2017; Saito et al., 2003; Scott et al., 2006; Strynar et al., 2012; Washington et al., 2010; Yoo et al., 2010) and biotic (Esparza et al., 2011; Houde et al., 2011; Murakami et al., 2011; Scott et al., 2006; Tittlemier et al., 2007) matrices at considerable concentrations (Cousins et al., 2022). Nonetheless, it remains unclear how many of the more than 6000 CAS-registered PFAS are actually present in the various environmental compartments and if so, at what levels (ITRC 2022). While the development of new analytical tools to detect the wide variety of PFAS has shown great progress (Jia et al., 2022), the application in environmental fate studies is lagging behind. Hence, there is an urgent need to study the environmental fate of the different chemical PFAS families.

Most PFAS have a polar head group and many of them are well soluble in water (>1000 mg/L) (ITRC 2022), making these compounds mobile in the environment.

Based on the amphiphilic properties of many PFAS, suiting their application as repellant coating, aqueous film forming agent, etc., they also tend to accumulate at water-solid and water-air interfaces. This complicates the determination of their bioavailability in complex environmental matrices such as soils and sediments (Reemtsma et al., 2016). Due to their surfactant-like properties, PFAS bioaccumulation is less well predicted by traditional indicators like lipophilicity (log K_{ow}) (Droge, 2019). Consequently, little is known about the bioavailability and bioaccumulation of PFAS (Pietrini et al., 2019), and their potential transfer across food webs (Liu et al., 2018). Research initiatives should therefore focus on an appropriate quantification of the bioavailability of PFAS, their bioaccumulation and trophic transfer. Moreover, efforts are also needed to investigate which chemical properties of PFAS can predict their bioavailability, bioaccumulation, and trophic transfer.

Compared to the highly debated (Green Science Policy Institute, 2022) and ambiguous information on human health risks based on detailed toxicological and epidemiological information for a limited number of PFAS, less is known about the hazards and risks of PFAS in the environment (Flynn et al., 2019; SETAC, 2019). Here we argue that the lack of ecotoxicity data for PFAS could to some extent relate to the scarcity of available standards at affordable prices, in combination with analytical challenges. Effects of PFAS on biota after long-term exposure (SETAC, 2019), and how these are affected by their intrinsic molecular properties and chemical structure are poorly understood (Ahrens & Bundschuh, 2014; Ding & Peijnenburg, 2013). This means that any established safe limits for PFAS in water, soil and sediment are preliminary, not based on thorough ecotoxicological considerations and prone to better scientific underpinning (Wintersen et al., 2019). The aim of this perspective was therefore to highlight current developments in PFAS environmental research, and to identify knowledge gaps and subsequent research needs to contribute to a comprehensive environmental risk assessment for PFAS. To this end, we consulted the open literature and databases on the environmental occurrence, hazard and risks of PFAS and integrated our findings

into the present critical perspective. Here, we defined PFAS according to the definition proposed by Buck et al., (2011), according to which PFAS are highly fluorinated aliphatic substances that contain one or more carbon (C) atoms on which all the hydrogen (H) substituents (present in the nonfluorinated analogues from which they are notionally derived) have been replaced by fluorine (F) atoms. When retrieving data from databases, the category PFAS was selected as provided by the database, which did not necessarily stick to the definition proposed by Buck et al., (2011). The way we explored literature and databases is elaborated in the Supplementary Information (Figure S1, Table S1).

2.2 Which PFAS are detected in the environment?

PFAS include thousands of per- and polyfluorinated substances, but it remains unclear how many of these compounds are actually present in the various environmental compartments and if so, at what levels. PFAS have been found in surface and ground waters and even in drinking water (Vughs et al., 2018), as well as in soils (Brusseau et al., 2020) and sediments (Bai & Son, 2021). Exposure pathways and environmental distribution processes of PFAS have been previously described by Panieri et al., (2022) and ITRC (2022). Nevertheless, a systematic inventory and detailed information on the occurrence and distribution of PFAS in the environment, both in terms of the presence of diverse types of PFAS (profiles) and the concentrations at reference sites and in polluted soils, sediments, and surface waters (levels), is virtually lacking. This becomes explicitly evident when evaluating the PFAS occurrence data for water and sediment available from the NORMAN database. Environmental occurrence data concerned freshwater (84.7%), brackish water (10.0%) and marine water (5.3%), and were available for 41 PFAS, visualized as the cumulative number of PFAS hits per year (to each consecutive year, the hits of the previous years were added) in Figure 2. This ranking showed that 50% of the cumulative occurrence data hits concerned 8 compounds, and 90% still concerned only 21 compounds. Dividing the number of PFAS for which environmental occurrence data were available (41) by the assumed total number of PFAS (6000)

revealed that the knowledge on their environmental fate is based on the analysis of less than 1% of the compounds that are currently categorized as PFAS. This is quite an alarming observation. Moreover, the data behind Figure 2 revealed a bias towards long-chained carboxylic acids, as defined by the OECD (2018). Hence, PFAS occurrence data is strongly biased towards a limited number of compounds and for the vast majority of PFAS reliable environmental occurrence data are still lacking. Although most legacy PFAS belong to the commonly studied PFAA family, the constant production of newly formulated emerging PFAS structures requires to broaden the spectrum of PFAS analyzed in environmental matrices. This argument is supported by the results of non-target screening and total oxidizable precursor (TOP) assay studies, which revealed that the number of quantified PFAS covers only a small fraction of what is actually present in the environment (Hensema et al., 2021; Göckener et al., 2021). As fundamental input for a reliable environmental risk assessment for PFAS, providing this missing information is urgently required.

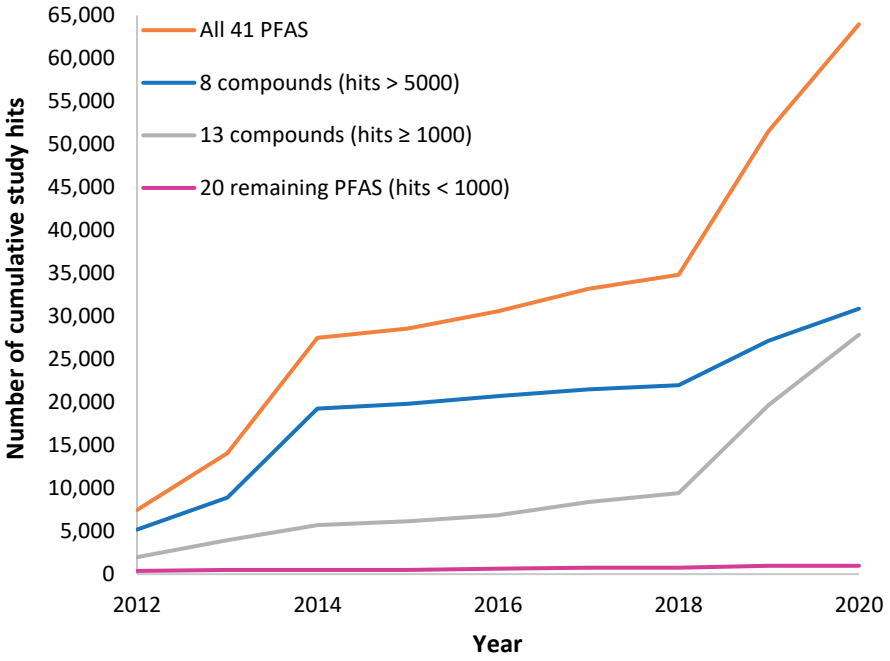


Figure 2: Cumulative number of published data on the occurrence of PFAS in the aquatic environment (water and sediment), shown for different PFAS, grouped based on the number of cumulative hits. Data were extracted from the NORMAN occurrence data base, which contains information from 2012 until 2020, by filtering for PFAS. See Table S2 in the Supplementary Information for a detailed overview of the chemicals that were included in this analysis.

The United States Environmental Protection Agency (USEPA) has documented standard methods for PFAS detection in drinking water (EPA 537.1 – Shoemaker & Tettenhorst, 2020; EPA 533 – Rosenblum & Wendelken, 2019), and non-potable water (EPA 8327 – U.S. EPA, (2021); EPA 1633 – U.S. EPA, (2024)), which can target up to 25 and 40 PFAS, respectively (Lewis et al., 2022; Simon et al., 2019). An ISO guideline (ISO, 2009) is also available for the determination of PFOS and PFOA in unfiltered samples of drinking water, ground water and surface water (freshwater and marine water) using high-performance liquid chromatography-

tandem mass spectrometry (HPLC-MS/MS). Within Europe some nations have standardized methods for PFAS analysis, such as the German DIN 38407-42 (2011) for water and DIN 38414-14 (2011) for sludge, compost and soil, but to the best of our knowledge there is no EU-wide unified protocol available. Protocols for PFAS detection in other matrices and for additional compounds have been reported, but these concern individual studies, all using slightly different extraction and analytical methods. Since these methods differ from laboratory to laboratory, there is no universal harmonized protocol for detecting a broader spectrum of PFAS. Deploying supplementary types of analysis, like the Total Organic Fluorine (TOF) method, as well as suspect- and non-target screening, can help broadening the spectrum of detected PFAS, although the results remain semi-quantitative or qualitative in some cases (Helmus et al., 2021).

By employing these diverse analytical methods, the cumulative number of detectable PFAS has increased over the years, up to 446 compounds in 2017 (Xiao, 2017). However, 435 of these could only be tentatively identified, due to the lack of corresponding analytical standards. Although this number is expected to increase over the years, with less than 80 reference standards available in 2022 (Androulakakis et al., 2022), the number of commercially available reference standards expressed as percentage of the known PFAS is still below 1.5%. Hence, the first step in enlarging the set of PFAS that can be analyzed would require further standardization of protocols, in combination with the extension of the number of available analytical standards, in order to increase the efficiency and reliability of suspect and non-target screening methods.

The available PFAS occurrence data for water and sediment used to construct Figure 2 were obtained from the NORMAN database, which, however, does not contain data for soil, immediately highlighting a bias towards the aqueous environment. Data of PFAS measurements in air are also missing from the NORMAN database, although it is a relevant matrix for some (semi)volatile PFAS structures and comprises an important transport pathway for many PFAS, mainly neutral and

volatile precursors (Ahrens et al., 2010; Armitage et al., 2009), along with the ocean currents (Zarfl et al., 2012). The bias towards water is further illustrated by compiling the available PFAS occurrence data from the published literature, showing that soils and suspended particulate matter (SPM) remain largely understudied, which we therefore identify as a pressing research priority. In contrast to the lack of air data in the NORMAN database, in the published literature we did find a considerable number of studies on PFAS measurements in air (gas or particle phase). Categorizing the literature data (Reference lists S1 and S2) per environmental compartment showed that 62.6% of the consulted studies analyzed PFAS in water phase and/or sediment, 27.9% measured PFAS in the air, while only 5.8% and 3.7% included soil and SPM respectively. The majority of aquatic studies concerned freshwater (69.4%), while much fewer studies considered marine (23.5%) and brackish water (7.1%). Historically, water received more attention than soil in chemical risk assessment, although soil is also an important compartment, which should not be neglected. It would therefore be a significant step forward if online databases like NORMAN would expand their PFAS occurrence data to also include the terrestrial environment.

2.3 Bioavailability, (bio)degradation, uptake, and bioaccumulation of PFAS

2.3.1 PFAS bioavailability and (bio)degradation

Contaminant bioavailability has been recognized as a critical metric for a proper exposure and effect assessment (Alexander, 2000; Cui et al., 2013). Bioavailability is strongly influenced by the intrinsic chemical properties of the compound, as well as by soil and sediment characteristics (Cui et al., 2013; Milinovic et al., 2015), affecting processes like sorption and diffusion (Reichenberg & Mayer 2006). The presence of cations and suspended solids can affect the overall sorption of anionic PFAS, consequently affecting the bioavailable fraction (Hammer et al., 2018). The freely dissolved concentration (C_{free}) is considered a useful metric of bioavailability, since it represents the contaminant concentration to which organisms are actually

exposed. But although PFAS are generally rather soluble in water, also for those that sorb to solids, C_{free} may not represent all potential uptake routes. Organisms interact with the abiotic environment through complex processes (Van der Meer et al., 2017). Some organisms might be simultaneously exposed to the freely available fraction of PFAS in the pore water, and the accessible fraction sorbed onto solids (Jager, 1998). The surfactant-like properties of PFAS, enabling them to form micelles (Alves et al., 2020; Kancharla et al., 2019) and to stick to interfaces, further complicate the determination of their bioavailability (Reemtsma et al., 2016). Properly assessing PFAS bioavailability, including investigating their interactions with the (a)biotic environment, is thus a prerequisite towards a comprehensive exposure assessment.

The (bio)degradation pathways of PFAS are poorly understood (Zhang et al., 2022b). Under ambient environmental conditions, abiotic processes that can transform precursors into non-degradable PFAS include hydrolysis, photolysis, and oxidation (ITRC 2022). Regarding biotic degradation, the majority of the reported reactions are limited to the removal of the non-fluorinated moieties, while complete defluorination seems less plausible (Zhang et al., 2022b). Moreover, after the removal of the functional groups, the degraded PFAS were even more persistent in the environment (Li et al., 2020). Certain enzymes, including transition metal-dependent enzymes, might be able to cleave the C-F bond through oxidation or reduction reactions (Shahsavari et al., 2021), however, not all PFAS are expected to be biodegraded through the same pathway, and a combination of several biodegradation pathways is more probable (Zhang et al., 2022b). Identifying these pathways and the resulting breakdown products including their environmental fate and (eco)toxicity is of high relevance for PFAS environmental risk assessment. (Bio)degradation is likely to further affect the composition of the PFAS mixture present in the environment, which can consequently affect the environmental exposure and thereby the ecotoxicity of these compounds.

2.3.2 Uptake and toxicokinetics of PFAS in biota

The accumulation of contaminants from the abiotic environment can take place through uptake from soil/sediment by ingestion, absorption from the water phase or a combination of these. There are, however, doubts about whether existing bioaccumulation metrics are applicable to PFAS (De Silva et al., 2021). One of the main considerations differentiating PFAS from hydrophobic organic compounds is their higher affinity for proteins and phospholipids over storage lipids (Armitage et al., 2017; Dassuncao et al., 2019), a property relevant for both short- and long-chained PFAS. Yet, the sorption affinity of different perfluoroalkyl acids (PFAAs) for proteins varies widely, suggesting site-specific interactions and facilitated transport of some compounds (De Silva et al., 2021; Henneberger et al., 2016). Therefore, protein or phospholipid normalization of measured PFAS concentrations seems worthwhile to consider (De Silva et al., 2021). For benthic invertebrates, sediment is governing PFAS exposure, while for pelagic organisms both water and sediment comprise important sources of PFAS exposure (Liang et al., 2022). For plants, the large root surface can favour the uptake of nutrients, which may potentially also favour the uptake of contaminants (Zhi et al., 2022). Similarly to other biota, the protein and lipid content of the roots may influence PFAS uptake kinetics and bioaccumulation (Wang et al., 2020a; Wen et al., 2018; Zhao et al., 2018). Once taken up by the roots, the Casparian strip comprises the main obstacle for PFAS translocation to other parts of the plant (Costello & Lee, 2024), although small and more water soluble PFAS can easily cross this barrier (Felizeter et al., 2012; Zhang et al., 2019a). According to two studies that tried to elucidate active transport mechanisms (Zhang et al., 2019; Zhi et al., 2022), the uptake of PFAAs seems to be an energy-dependent active uptake process, mediated by carrier proteins. Depending on the specific compound and plant species, different channels may be involved in these transport processes (Costello & Lee, 2024; Wang et al., 2020a; Zhang et al., 2019b). Although knowledge of PFAS bioaccumulation steadily increases, the processes governing the uptake of PFAS by organisms and their partitioning across tissues remain poorly understood, even for commonly studied PFAS (De Silva et al.,

2021). Filling this knowledge gap is required for the development of mechanistic models for the environmental risk assessment of PFAS (De Silva et al., 2021).

While it is assumed that most PFAS cannot be further be degraded, certain subclasses can undergo limited metabolism (Kolanczyk et al., 2023; Brase, et al., 2021). Flurotelomer alcohols (FTOHs) and perfluoroalkyl phosphate esters (PAPs) for instance can be transformed into PFCAs (Butt et al., 2014; D'eon and Mabury 2011). Other precursors include perfluoroalkyl sulfonamides sulfonamides (e.g. FBSA, FOSA), which can result in the formation of PFSAs (Ross, et al., 2012). It is important to deepen our understanding of PFAS metabolic pathways and biotransformation products across species since they could play a major role in the exerted toxicity.

2.3.3 Which PFAS are analyzed in biota?

Several studies reported the accumulation of PFAS in aquatic (in)vertebrates, including amphibians (Abercrombie et al., 2020), zebrafish (Menger et al., 2020), chironomids (Wen et al., 2016) and daphnids (Dai et al., 2013; Xia et al., 2013; 2015), sometimes even reaching high concentrations, like in crabs (Choi et al., 2020). Nonetheless, since bioaccumulation of PFAS is often not considered on forehand, this remains largely understudied for the majority of the fluorinated compounds, which is not justified by the literature cited here. A list of the compounds analyzed in biota can be found in the Supplementary Information.

Most bioaccumulation studies focused on linear isomers of compounds like PFOS and PFOA and other perfluoroalkyl acids (PFAAs), while branched isomers and other PFAS families remain largely understudied, including the compounds that are currently replacing PFOS and PFOA (Karnjanapiboonwong et al., 2018). This is reflected by data from the open literature published from 2012 until May 2022 compiled in Figure 3, highlighting a bias towards a limited spectrum of PFAS analyzed in biota. Based on these data, 50% of the studies that included measurements in biota concerned only 8 PFAS, while 90% of the studies still concerned only 34 out of the 164 PFAS for which bioaccumulation data were

available. PFOS and PFOA are the most commonly studied PFAS in biota, accounting for 13% of all studies. Moreover, 14 PFAS were investigated in only two studies (28 hits in total), and 83 PFAS in just one, pooled together in the orange bars in Figure 3. Taking into consideration the number of PFAS measured in biotic and abiotic matrices according to the open literature and the NORMAN database, this number still remains only a small fraction of the total number of PFAS.

2.3.4 Which organisms are studied for PFAS bioaccumulation?

PFAS analyses in biota show the same limitations as those in the abiotic environmental compartments, with a strong bias towards aquatic biota, leaving terrestrial organisms largely understudied. This becomes evident when consulting the available literature, since most of the studies published from 2012 until May 2022 that quantified PFAS in various biotic matrices focused on aquatic organisms (Table 1). It should be noted that the consulted literature included studies performed under laboratory conditions, as well as measurements in field-collected biota, but did not consider bioaccumulation measurements performed within ecotoxicity studies. Among aquatic organisms, fish were predominantly studied (36% of cases), with aquatic invertebrates and macrophytes being included less frequently. Considerable research has been performed on terrestrial biota, but most of these data concern agricultural crop species, studied under laboratory conditions, possibly due to their higher relevance for human exposure. More information regarding the studies included in this analysis, can be found in the Supplementary Information (Reference list S4).

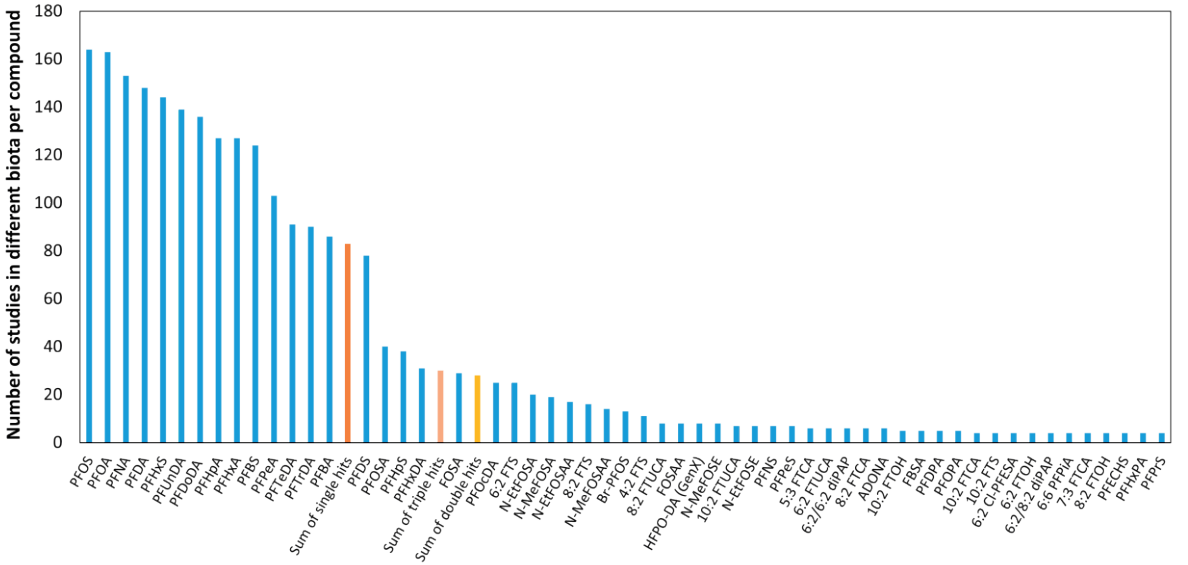


Figure 3: Number of studies ("study hits") for different PFAS in studies that measured concentrations in biotic matrices, including fish, aquatic and terrestrial invertebrates, mammals, macrophytes, periphytic biofilms, birds, reptiles, amphibians and eggs. All PFAS with a single hit were grouped together into the orange bar, to facilitate graphical visualization. Studies on PFAS bioaccumulation performed under laboratory conditions were also considered (e.g., mesocosm/mechanistic studies). Data are based on literature published from 2012 until May 2022. A complete list of the studies included, as well as the PFAS with one hit can be found in the Reference list S3 and in Table S3, respectively

In laboratory toxicity tests, sometimes also bioaccumulation of PFAS is measured. Such bioaccumulation data is usually calculated from quantified exposure concentrations in media and concentrations within organisms at the end of the toxicity tests. Hence, data on PFAS bioaccumulation in toxicity tests from 2012 to 2022 (latest update: March 15th 2023) was retrieved from the ECOTOX Knowledgebase, an open-access database, which is updated quarterly and is maintained by the US EPA that compiles data from peer-reviewed ecotoxicity studies (Olker et al., 2022) (Table 1). Data entry requirements of the ECOTOX Knowledgebase stated that publications must report biological effects on living organisms (US Environmental Protection Agency, 2022). To this end, studies that only focus on bioaccumulation of PFAS into organisms are not part of the ECOTOX Knowledgebase, in our case preventing overlap with the bioaccumulation only studies mentioned above. We extracted PFAS bioaccumulation data points reported for algae, amphibians, crustaceans, fish, higher plants, fungi, insects, spiders, mollusks, worms and other invertebrates. Subsequently, these data, listed separately in Table 1, were added as complementary information to the studies that focused on assessing PFAS accumulation in the laboratory and the field, consisting of studies where no toxicological endpoints were assessed. The available bioaccumulation data retrieved from the ECOTOX knowledgebase from the past 10 years are strongly biased towards fish and terrestrial higher plants, mainly crops. Since fish are one of the dominant groups used for toxicity testing (as discussed in the next chapter), the number of accumulation data generated from toxicity tests with fish is also higher compared to the other groups. Since it is argued that PFAS bioaccumulation can be species- and compound-specific (Johnson et al., 2021; Land et al., 2018), there is a clear need to catch up in assessing the bioaccumulation potential of the wide variety of PFAS structures in a broader range of organisms than studied so far.

Table 1: Total number of studies on PFAS bioaccumulation. Aquatic and terrestrial biota include both invertebrates and plants. The category 'other groups' includes aquatic and terrestrial mammals, birds, reptiles, amphibians and eggs. Studies on PFAS bioaccumulation under laboratory conditions were also considered (e.g., mesocosm/mechanistic studies). Data in the first row are based on literature published from 2012 until May 2022. A complete list of the studies included can be found in the Reference list S4. Data points on PFAS bioaccumulation studied in ecotoxicity studies (second row) were retrieved from the ECOTOX Knowledgebase.

	Fishes	**Aquatic Invertebrates and Macrophytes**	**Terrestrial Higher Plants**	**Terrestrial Invertebrates**	**Other groups**
Number of PFAS studies (laboratory and field studies)	75	45	24	15	47
Data points on PFAS accumulation from ecotoxicity studies (ECOTOX Knowledgebase)	239	117	581	51	N.A.[1]

[1] Not applicable

2.3.5 Trophic transfer of PFAS

Despite the increasing number of studies on PFAS transfer along food chains during recent years, knowledge on this topic remains limited (Liu et al., 2018). As observed for environmental fate and bioaccumulation, also the focus of food web studies is restricted to a limited number of PFAS, mostly long-chained PFAAs (OECD definition) leaving emerging PFAS families understudied (Figure 4). Based on studies published from 2012 until May 2022, we found 33 compounds with only a single hit in food web studies. Moreover, 50% of the food web studies concerned 10 PFAS, while 90% still concerned only 35 out of the 72 PFAS for which we found food web studies. PFOS was the most studied compound, while five long-chained perfluoro carboxylic acids (PFOA, PFDA, PFNA, PFUnDA, PFDoDA) and one medium-chained perfluoro sulfonic acid (PFHxS) shared the second place.

Strikingly, to the best of our knowledge, only one terrestrial food web study covering more than 2 distinct trophic levels has been performed (Huang et al., 2022b), emphasizing again a bias towards aquatic ecosystems. Concerning specific organism groups, higher plants were included in only 25% of the studies on PFAS food web distribution (Table 2).

Table 2: Categorization of food web studies per ecosystem type (first row) and inclusion of plants in these studies (second row). Data are based on literature published until May 2022. The list of publications is the same as the one from Figure 4 (Reference list S5).

	Aquatic	**Riparian**	**Estuary**	**Terrestrial**
Number of studies per ecosystem type	23	2	2	1
Number of studies including higher plants	4	2	1	0

2.4 Ecotoxicity of PFAS

2.4.1 Which PFAS are tested for ecotoxicity?

The need for a comprehensive ecological risk assessment for PFAS calls for an improved understanding of their ecotoxicity. Several studies have concluded, however, that the current information on the ecotoxicity of PFAS is scattered, ambiguous, and strongly biased toward a limited spectrum of compounds and test organisms (ITRC 2022; Van Keer et al., 2020; Zodrow et al., 2021). To address this knowledge gap, an analysis was conducted using data from studies spanning 2012 to 2022, focusing on algae, higher plants, fungi, amphibians, fish, insects, crustaceans, spiders, molluscs, worms, and other invertebrates. This data was retrieved from the ECOTOX Knowledgebase on May 17th, 2023. Prior to analysis, a data filtering process was implemented, excluding studies that failed to report results or relevant endpoints.

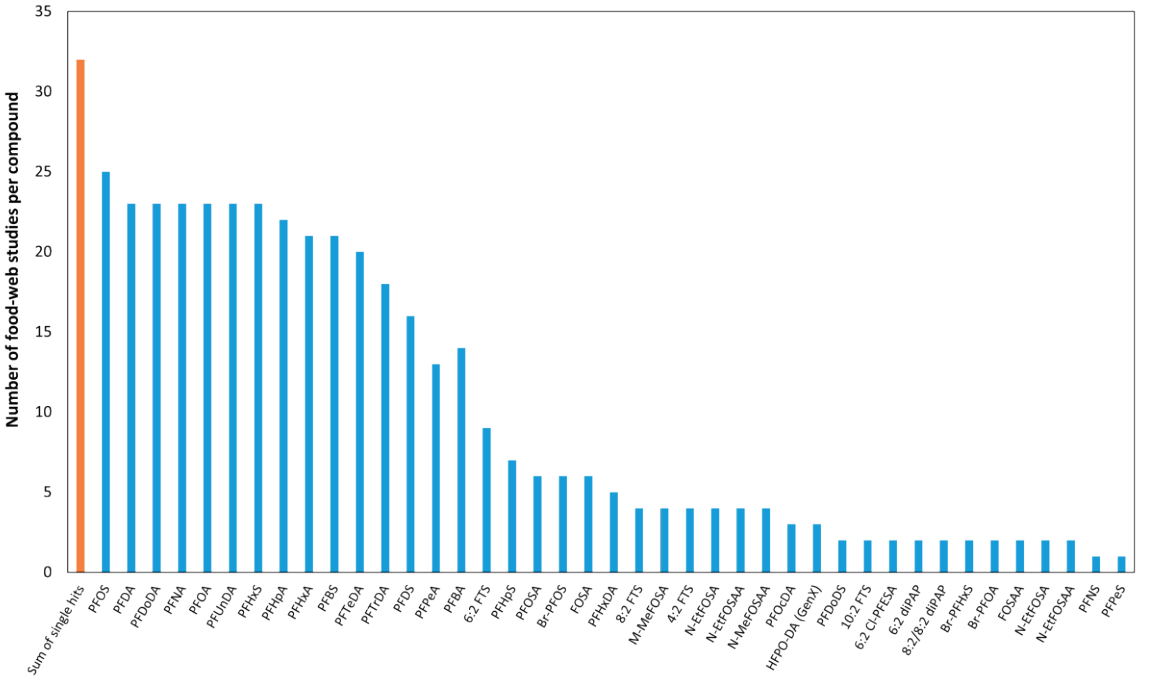


Figure 4: Number of study hits for different PFAS measured in food web studies. Only studies that covered more than 2 distinct trophic levels were included. Data are based on studies published until May 2022. All compounds with a single hit were grouped together and summed into the orange bar. A list of these single-hit compounds, as well as the list of publications from which the data were retrieved are available in Table S4 and the Reference list S5 respectively.

This inventory revealed that research on PFAS ecotoxicity has grown drastically over the past 10 years, resulting in an extensive dataset presently containing over 22,000 ecotoxicity data points. Yet, this dataset covers only a limited selection of 135 PFAS, representing 2.3% of the compounds currently defined as PFAS. PFOS and PFOA accounted for 45.0% of the entire data set, while the four following most studied compounds collectively represented 19.6%. The remaining 35.4% of the ecotoxicity data comprised only 129 compounds (Figure 5). All studied compounds were categorized into distinct PFAS families following the Organization for Economic Co-operation and Development [OECD] risk assessment report No. 39 (OECD, 2018). This report distinguished 134 PFAS categories based on their molecular structures, however, compounds from only 31 PFAS categories have been tested for ecotoxicity. The most studied groups were PFCAs (perfluoroalkyl carboxylic acids, their salts and esters; 11 compounds) and PFSAs (perfluoroalkane sulfonic acids, their salts and esters; 10 compounds). From 29 other PFAS families less than 5 compounds have been tested for ecotoxicity, while 56 PFAS were not classified into any group. The full list of PFAS and their classification can be found in the Supplementary Information (Tables S5 and S6). The present evaluation of the available ecotoxicity data underlines that among the thousands of PFAS that are known to be produced, only a few compounds of a limited number of PFAS categories have been well-studied for their environmental hazards. Since there is no clear common mode of action of PFAS (ITRC 2022), this limited focus impedes a detailed evaluation of PFAS ecotoxicity. Therefore, expanding the compound groups beyond PFSAs and PFCAs and broadening the number of individual PFAS tested in ecotoxicity studies are crucial steps towards a more comprehensive understanding of PFAS ecotoxicity, considering the diversity of molecular structures of PFAS.

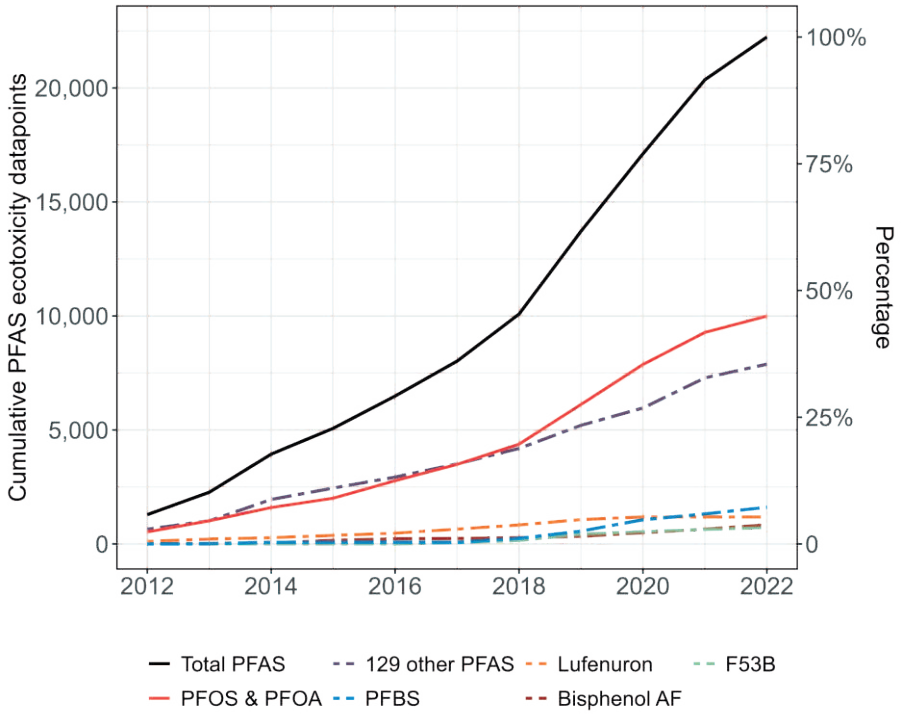


Figure 5: Cumulative number of data points for the ecotoxicity of PFAS. The total number of ecotoxicity data is shown for all PFAS, along with the number of data points for the most frequently investigated compounds (PFOS+PFOA, PFBS, Lufenuron and F53B), and for the other PFAS (129 compounds). See Tables S5 and S6 for a detailed overview of the PFAS compounds studied.

2.4.2 PFAS ecotoxicity to various organism groups

For both the aquatic and the terrestrial compartment, we categorized the available ecotoxicity data into two species groups, ranking these based on the highest number of available data points. The remaining data were labeled as other species (Figure 6, left). Subsequently, per environmental compartment we analyzed the distribution of the available ecotoxicity data over acute and chronic tests, following the criteria summarized by Davey et al., (2022) (Table S7). Next, we investigated the five most

extensively studied endpoints and grouped the remaining endpoints within each species group, labelling these as 'other effects' in Figure 6 (right).

Our evaluation showed that ecotoxicity studies on PFAS over the past 10 years generated a higher proportion of acute data for aquatic organisms, but in contrast a greater emphasis on chronic ecotoxicity was observed for terrestrial organisms (Figure 6, left). The main conclusion, however, is that the available data is strongly biased towards aquatic biota and a limited number of species groups.

A large number of PFAS ecotoxicity data for aquatic organisms was generated in fish tests, which accounted for 78.0% of the acute data and 60.0% of the chronic data, respectively. The acute fish tests were largely restricted to the fish embryo test. This test focusses on developmental effects during the first 5 days post-hatching, which is used as an indicator of the modes of action of compounds, and of mechanisms of environment-related diseases in vertebrates, including humans (Hill et al., 2005; Bambino & Chu, 2017). Moreover, over half of the fish tests focused on genetic, biochemical, and morphological endpoints (Figure 6a). Although many valuable data have been obtained by employing these tests, they primarily addressed compound toxicity rather than ecotoxicity, leaving the environmental hazards of PFAS relatively understudied. The second most studied species group in the aqueous environment was crustaceans, although they only comprised 7.0% of the acute data and 9.6% of the chronic data. Among the five most assessed effects, biochemical and genetic endpoints accounted for 34.0% of the data, and population, immobilization, and reproduction tests collectively accounted for 51.0% of the data (Figure 6b). Seven other species groups, including algae, amphibians, molluscs, insects, spiders, worms, and plants, were also studied for PFAS ecotoxicity in the aqueous environment. In these studies, 34.7% of the data concerned genetical, enzymatic, and biochemical endpoints, while only 25.0% addressed the effects of PFAS on the population growth of these organisms (Figure 6c). Additionally, among the 135 PFAS for which ecotoxicity data was available, fish studies covered 102 compounds, while only 31 compounds were tested on crustaceans and studies on the other 7 species groups collectively covered only 56 compounds. Furthermore,

76.8% of the present aquatic toxicity tests were performed in freshwater media, while only 10.7% and 12.5% of the tests were performed in saltwater media and other types of culture, respectively.

PFAS ecotoxicity testing on terrestrial organisms mainly focused on higher plants, and insects/spiders, leaving PFAS ecotoxicity to other organisms comparatively unknown. In addition to significantly less data being generated for terrestrial organism compared to aquatic organisms, the number of PFAS studied for terrestrial ecotoxicity was also severely limited. The ecotoxicity of 34 PFAS has been studied on higher plants, while the toxicity to insects/spiders was investigated for only 17 compounds. Studies on 6 other terrestrial species groups covered 33 PFAS. It is noteworthy that data on PFOS and PFOA collectively represented 69.0% and 66.0% of the data on higher plants and the 6 other species groups, respectively. In contrast to the other organism groups, data on the toxicity of 13 compounds, specifically manufactured as pesticides or fungicides, dominated the ecotoxicity dataset for insects/spiders, representing 97.0% of the data. In studies concerning higher plants, 47.7% of the data focused on biochemical, genetic, and enzymatic endpoints, while 28.6% and 15.7% of the data focused on PFAS accumulation and plant growth, respectively (Figure 6d). The endpoints studied for insects/spiders included mortality (38.6%), reproduction and population (33.7%), and behavioral and developmental effects (13.6%) (Figure 6e). Biochemical, genetic, and enzymatic endpoints collectively represented 55.9% of the data on 6 other terrestrial species groups, while population and reproduction data covered 24.9% of the dataset (Figure 6f).

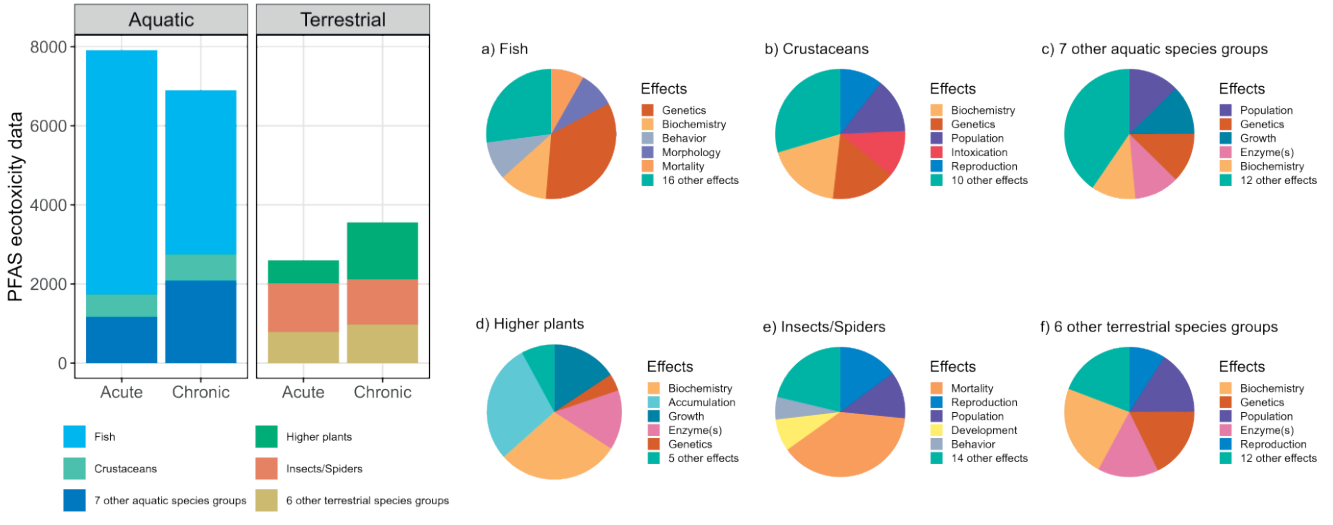


Figure 6: Distribution of ecotoxicity data on PFAS over acute and chronic tests for aquatic and terrestrial organisms (left), and over the 5 most studied endpoints in the respective species groups (right).

It has been argued that the concentrations at which PFAS show lethal effects in laboratory toxicity tests are unrealistically high and therefore not very environmentally relevant (Banyoi et al., 2022; Sinclair et al., 2020). Yet, considering the persistency of PFAS, organisms in PFAS-contaminated areas may be chronically exposed to substantially lower concentrations, potentially causing a wide range of more subtle sublethal effects (ITRC 2022; Sinclair et al., 2020). Thus, chronic studies focusing on the effects of PFAS on endpoints relevant to the population level, such as growth and reproduction, are considered to be much more important than acute tests with mortality as the only endpoint. However, according to the evaluation above, data for such relevant chronic endpoints is virtually lacking. Moreover, given the persistent and bio-accumulative nature of PFAS (Ankley et al., 2021), it is highly likely that organisms are exposed to and accumulate PFAS over multiple generations, asking for multigeneration studies in order to obtain a more realistic assessment of the environmental hazards of PFAS (Stefani el al., 2014; Guimarães et al., 2023).

The present inventory of PFAS ecotoxicity data underlined 1) the severe imbalance between the aqueous and terrestrial environment, 2) the severely insufficient knowledge of PFAS ecotoxicity across a broader range of compound families to a broader range of organisms, and 3) the severely insufficient knowledge of PFAS ecotoxicity regarding chronic ecologically relevant endpoints. As a result, current safe limits for PFAS in surface waters, soils and sediments are rather preliminary and primarily derived from human risks instead of ecotoxicological considerations (Wintersen et al., 2019; Smit & Verbruggen., 2022). Therefore, we identify these knowledge gaps as an urgent research priority for a realistic and reliable environmental risk assessment of PFAS.

2.5 The influence of molecular properties on PFAS environmental fate and adverse biological effects

For many organic chemicals, relationships have been documented between physicochemical properties and molecular descriptors on the one hand and fate (e.g.,

sorption, degradation, bioaccumulation) (Nguyen et al., 2020) and effects (e.g., ecotoxicity) on the other (Bleeker et al., 1998; Kraak et al., 2009). Such relationships, for instance, predict the potential bioaccumulation of chemicals in aquatic, benthic and terrestrial organisms from the octanol-water partition coefficient (K_{ow}), or the potential for biodegradation and biotransformation from molecular descriptors (Arnot et al., 2009; Gossett et al., 1983). Promising results have been obtained in predicting PFAS sorption based on such descriptors (Jiang et al., 2022). Nevertheless, currently available tools like EPI Suite™ (2025) seem not applicable for predicting the environmental fate of all PFAS, due to their amphiphilic structure, consisting of a polar head and a lipophilic tail. Similarly, predictions of the bioaccumulation of PFAS in aquatic and terrestrial organisms are not reliable, due to a lack of mechanistic understanding of the influence of the physicochemical and molecular properties on sorption behavior in soils and sediments and on the uptake of PFAS in biota. Moreover, recent publications reported the absence of significant correlations between traditional molecular descriptors, such as chain length, and bioaccumulation or biomagnification of PFAS (Byns et al., 2022; Mazzoni et al., 2020). Phospholipid membrane−water partition coefficients (K_{mw}) could serve as an alternative or complementary predictor of PFAS bioaccumulation potential (Droge, 2019; Ebert et al., 2020). The K_{mw} has been shown to better predict fish BCFs than K_{ow}, which led to opposite conclusions (Inoue et al., 2012). Nonetheless, the variety in structures, speciation and biodegradation potential make generalized predictions on the environmental fate of PFAS almost impossible and requires the screening of a wide diversity of compounds, in combination with mechanistic studies under realistic exposure scenarios and different environmental conditions. Above all, we foresee the need to evaluate the potential of novel molecular descriptors to serve as a better proxy for PFAS environmental fate, like membrane-water (K_{mw}) and protein-water ($K_{albumin\text{-}water}$) partition coefficients, in addition to traditional parameters like K_{ow} (Allendorf et al., 2019; Droge et al., 2016).

Apart from the intrinsic chemical properties, environmental conditions can also affect the environmental fate of PFAS. Sorption to soils and sediments seems to be

related to the organic matter content but is also highly dependent on other physical (clay content, grain size) and chemical (pH, presence of specific cations) characteristics (Milinovic et al., 2015). For example, pH could change PFAS speciation and soil chemistry, which both affect PFAS mobility (Nguyen et al., 2020). At severely contaminated sites, PFAS environmental fate could also be impacted by the amount initially released into the environment, in combination with their bioavailability, since competitive sorption of PFAS has previously been reported (Huang et al., 2022a; Maimaiti et al., 2018). This interplay between intrinsic chemical properties and external environmental conditions makes the prediction of PFAS environmental fate challenging.

Few attempts have been made to relate PFAS toxicity to chemical properties, mainly for human health related endpoints like the acute LD_{50} for rats (e.g., Feinstein et al., 2021). However, no such attempts have been made for sublethal effects and neither for the ecotoxicity of PFAS to aquatic and terrestrial organisms. QSAR tools are available to predict LC_{50} and EC_{50} values of chemicals from their molecular descriptors. Such relationships have been included in tools like the OECD QSAR Toolbox (2025) and EPI Suite™ (2025) which can be used for predicting the fate and effects of new chemicals submitted within regulatory frameworks like the Toxic Substances Control Act (TSCA) in the United States of America or REACH in the European Union. However, QSAR-like modelling requires large quantities of toxicity data, for which the conventional toxicity tests are not qualified, due to their resource- and labor-intensity. This calls for high-throughput toxicity, such as the TTR-TR CALUX test, the fish embryo tests (FET), the rapid daphnid immobilization tests etc. Such tests should be accompanied with exposure and bioavailability assessment, so that the outcome can be translated to relevant environmental conditions. In addition, machine learning techniques have already been incorporated into QSAR modelling to screen 1012 PFAS from the OECD list for 26 toxicity tests, but these were mainly related to human health rather than to ecotoxicity (Cheng & Ng, 2019). Hence, there is an urgent need to include environmentally relevant ecotoxicity tests in PFAS hazard assessment and to screen

a wide diversity of PFAS for fate and effects, in order to relate these to the chemical properties of the compounds.

2.6 Research priorities for an improved environmental risk assessment of PFAS

2.6.1 What is hampering PFAS environmental risk assessment?

Environmental risk assessment consists of exposure and hazard assessment. In an attempt to distinguish which of these two pillars comprises the limiting factor for PFAS environmental risk assessment, we investigated the overlap between available occurrence and ecotoxicity data. Data points on aquatic occurrence were obtained from the NORMAN database, which was visited in May 2022. For soil the publicly available literature was consulted, due to the lack of soil occurrence data in the NORMAN database and in this case the number of studies ("study hits") was counted. The ECOTOX Knowledgebase was used to gather relevant ecotoxicity datapoints for aquatic organisms and study hits for terrestrial organisms. The dataset was the same as the one used in Chapter 4. Since sediment inhabiting benthic organisms are not listed as a specific category in the ECOTOX Knowledge base, we only examined the occurrence-ecotoxicity overlap for the aqueous and soil compartments (Tables S8 and S9).

A visual representation of the occurrence-ecotoxicity overlap investigation is shown in Figure 7a for water and Figure 7b for soil. For the aqueous environment, there were 29 PFAS with both occurrence and ecotoxicity data available, although several compounds remained seriously understudied with very few study hits. There were four precursor PFAS, for which no ecotoxicity data on aquatic organisms were found and 91, for which no occurrence data were found. This discrepancy could be attributed to the different PFAS categorization by NORMAN database and ECOTOX knowledge base. For occurrence, most research has been performed on PFAAs, while the top compounds studied for ecotoxicity were by far PFOS and PFOA. For soil, both occurrence and ecotoxicity data were available for 28 PFAS and within those all but two (PFOS and PFOA) had less than 10 ecotoxicity study

hits. For a total of 40 PFAS belonging to different families no ecotoxicity data for terrestrial organisms were found in the ECOTOX Knowledge base, while for 24 compounds (mostly fluorinated pesticides), no soil occurrence data were found in the consulted literature. As mentioned previously, this could also relate to different definitions used for PFAS by the ECOTOX Knowledge base and the open literature that was consulted. Among the mostly studied PFAS for soil occurrence were several PFAAs, while for ecotoxicity, five pesticides along with PFOS and PFOA were studied far more than the other compounds.

Based on our findings, we identified a bias favoring the aquatic over the soil compartment and a serious knowledge gap on ecotoxicity, with only very few PFAS having a sufficient number of data points to enable a hazard assessment, especially for terrestrial organisms. Only for 29 and 28 PFAS we found both occurrence and ecotoxicity data for the aquatic and the terrestrial compartment respectively, and moreover these compounds were not the same for the two compartments. Only 18 compounds had both occurrence and ecotoxicity data available for both compartments, including PFAAs, GenX and F-53B. This implies that compounds belonging to other PFAS families are seriously understudied in terms of their aquatic or soil occurrence and ecotoxicity or both. More importantly, the total number of compounds for which we found both occurrence and ecotoxicity data in the two databases and the open literature, still represents only a very small fraction (below 0.5%) of the compounds that are currently categorized as PFAS. Even if this small fraction would concern the most abundant and the most toxic PFAS, this finding still highlights that the environmental risk assessment for this group of compounds contains some serious knowledge gaps.

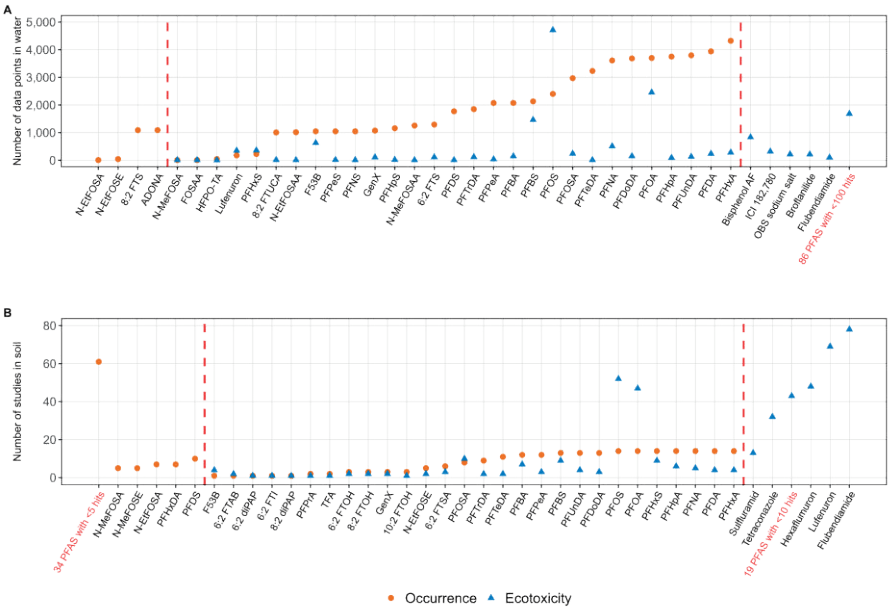


Figure 7: Number of data points on the occurrence of PFAS and the number of studies on the ecotoxicity of PFAS in water (a) and soil (b). In (a), PFAS with less than 100 ecotoxicity data points were grouped together and are highlighted in red. In (b), compounds with less than 10 occurrence and ecotoxicity study hits were combined in two different groups and are highlighted in red. See Tables S8 and S9 for a detailed overview of the data points and Reference list S6 for the soil study hits per compound.

2.6.2 Research priorities

To move towards an improved environmental risk assessment of PFAS, the present critical perspectives identified the following research priorities:

- To increase the efficiency and reliability of target, suspect and non-target screening methods for detecting and quantifying PFAS concentrations in different environmental compartments, with the emphasis on understudied PFAS, independent of family and chain length. This calls for further standardization of analytical protocols, in combination with the extension of the available analytical standards.

- To provide detailed information on the occurrence of PFAS in different environmental compartments, both in terms of chemical composition (profile) and concentrations (total and (bio)available levels). To compensate for the bias towards water and sediment, special focus on concentrations in soils and suspended particulate matter is required.
- To expand the limited spectrum of PFAS analyzed in biota and food web studies, including wild plant species and to catch up in the terrestrial environment to compensate for the bias towards aquatic organisms and aquatic food webs.
- To put emphasis on ecotoxicity testing of representatives of the different PFAS families with terrestrial organisms to compensate for the bias towards aquatic organisms, while including chronic and multigeneration ecotoxicity studies on relevant ecological endpoints like growth and reproduction.
- To relate the environmental fate and ecotoxicological effects of PFAS to their molecular properties to allow for extrapolation to untested compounds.
- Develop and evaluate methods to translate exposure in complex matrices like soil and sediment vs. water-only testing.

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Supplementary Information

The Supplementary Information for this chapter is available at: https://doi.org/10.1002/etc.5729



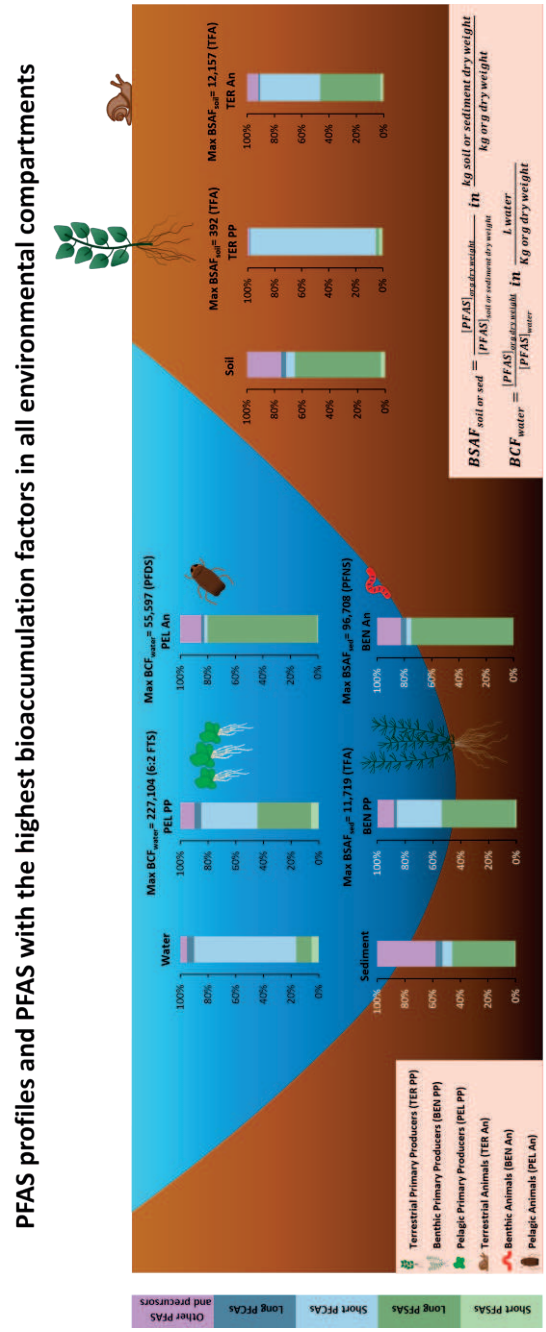
Chapter Three

Strong Bioaccumulation of a Wide Variety of PFAS in a Contaminated Terrestrial and Aquatic Ecosystem

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Ioanna S. Gkika, J. Arie Vonk, Thomas L. Ter Laak, Cornelis A.M. van Gestel, Jildou Dijkstra, Thimo Groffen, Lieven Bervoets, Michiel H.S. Kraak

Graphical Abstract



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 a 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.

Keywords: Bioaccumulation, primary producers, invertebrates, PFAS-contaminated ecosystem, terrestrial, aquatic

Highlights

- Understudied terrestrial and aquatic taxa were screened for 44 PFAS.
- A wide and diverse range of PFAS had high concentrations in soil, sediment and water.
- A wide range of PFAS strongly bioaccumulated in primary producers and animals.
- Several PFAS showed very high bioaccumulation and/or bioconcentration factors.
- Alternative molecular descriptors like K_{mw} may explain bioaccumulation patterns.

3.1 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 PFAS are also commonly found in many organisms (Huang et al., 2022b). 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., 2022; 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.

3.2 Materials and methods

3.2.1 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) (Figure 8). The location was chosen because it is a contamination hotspot in close proximity to the 3M 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 3M 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.

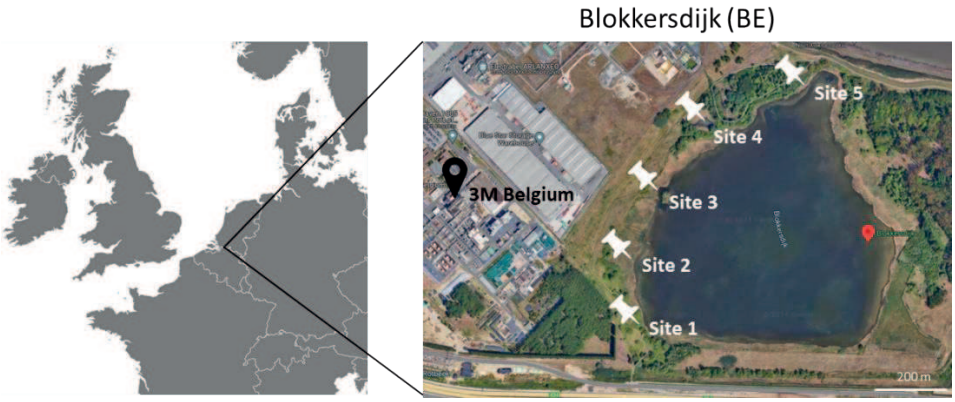


Figure 8: The location of Lake Blokkersdijk in Belgium and the five sampling sites (1-5) (Site 1; 51°13'46.2"N 4°20'29.6"E. Site 2; 51°13'56.0"N 4°20'28.9"E, Site 3; 51°14'05.4"N 4°20'40.1"E, Site 4; 51°14'09.3"N 4°20'43.0"E, Site 5; 51°14'09.9"N 4°20'51.8"E).

3.2.2 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 (Figure 8). 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).

3.2.3 PFAS extraction and analysis

All environmental and biota samples were analyzed for 44 PFAS 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).

3.2.4 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).

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

$$\text{AVG BSAF}_{\text{organism category}} = \frac{\sum_{n=1}^{n=x} \text{BSAF}_{\text{taxon}}}{\#\ \text{taxa}} \left[\frac{\text{kg}_{\text{soil or sediment}}\ \text{dw}}{\text{kg}_{\text{org}}\ \text{dw}}\right] \text{(1B)}$$

$$\text{BCF}_{\text{taxon}} = \text{AVG}_{\text{site=1}}^{5} \frac{[\text{PFAS}]_{\text{taxon}}}{[\text{PFAS}]_{\text{water}}} \left[\frac{\text{L}_{\text{water}}}{\text{kg}_{\text{org}}\ \text{dw}}\right] \text{(2A)}$$

$$\text{AVG BCF}_{\text{organism category}} = \frac{\sum_{n=1}^{n=x} \text{BCF}_{\text{taxon}}}{\#\ \text{taxa}} \left[\frac{\text{L}_{\text{water}}}{\text{kg}_{\text{org}}\ \text{dw}}\right] \text{(2B)}$$

In Equations (1A) and (2A), $[\text{PFAS}]_{\text{taxon}}$ is the PFAS concentration in each organism at each of the five sites, $[\text{PFAS}]_{\text{soil or sediment}}$ and $[\text{PFAS}]_{\text{water}}$ are the PFAS concentrations in the soil, sediment or water at the same site. In Equations (1B) and (2B) the $\sum_{n=1}^{n=x} \text{BSAF}_{\text{taxon}}$ and $\sum_{n=1}^{n=x} \text{BCF}_{\text{taxon}}$ 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)).

3.2.5 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 ($p < 0.05$).

3.3 Results

3.3.1 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 (Figures 9A-9B). 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) (Figures 9A-9B). 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 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%) (Figure 9A). 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 (Figure 9B).

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, Figure 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

3.3.2 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%) (Figure 10A). 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) (Figure 10B). 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 (Figure 10A). Average total ∑PFAS concentrations were similar in benthic and pelagic primary producers (3,207 and 3,522 ng/g dw, respectively) (Figure 10B) 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%) (Figure 10C). 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) (Figure 10D). 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) (Figure 10C). 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 (Figure 10D). 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).

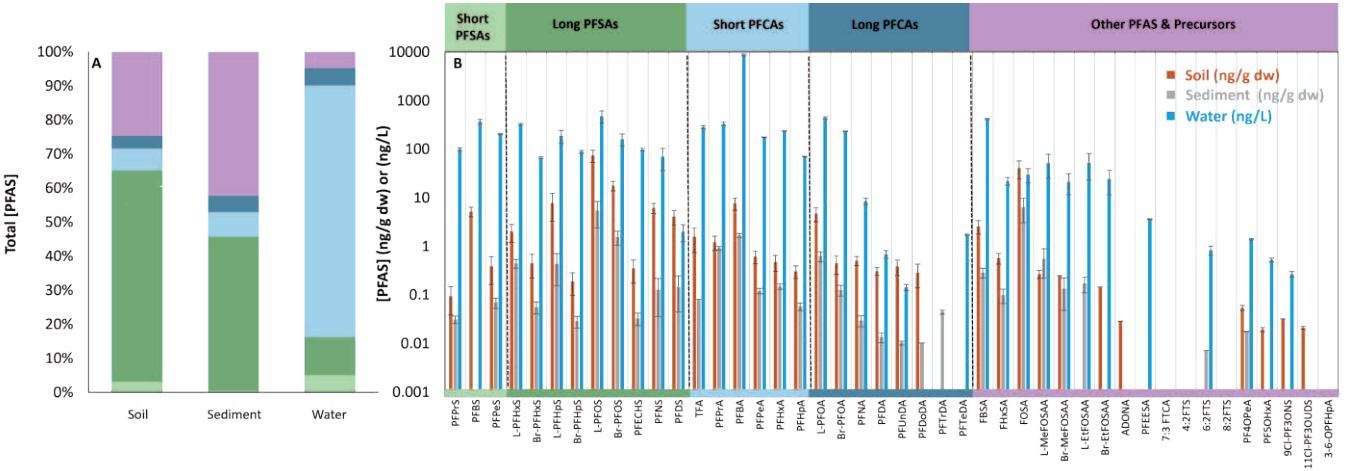


Figure 9: Relative PFAS profiles per subclass (weight basis) and average (n = 5 sites) individual PFAS concentrations in soil, sediment and water sampled from Lake Blokkersdijk, reported in ng/g soil dw, ng/g sediment dw and ng/L water, respectively. In panel A, the different colors correspond to the 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.

3.3.3 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 (Figure 11), 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 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 (Figure 11). 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 (Figure 11). 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).

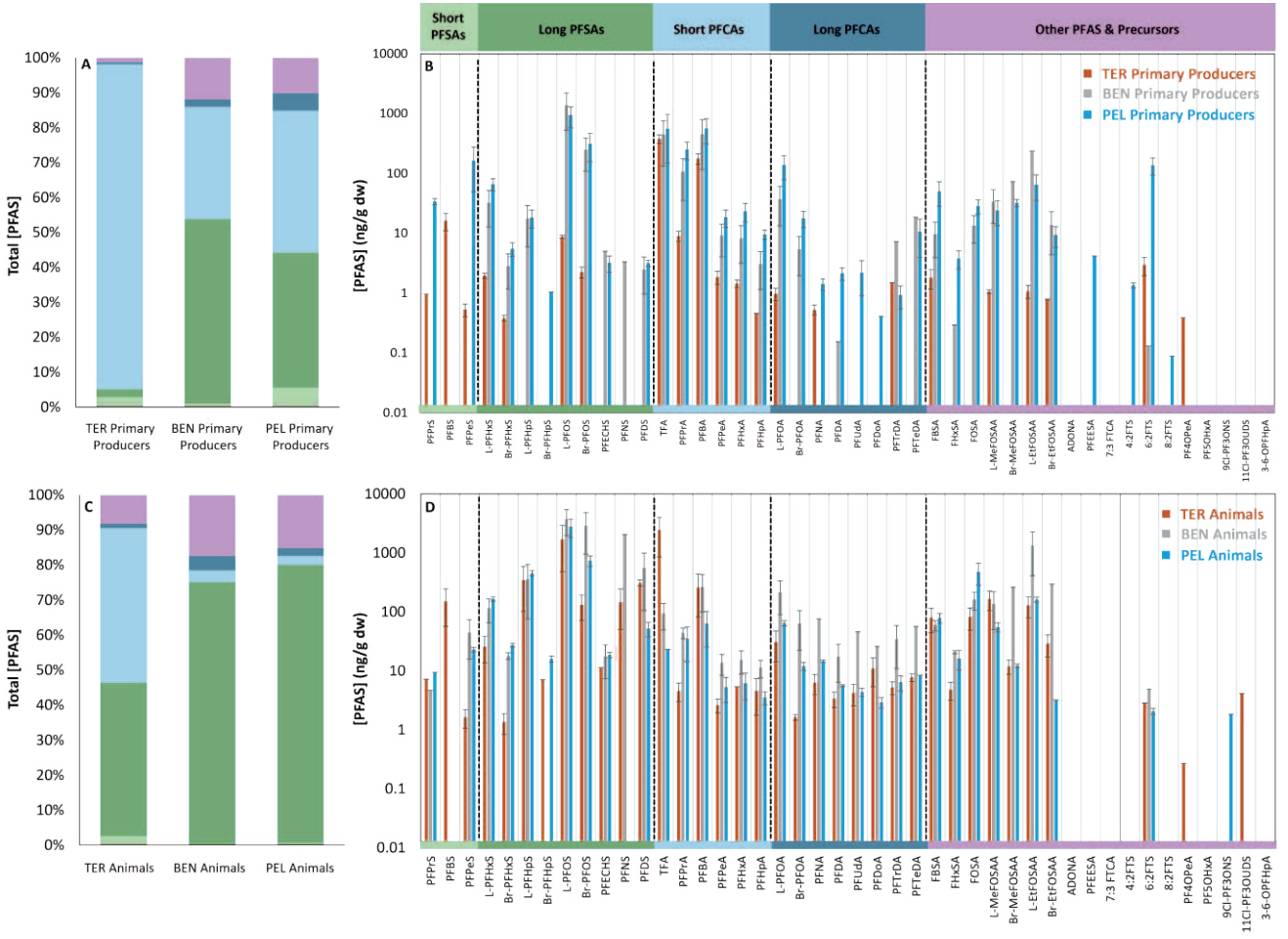

A
Total [PFAS]
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
TER Primary Producers
BEN Primary Producers
PEL Primary Producers
B
[PFAS] (ng/g dw)
10000
1000
100
10
1
0.1
0.01
Short PFSAs
Long PFSAs
Short PFCAs
Long PFCAs
Other PFAS & Precursors
TER Primary Producers
BEN Primary Producers
PEL Primary Producers
PFPrS
PFBS
PFPeS
L-PFHxS
Br-PFHxS
L-PFHpS
Br-PFHpS
L-PFOS
Br-PFOS
PFECHS
PFNS
PFDS
TFA
PFPrA
PFBA
PFPeA
PFHxA
PFHpA
L-PFOA
Br-PFOA
PFNA
PFDA
PFUdA
PFDoA
PFTrDA
PFTeDA
FBSA
FHxSA
FOSA
L-MeFOSAA
Br-MeFOSAA
L-EtFOSAA
Br-EtFOSAA
ADONA
PFEESA
7:3 FTCA
4:2FTS
6:2FTS
8:2FTS
PF4OPeA
PF5OHxA
9Cl-PF3ONS
11Cl-PF3OUDS
3-6-OPFHpA
C
Total [PFAS]
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
TER Animals
BEN Animals
PEL Animals
D
[PFAS] (ng/g dw)
10000
1000
100
10
1
0.1
0.01
TER Animals
BEN Animals
PEL Animals
PFPrS
PFBS
PFPeS
L-PFHxS
Br-PFHxS
L-PFHpS
Br-PFHpS
L-PFOS
Br-PFOS
PFECHS
PFNS
PFDS
TFA
PFPrA
PFBA
PFPeA
PFHxA
PFHpA
L-PFOA
Br-PFOA
PFNA
PFDA
PFUdA
PFDoA
PFTrDA
PFTeDA
FBSA
FHxSA
FOSA
L-MeFOSAA
Br-MeFOSAA
L-EtFOSAA
Br-EtFOSAA
ADONA
PFEESA
7:3 FTCA
4:2FTS
6:2FTS
8:2FTS
PF4OPeA
PF5OHxA
9Cl-PF3ONS
11Cl-PF3OUDS
3-6-OPFHpA

Figure 10: 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 line. All raw data behind the plots, as well as PFAS concentrations in all individual taxa are included in Tables S8-S13.



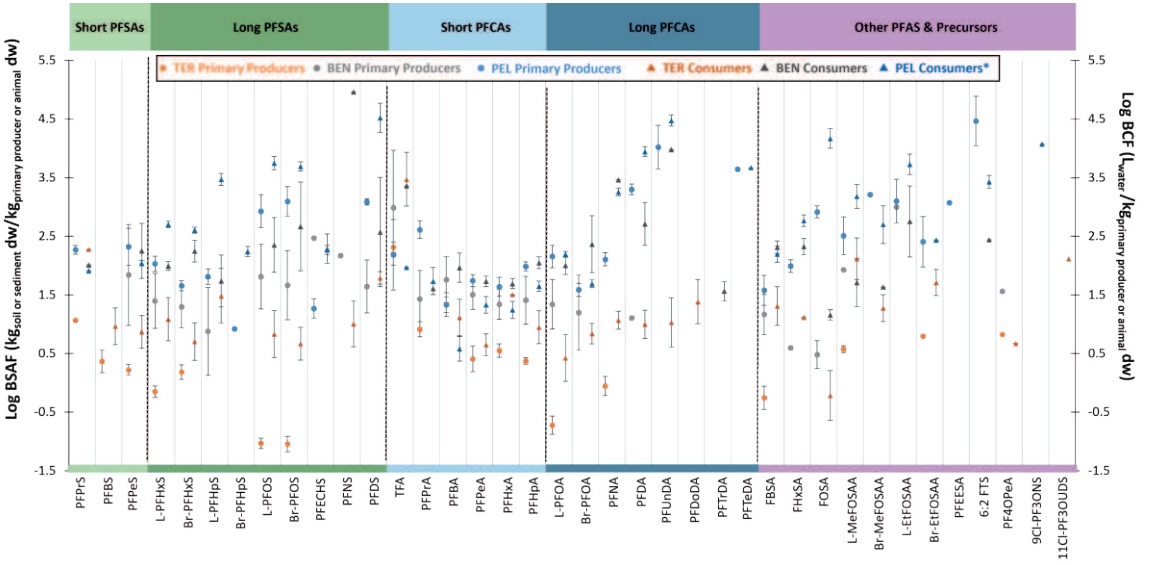


Figure 11: Logarithmically transformed biota-to-soil and biota-to-sediment bioaccumulation factors (BSAFs) for the uptake of PFAS from soil and sediment into terrestrial (TER) and benthic (BEN) primary producers and animals (left Y axis), respectively, reported in kg soil or sediment dw/kg org dw, and 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).

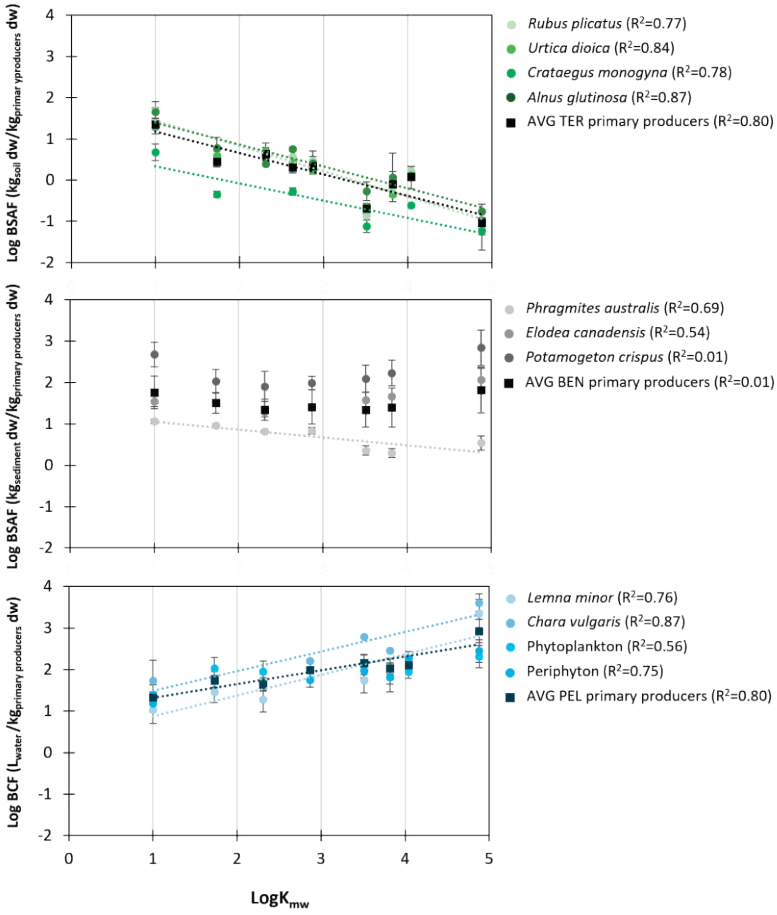


Figure 12: Correlation between average bioaccumulation factors in primary producers and membrane-to-water partition coefficients (K_{mw}) of PFAS. For the uptake of PFAS from soil and sediment into terrestrial (A) and benthic (B) primary producers, the logarithmically transformed biota-to-soil or biota-to-sediment 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).

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 (Figure 11). 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) (Figure 11). 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 (Figure 11). BCFs for pelagic animals were calculated for 31 PFAS, yet based on only one taxon (Corixidae) (Figure 11). 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).

3.3.4 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, Figure 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* (Figure 12). 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).

3.4 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 this 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 primary producers compared to terrestrial and pelagic primary producers that 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.

3.4.1 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 & 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 & Mabury, 2014) and the use of larger quantities to obtain similar product performance, as the technical performance of 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 all 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 & 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 & 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.

3.4.2 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., 2020a; 2021a) 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., 2019b). 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., 2019a). Earlier studies have also found significantly higher concentrations in SPM compared to sediment (Borthakur et al., 2021; Liu et al., 2019a), 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.

3.4.3 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 & Landberg, 2024; Griffin et al., 2023; Mei et al., 2021; Prosser et al., 2016; Wen et al., 2013; Zhang et al., 2021a). This may explain the differences between the bioaccumulation factors from various studies (Arnot & Gobas, 2006; Burkhard & Votava, 2023a; 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.

3.4.4 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 & van der Heijden, 2007). With most PFAS being present in their anionic form in natural environments (Ding & 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 & 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 & 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 & Votava, 2023a), 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 & 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 & 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., 2010a; Xie et al., 2010b; 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}.

3.5. 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.

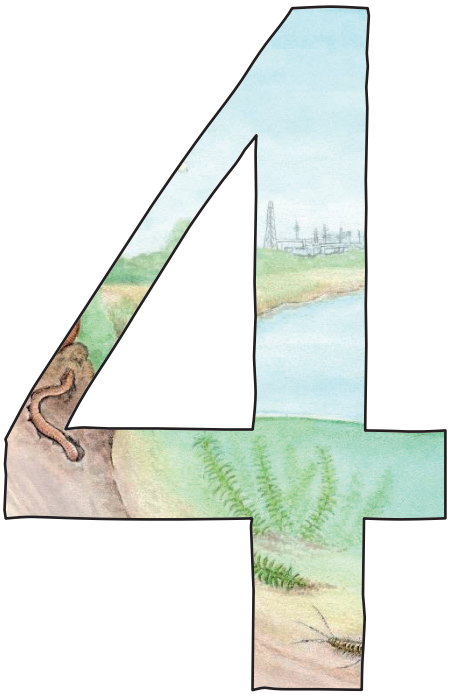
Acknowledgements

The authors declare no conflicts of interest

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Supplementary Information

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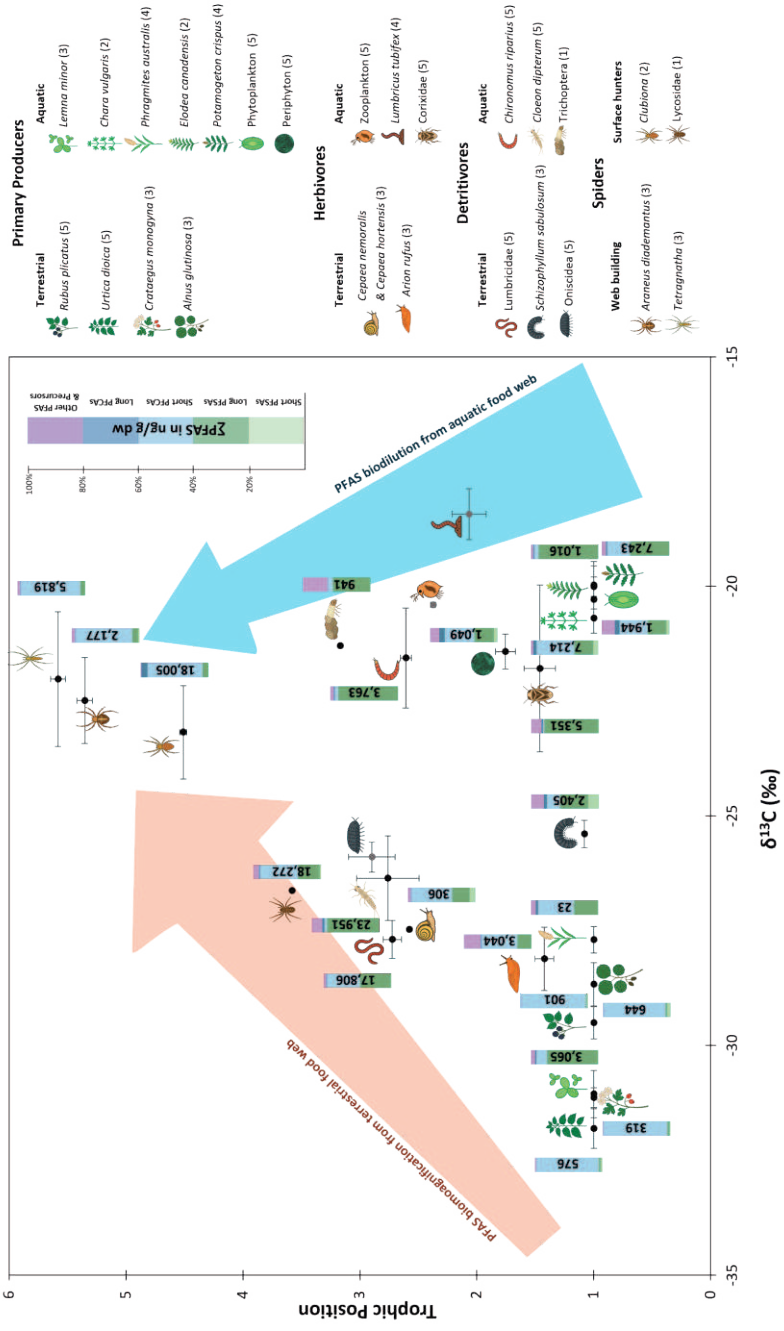
Chapter Four

Differential PFAS Transfer Through the Terrestrial and Aquatic Food Web to Predatory Spiders

Under review

Ioanna S. Gkika, J. Arie Vonk, Thomas L. Ter Laak, Cornelis A.M. van Gestel, Jildou Dijkstra, Thimo Groffen, Lieven Bervoets, Michiel H.S. Kraak

Graphical Abstract



Abstract

The great environmental persistence of Per- and Poly Fluorinated Substances (PFAS) causes prolonged exposure of organisms, leading to bioaccumulation and trophic transfer. However, if trophic transfer is studied at all, the different ecosystems are generally still examined in isolation. The aim of this study was therefore to unravel the PFAS transfer through the terrestrial and aquatic food web, ending up in predatory spiders. To this end, organisms from the aquatic and terrestrial compartments of a PFAS-contaminated ecosystem were collected and screened for 44 PFAS. The trophic position of the organisms was investigated using stable isotopes. PFAS were present in organisms from all trophic levels (primary producers, herbivores, detritivores, predators), albeit with varying total concentrations and profiles. Several PFAS tended to biomagnify in the spiders through the terrestrial food web, but biodiluted through the aquatic food web. For some PFAS-organism combinations, very high concentrations were observed, and moreover, there is now evidence that some PFAS have the potential to biomagnify. The apparent arbitrariness of which substances biomagnify and which biodilute, in combination with the trophic transfer of TFA, the smallest and most polar PFAS, emphasizes that we still lack insight into the drivers of environmental PFAS distribution, uptake and transfer.

Keywords: trophic transfer, primary producers, invertebrates, PFAS-contaminated ecosystem, stable isotopes, integrated approach

Synopsis: This study reports the ubiquitous presence of various PFAS in many organisms and their trophic transfer in a combined terrestrial and aquatic ecosystem.

4.1. Introduction

Per- and Poly Fluorinated Substances (PFAS) are a widely used group of synthetic chemicals, characterized by their high stability and great environmental persistence (Parsons et al., 2008). These properties make them suitable for numerous industrial and consumer applications but also render them hazardous environmental contaminants. Their widespread use in combination with their general high mobility results in the ubiquitous global detection of PFAS in many environmental compartments (Brunn et al., 2023; Wang et al., 2025). Once in the environment, their persistency causes prolonged exposure of organisms, leading to bioaccumulation, trophic transfer, and consequent adverse effects on human and environmental health (Fiedler et al., 2019; Guo et al., 2019).

The omnipresence of PFAS, in combination with their worrisome properties, has initiated extensive research on their occurrence, distribution, bioaccumulation and trophic transfer (Gkika et al., 2023). Yet, these studies still cover only a small fraction of the over 4,700 Chemical Abstracts Service (CAS)-registered PFAS that have been identified on the global market (OECD, 2018) and also cover only a limited number of organisms and ecosystems (Brunn et al., 2023; Gkika et al., 2025; Lewis et al., 2022; Wang et al., 2025). Nonetheless, the limited available information so far already shows varying PFAS bioaccumulation and biomagnification between different ecosystems (Dai et al., 2025; Huang et al., 2022b; van den Brink et al., 2016).

The complex and constant interplay between terrestrial and aquatic ecosystems and the potential transfer of PFAS from aquatic to terrestrial biota (Daley et al., 2011; Koch et al., 2020; Yu et al., 2013) highlight the need to study the fate of PFAS in different ecosystems in an integrated holistic approach. Yet, if trophic transfer is studied at all, the different ecosystems are still examined in isolation, albeit the number of studies employing an integrated approach is increasing (Daley et al., 2011; Endicott et al., 2025; Gkika et al., 2025; Hopkins et al., 2023; Ibor et al., 2025; Koch et al., 2020; 2021). Here, we argue that terrestrial spiders offer the unique possibility to integrate the fate of PFAS in the aquatic and terrestrial environment, since they

are among the few primarily terrestrial invertebrate predators that incorporate aquatic resources into their diets, thereby linking aquatic and terrestrial food webs (Middendorf et al., 2025; Paetzold et al., 2006).

The aim of this study was therefore to unravel the PFAS transfer through the terrestrial and aquatic food web in a contaminated ecosystem, ending up in predatory spiders. To this end, the structure of the integrated terrestrial-aquatic food web was unraveled using stable isotopes assessing the trophic position of each organism. Sampled organisms were grouped according to their trophic position into primary producers, consumers (including herbivores and detritivores), and spiders as top predators. The concentrations and profiles of 44 PFAS were determined in all individual organisms. PFAS trophic transfer, expressed as either trophic magnification or trophic dilution factors, was examined by first excluding and then including the spiders as part of the food web. By doing so, the PFAS fluxes through the terrestrial and aquatic food web could initially be examined separately, while by adding the spiders the effect of a predator that feeds on both terrestrial and aquatic resources on the trophic transfer of PFAS was assessed.

4.2. Materials and methods

4.2.1 Field sampling

For a detailed description of the study area and sampling sites, we refer to Gkika et al., (2025), since the same samples were analyzed and exactly the same setup as for the current study was used. Briefly, the most common organisms from the terrestrial and aquatic ecosystem were sampled at five sites, between 200 and 300 m apart, along the western bank of Lake Blokkersdijk (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), nearby a fluorochemical plant close to Antwerp (Belgium). Sampling included primary producers, herbivores, detritivores and predators, all identified to the lowest possible taxonomic level. For terrestrial primary producers, stems with leaves were hand-picked from four plant species (*Rubus plicatus, Urtica dioica, Crataegus monogyna* and *Alnus glutinosa*). Upon

collection, plant samples were rinsed with ambient water to remove any particles. Terrestrial invertebrates were manually collected from the soil surface and the surrounding vegetation and included the Gastropoda *Arion rufus* and *Cepaea* spp. (including both *Cepaea nemoralis* and *Cepaea hortensis*), Oligochaeta/Lumbricidae, the diplopod (Julidae) *Schizophyllum sabulosum,* and Isopoda/Oniscidea. Aquatic primary producers included phytoplankton (size class 41-335 μm; collected by filtering water through a 335 and 41 μm mesh plankton net, subsequently), periphyton scraped from reed stems, and whole plants of three submerged (*Potamogeton crispus, Elodea canadensis, Chara vulgaris),* one floating (*Lemna minor*), and young shoots (50 cm) of one emergent *(Phragmites australis*) macrophyte species. Upon collection, the macrophyte samples were rinsed with ambient water to remove any debris. Zooplankton was collected by filtering water through a 335 μm mesh plankton net and consisted almost exclusively of *Daphnia magna*. Aquatic macroinvertebrates were collected using a 0.5-mm mesh sweeping net through the aquatic vegetation as well as on top of the sediment layers and included Corixidae and *Lumbricus tubifex* and three taxa of (epi)benthic insect larvae (*Chironomus riparius, Cloeon dipterum, Mystacides longicornis*). Predatory spiders were collected from the littoral and terrestrial vegetation and included two web building species (*Araneus diadematus, Tetragnatha sp.*) and two surface hunters (*Clubiona sp.*, Lycosidae). Further details on the replication of field samples are included in the Supplementary Information (Section S1, Table S1).

4.2.2 Construction of the terrestrial-aquatic food web by stable Isotope analysis

In order to construct the food web and assign a trophic position to each organism, stable isotope analysis was performed. Prior to the analysis all samples were freeze dried. Pooled *C. nemoralis*/*C. hortensis* samples were acidified with sufficient HCl (3 mM) to remove any inorganic carbon and subsequently freeze-dried again. Samples were homogenized using a mortar and pestle or an herb grinder mill for the reed *P. australis*. When there was enough dry weight of the sampled organisms to allow for technical replicates, triplicate samples per taxon (2-7 mg of dry material) were

weighed in tin capsules. Otherwise, one sample per taxon was analyzed. Total C, total N, $\delta^{13}C$ and $\delta^{15}N$ were measured using a Vario Isotope cube, Elementar GmbH (Langselbold, Germany) and sequentially a BioVision isotope ratio mass spectrometer (Elementar, UK Ltd., Manchester UK). Isotope ratios were defined following Equation (3), where R_{sample} is the ratio between the heavy and light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in each sample and $R_{standard}$ is the same ratio in the reference material (^{13}C: IAEA-CH_6, sucrose and IAEA-600, caffeine; ^{15}N: USGS-25, ammonium sulfate and IAEA-N_2, ammonium sulfate). A higher $\delta^{13}C$ or $\delta^{15}N$ value (‰) means that the sample is more enriched with the heavy isotope, so with either ^{13}C or ^{15}N (van der Lee et al., 2021).

$$\delta_x = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1{,}000 \quad (3)$$

The trophic position of all taxa, except for Lumbricidae, was determined based on the model proposed by Post (2002) (Equation (4)) for each consumer taxon sample separately ($\delta^{15}N$consumer). Following Borgå et al., (2012) and Post, (2002), the $\delta^{15}N$ value of herbivores, representing trophic position 2, was selected as the baseline ($\delta^{15}N$base) (Borgå et al., 2012; Post, 2002). For the terrestrial taxa, the $\delta^{15}N_{base}$ was the average $\delta^{15}N$ value of the Gastropoda, while for the aquatic taxa it was the average $\delta^{15}N$ value of the zooplankton, *L. tubifex* and Corixidae. The Δn represents the expected enrichment of $\delta^{15}N$ with each trophic position and was set to 2.55 for both terrestrial and aquatic taxa, based on the meta-analysis by Matthews and Mazumder, (2008). The trophic positions of all primary producers, except for periphyton, were set to one, since periphytic biofilms include both autotrophs and heterotrophs, and so its overall $\delta^{15}N$ signature is expected to reflect mixed trophic contributions. The trophic positions of the predatory spiders were calculated based on the baseline of the terrestrial ecosystem. For Lumbricidae, trophic position was calculated according to the regression proposed by Potapov et al., (2019) (Equation (5)), since both anecic and epigeic earthworm species were sampled. Trophic positions were calculated for each taxon individually as further elaborated in Section S2 (Text S1).

$$TP = 2 + \left(\frac{\delta^{15}N_{consumer} - \delta^{15}N_{base}}{\Delta_n}\right) \quad (4)$$

$$TP_{Lumbricidae} = 2.05 + 0.0658 * \delta^{15}N \quad (5)$$

4.2.3 PFAS extraction and analysis

All organisms were analyzed for 44 PFAS covering a wide range of structures, including six isomer pairs, (Section S3, Text 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". Short-chain PFCAs included compounds with up to six fluorinated carbons, while short-chain PFSAs included compounds with up to seven fluorinated carbons (ITRC 2023). When showing PFAS profiles, herbivores and detritivores were merged into the group of consumers and their concentrations were plotted together. The protocols used for PFAS extraction, analysis and quality assurance/quality control (QA/QC) assessment have been described previously (Gkika et al., 2024; 2025; Sadia et al., 2020; 2023). Briefly, 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 Gkika et al., (2025), since results from the same samples were used in the present study. Some biota samples did not fulfill the QA/QC criteria and were therefore excluded from further analysis (Section S3, Text S2).

The concentrations of 44 PFAS were measured in the sampled terrestrial and aquatic organisms. In the aquatic biota, 42 PFAS were measured since two compounds (PFBS and 7:3 FTCA) did not fulfill the QA/QC criteria and were therefore excluded from further analysis. Concentrations in organisms from the five sampling sites were averaged per species for the whole study area. These average concentrations were then summed per PFAS subclass to determine the PFAS

profiles in each of the taxa on a dry weight basis (ng/g dw). 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 (Section S1, Table S1).

4.2.4 Trophic magnification factors

To evaluate the trophic transfer of PFAS through both the terrestrial and aquatic food web up to the predatory spiders, a compound-specific regression analysis between the calculated trophic positions and the log-transformed PFAS concentrations of all terrestrial and all aquatic taxa separately was performed, similar to Jones et al., (2014) for metals. Trophic magnification was examined first by including only the primary producers, herbivores and detritivores of the aquatic and terrestrial food web separately, excluding the spiders. Next, trophic magnification was calculated again, now also including the spiders as predators, catching prey from both terrestrial and aquatic origin. Linear regressions to determine trophic magnification factors (TMFs) and trophic dilution factors (TDFs) of individual PFAS were performed only when more than 50% of the included organisms had concentrations > LOQ (LOQ criterion). For these PFAS, the values for organisms with concentrations < LOQ were substituted by 0.5*LOQ. For statistically significant regressions (Pearson correlation test; α=0.05) of the TMF (Equation 6), we distinguished three types of results based on the slope (b). A slope > 0 indicated biomagnification and subsequently TMFs were calculated (Equation (6)). A slope < 0 pointed towards biodilution and subsequently, trophic dilution factors (TDFs) were calculated using the same equation. Non-significant regressions indicated that neither biomagnification nor biodilution took place.

$$\text{TMF or TDF} = 10^{b} \quad (6)$$

4.3. Results

4.3.1 Aquatic-terrestrial food web structure

The food web structure of the joint terrestrial and aquatic ecosystem of Lake Blokkersdijk was evaluated using $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures (Figure 13; Section S4, Table S2). In general, the aquatic primary producers had higher $\delta^{13}C$ (± SEM (Standard Error of the Mean)) values compared to the terrestrial ones, except for *Lemna minor*, which had among the lowest $\delta^{13}C$ signatures. The $\delta^{13}C$ values of terrestrial primary producers ranged from -31.8 ± 0.43‰ (*Urtica dioica*) to -28.7 ± 0.47‰ (*Alnus glutinosa*), while those of aquatic primary producers, excluding *Lemna minor*, ranged from -20.7‰ (*Chara vulgaris*; n=1) to -20.0 ± 0.52‰ (*Potamogeton crispus*). Also, most of the terrestrial herbivores and detritivores showed more negative $\delta^{13}C$ values compared to the aquatic ones. Concerning the two terrestrial herbivores, *Arion rufus* had the lowest $\delta^{13}C$ value (-28.1 ± 0.69‰) and (pooled) *Cepaea nemoralis* and *Cepaea hortensis* the highest (-27.5 ± 0.04‰), while $\delta^{13}C$ values of aquatic herbivores ranged from -21.8 ± 1.8‰ (Corixidae) to -18.4 ± 0.56‰ (*Lumbriculus tubifex*). The three terrestrial detritivores had similar $\delta^{13}C$ values, ranging from -27.7± 0.41‰ (Lumbricidae) to -25.4 ± 0.30‰ (*Schizophyllum sabulosum*), while aquatic detritivores ranged from -26.4 ± 0.92‰ (*Cloeon dipterum*) to -21.3‰ (*Mystacides longicornis*; n=1). All predatory spiders had $\delta^{13}C$ values similar to the aquatic organisms, ranging from -23.2 ± 1.0‰ (*Clubiona sp.*) to -22.0 ± 1.5‰ (*Tetragnatha sp.*), except for Lycosidae which appeared more depleted (-26.6‰; n=1), with a value closer to that of the terrestrial organisms.

Average isotopic $\delta^{15}N$ signatures of terrestrial herbivores and detritivores (range -1.92 to 14.6‰) were generally lower than those of the aquatic ones (range 6.99 to 12.9‰). After calculating the trophic position of both food webs separately based on the base $\delta^{15}N$ signatures of the main herbivores in each system, the trophic position (± SEM) of the consumers showed similar ranges between the two parts of the food web. For the herbivores, the trophic position ranged from 1.4 ± 0.08 (*A. rufus*) to 2.6 ± 0.01 (pooled *C. nemoralis* and *C. hortensis*) for the terrestrial and from

1.5 ± 0.13 (Corixidae) to 2.4 ± 0.02 (zooplankton) for the aquatic organisms. The trophic position of the detritivores was also similar, except for the millipede *S. sabulosum*, which had the lowest trophic position of 1.1 ± 0.003. Trophic position values of the other two terrestrial detritivores were 2.7 ± 0.08 (Lumbricidae) and 2.9 ± 0.2 (Oniscidea), while for the aquatic detritivores they ranged from 2.6 ± 0.05 (*Chironomus riparius*) to 3.2 (*Mystacides longicornis*; n=1). The trophic position of all spiders was higher compared to all other organisms, extending from 3.6 (Lycosidae; n=1) to 5.6 ± 0.06 (*Tetragnatha sp.*).

4.3.2 PFAS profiles in the organisms

The numbers of unique PFAS quantified in each of the five organism groups ranked from highest to lowest were: 37 (aquatic primary producers) > 36 (terrestrial consumers) > 34 (aquatic consumers) > 26 (spiders) > 22 (terrestrial primary producers) (Section S5, Figures S1-S5; Table S3). PFAS were then grouped into five subclasses, leading to the PFAS profiles described below. All PFAS concentrations are reported on a dry weight basis (ng/g dw). The concentrations and profiles of PFAS in the abiotic environment are reported by Gkika et al., (2025).[8]

Primary producers

All primary producers contained numerous PFAS, with varying profiles and individual PFAS concentrations (Figure 14A-14B). In the terrestrial primary producers, the number of quantified PFAS ranged from 13 (*Crataegus monogyna;* 30% of quantified PFAS) to 20 (*Urtica dioica*; 46%). All terrestrial primary producers had very similar PFAS profiles, clearly dominated by short-chain PFCAs (> 92% in all cases) (Figure 14A). The ∑PFAS concentrations in terrestrial primary producers were within the same order of magnitude, ranging from 319 (*C. monogyna*) to 901 ng/g dw (*Rubus plicatus*). TFA and PFBA always had the highest concentrations, ranging from 219 (*C. monogyna*) to 570 ng/g dw (*R. plicatus*) for TFA and from 79 (*C. monogyna*) to 281 ng/g dw (*R. plicatus*) for PFBA.

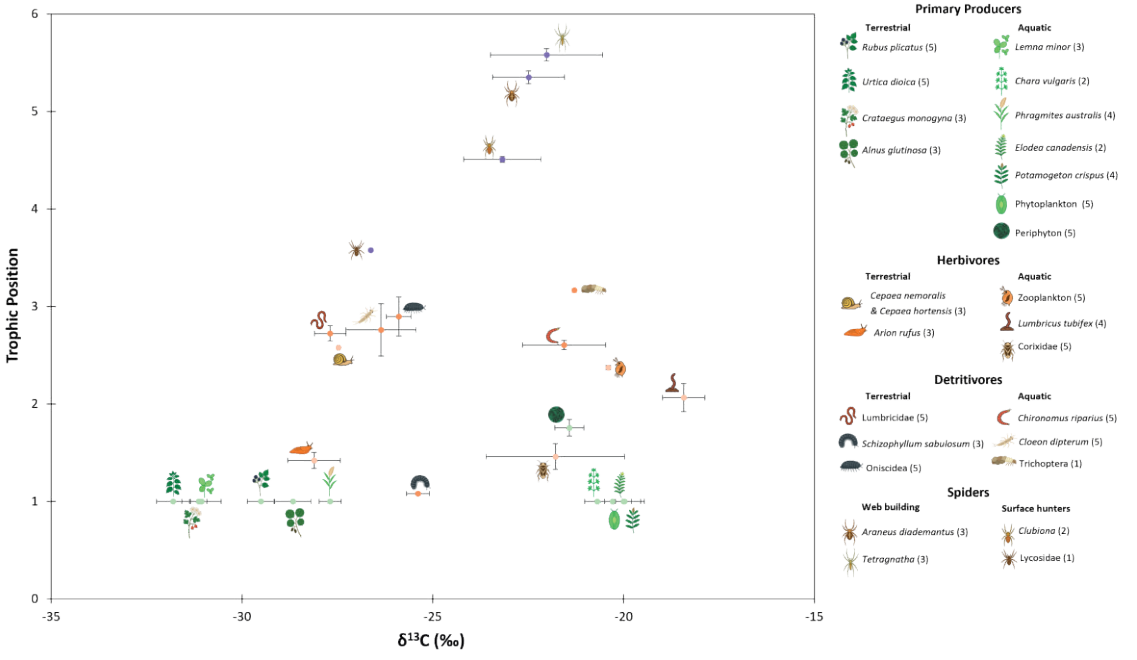


Figure 13: Structure of the terrestrial and aquatic food web of the Lake Blokkersdijk ecosystem. Data points show average Trophic Position (TP) and δ13C values and error bars represent the corresponding Standard Error of the Mean (SEM) for organisms from the five different sample sites in the area. TPs were calculated using the δ15N stable isotope signatures, as explained in section 2.2. The different colors of the data points correspond tothe differenttrophic categories of the organisms, including: primary producers (green), herbivores (light peach), detritivores (orange) and predatory spiders (purple). The legend on the right indicates the taxa that correspond to each icon and the number of field replicates is shown in parenthesis.

In the aquatic primary producers, the number of quantified PFAS ranged from 12 in the macrophyte *Elodea canadensis* (29%) to 31 in both periphyton and phytoplankton (74%). The PFAS profiles were more variable in the aquatic compared to the terrestrial primary producers (Figure 14B). The PFAS profiles of *E. canadensis*, *Lemna minor* and *Potamogeton crispus* were dominated by long-chain PFSAs (88%, 75% and 57% respectively), with short-chain PFCAs also contributing considerably to the PFAS load in *P. crispus* (37%). In the remaining aquatic primary producers, short-chain PFCAs accounted for the majority of the total PFAS concentration (*Chara vulgaris*: 56%; *Phragmites australis*: 53%; periphyton: 44%; phytoplankton: 42%), but still with a considerable contribution of long-chain PFSAs (range 27% to 37%). A higher number of detected PFAS did not always translate into higher ∑PFAS concentration. The ∑PFAS concentrations varied more than two orders of magnitude between the different aquatic primary producers, ranging from 59 (*P. australis*) up to 7,243 ng/g dw (*P. crispus*). The long-chain PFAS L-PFOS and PFBA were always among the top three compounds with the highest concentrations, ranging from 11 (*P. australis*) to 3,411 ng/g dw (*P. crispus*) for L-PFOS, and from 20 (*P. australis*) to 1,446 ng/g dw (*C. vulgaris*) for PFBA. The short-chain PFCAs TFA and PFBA were also among the compounds with the highest concentrations in some aquatic primary producers, with concentrations reaching 1,985 and 1,446 ng/g dw in *C. vulgaris*, respectively.

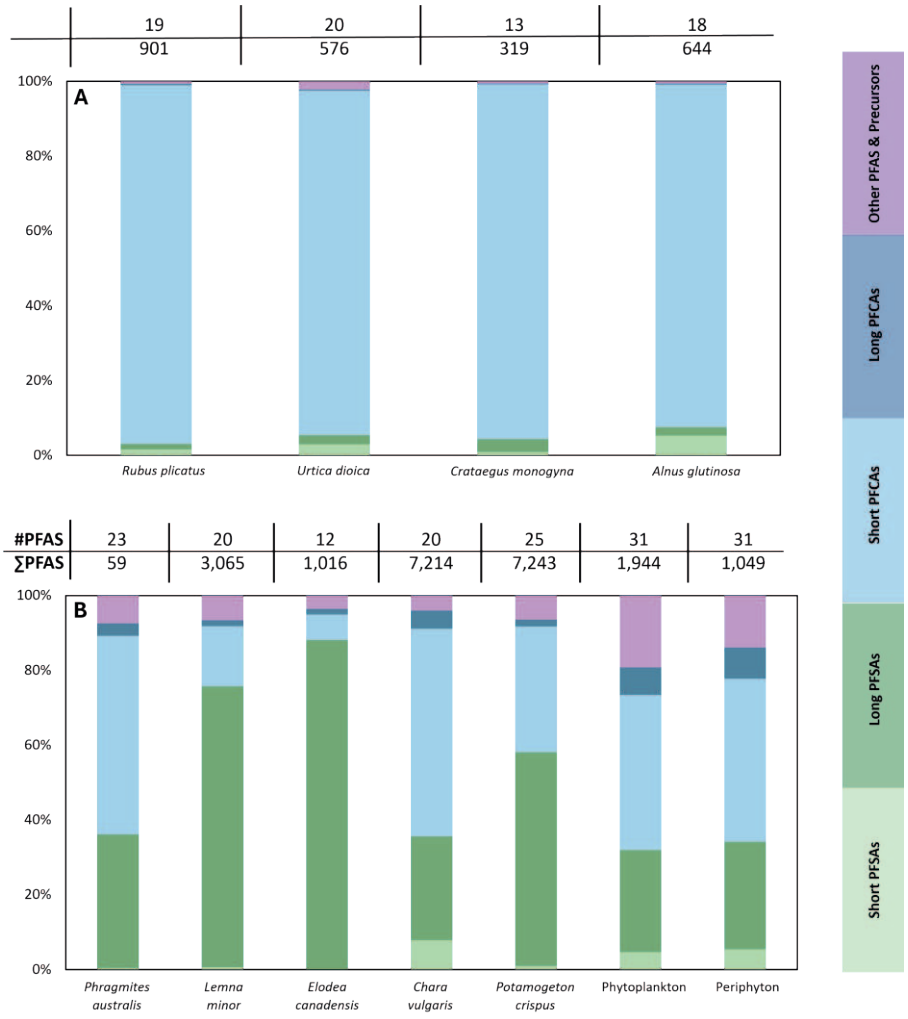


Figure 14: Relative PFAS profiles per subclass (dry weight basis) in terrestrial (A) and aquatic (B) primary producers sampled from the Lake Blokkersdijk ecosystem. The different colors correspond to the five different PFAS subclasses defined in the legend on the right. Above each bar, the number of quantified PFAS (#PFAS) and the total average PFAS concentration (∑PFAS; in ng/g dry weight) are indicated.

Consumers

The number of quantified PFAS in the terrestrial consumers ranged from 12 in the pooled snails *Cepaea nemoralis* and *Cepaea hortensis* (27%) to 34 in the millipede *Schizophyllum sabulosum* (77%). Short-chain PFCAs was the most represented subclass in *C. nemoralis* and *C. hortensis* (60%), *Arion rufus* (53%), and Lumbricidae (49%), followed by precursors (*A. rufus*; 25%) or short-chain PFCAs, which had a considerable contribution to the total PFAS load, especially in Lumbricidae (45%) (Figure 15A). The PFAS profile in the millipede *S. sabulosum* was dominated by long-chain PFSAs (41%), followed by short-chain PFCAs and precursors with comparable contributions (~20%). The ∑PFAS concentrations differed substantially between the terrestrial consumers and ranged from 306 (pooled *C. nemoralis* and *C. hortensis*) to 17,806 ng/g dw (Lumbricidae). TFA and L-PFOS were the two compounds with the highest concentrations, except in *A. rufus*, where TFA was followed by L-EtFOSAA. TFA concentrations ranged from 164 (pooled *C. nemoralis* and *C. hortensis*) to 7,898 ng/g dw (Lumbricidae), while L-PFOS concentrations ranged from 66 (pooled *C. nemoralis* and *C. hortensis*) to 5,839 ng/g dw (Lumbricidae).

The number of quantified PFAS in aquatic consumers ranged from 14 in *Mystacides longicornis* (33%) to 33 in Corixidae (79%). PFAS profiles were quite consistent between these taxa, with long-chain PFSAs making up more than 75% in all cases, except for *M. longicornis* (56%), followed by precursors, a subclass that had a considerable contribution to the total PFAS load in *M. longicornis* (36%) (Figure 15B). Despite the rather homogenous profiles, ∑PFAS concentrations varied over an order of magnitude between the aquatic consumers, ranging from 941 (*M. longicornis*) to 23,951 ng/g dw (mayfly *Cloeon dipterum*). L- and Br-PFOS together with some sulfonamide-based precursors (FBSA, FOSA, or L-EtFOSAA) were among the compounds with the highest concentrations in all aquatic consumers, except for *Chironomus riparius*, where instead of Br-PFOS, PFBA dominated (102 ng/g dw).

Concentrations of L- and Br-PFOS ranged from 310 (*M. longicornis*) to 7,696 ng/g dw in *C. dipterum* and from 148 in *Mystacides longicornis* to 5,634 ng/g dw in *C. dipterum*.

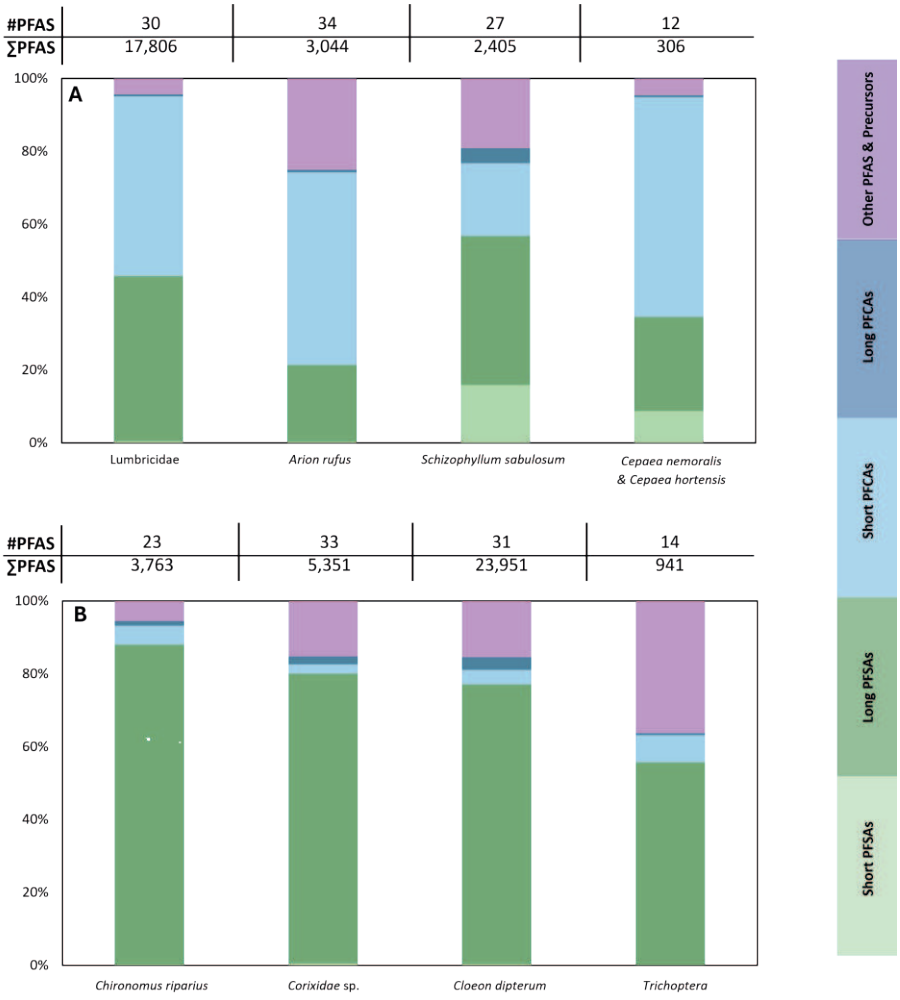


Figure 15: Relative PFAS profiles per subclass (dry weight basis) in terrestrial (A) and aquatic (B) consumers (herbivores + detritivores) sampled from the Lake Blokkersdijk ecosystem. The different colors correspond to the five different PFAS subclasses defined in the legend on the right. Above each bar the number of quantified PFAS (#PFAS) and the total average PFAS concentration (∑PFAS; in ng/g dry weight) are indicated.

Spiders

In predatory spiders the number of quantified PFAS ranged from 15 in *Clubiona sp.* (34%) to 22 in *Araneus diademantus* (50%). In all cases, profiles were dominated by short-chain PFCAs, reaching up to more than 80% in three out of the four spider taxa (Figure 16). In Lycosidae, long-chain PFSAs contributed considerably to the PFAS load (32%), while the subclass of long-chain PFCAs was only present in the *Clubiona sp.*. The ∑PFAS concentrations ranged from 2,177 (*A. diademantus*) up to 18,272 ng/g dw (Lycosidae). TFA and L-PFOS showed the highest concentrations in all individual spider taxa, ranging from 1,810 to 14,453 ng/g dw for TFA and from 92 to 10,170 ng/g dw for L-PFOS.

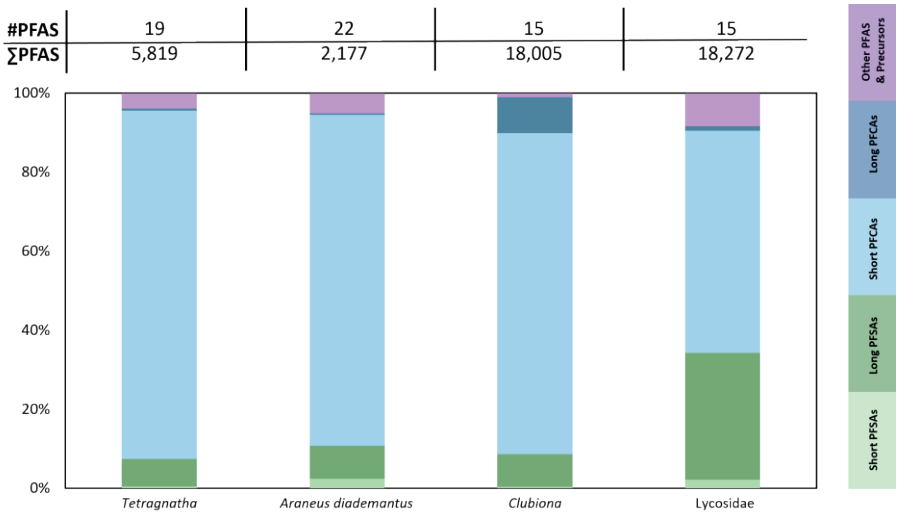


Figure 16: Relative PFAS profiles per subclass (dry weight basis) in predatory spiders sampled on the banks of Lake Blokkersdijk. The different colors correspond to the five different PFAS subclasses defined in the legend on the right. Above each bar the number of quantified PFAS (#PFAS) and the total average PFAS concentration (∑PFAS; in ng/g dry weight) are indicated.

4.3.3 Trophic magnification

Trophic magnification was investigated for 44 and 42 PFAS in the terrestrial and aquatic part of the ecosystem, respectively. The results were categorized based on whether they indicated significant trophic magnification (TMF), trophic dilution (TDF) or none of the two when there was no significant relationship between PFAS concentration and trophic level (N.S.). First, the terrestrial and aquatic PFAS fluxes were examined in the absence of the spiders as top predators and then including the spiders as shared predators of both parts of the ecosystem. All significant regressions for the two parts of the ecosystem in the absence and presence of the spiders can be found in the SI (Section S6, Figures S6-S7). Concerning the food web without the spiders, 16 and 20 PFAS met the LOQ criterion, but only four and two PFAS fulfilled the significance criterion, for the terrestrial and aquatic food web, respectively. When examining the complete food web, including the spiders, 14 and 20 PFAS fulfilled the LOQ criterion, while nine and six met the significance criterion for the terrestrial and aquatic food web, respectively (Figure 17; Table 3).

For the terrestrial food web, for both scenarios, all PFAS with significant results showed trophic magnification, except for PFPrA in the absence of spiders that showed trophic dilution (TDF: 0.21). In contrast, for the aquatic food web most significant regressions indicated trophic dilution. Moreover, very little overlap was observed between the PFAS that biomagnified in the terrestrial and aquatic food webs, with only TFA and L-MeFOSAA showing consistent trophic magnification in both food webs in the presence of spiders with TMFs of 1.79 and 4.79 for TFA and 2.06 and 1.71 for L-MeFOSAA, for the terrestrial and aquatic ecosystem, respectively. Long-chain PFSAs and sulfonamide-based precursors, which were the subclasses with the highest TMFs in the terrestrial food web, showed no significant biomagnification in most cases in the aquatic food web. Nonetheless, results were consistent for individual compounds, meaning that PFAS that were biomagnifying in the terrestrial food web were not found to biodilute in the aquatic food web and *vice versa*. When including the spiders, more PFAS exhibited significant trophic magnification or dilution, but with lower TMF and TDF values. For the aquatic food

web, adding the spiders resulted in more PFAS with significant regressions, most of them exhibiting trophic dilution, while in the absence of spiders the two PFAS with significant regressions both showed trophic magnification (FHxSA: 3.11; PFDA: 2.48). The highest TMFs were found in the terrestrial food web in the absence of spiders for L-PFOS (11.1), Br-PFOS (6.27) and FBSA (5.35). The lowest TMFs were found in the terrestrial food web in the presence of spiders, for PFPeA (1.46), Br-PFHxS (1.53) and FBSA (1.63), which all belong to different PFAS subclasses. The highest TDF was observed in the aquatic food web in the presence of spiders for PFBA and PFHxA (TDF = 0.68 for both). Four out of the five cases of trophic dilution were found in the aquatic food web in the presence of spiders and only one in the terrestrial food web in the absence of spiders.

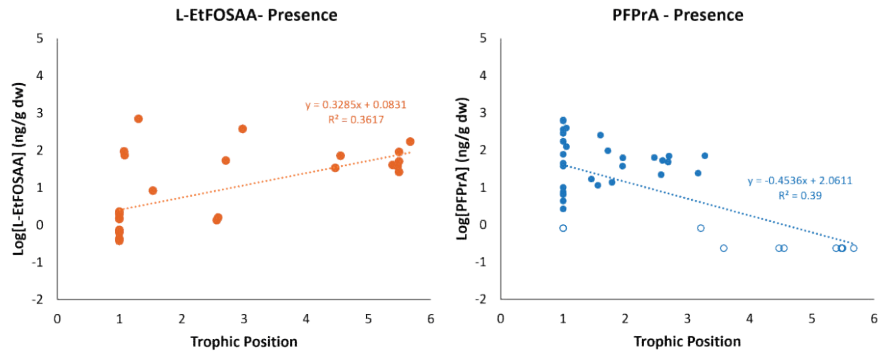


Figure 17: Examples of two regressions, one with a positive slope, indicating trophic magnification (left: terrestrial food web - presence of spiders) and one with a negative slope, indicating trophic dilution (right: aquatic food web - presence of spiders). Hollow symbols indicate cases where PFAS concentrations were < LOQ and substituted with 0.5 * LOQ, while closed symbols are actual measured PFAS concentrations.

Table 3: The trophic magnification (TMF; green) and trophic dilution factors (TDF; peach) expressed as 10 to the power of the slope of the log-transformed regression of PFAS concentration against trophic position, determined for different PFAS in the terrestrial (TER) and aquatic (AQ) food webs of the Lake Blokkersdijk ecosystem in two trophic transfer scenarios (absence and presence of spiders). Only PFAS that had more than 50% of data points above the LOQ in at least one of the four examined scenarios (terrestrial without spiders; terrestrial with spiders; aquatic without spiders; aquatic with spiders) are shown in the table. N.S. indicates that there was no significant relationship between PFAS concentration and trophic level, Hatched indicate that the 50% > LOQ criterion was not fulfilled. PFAS were grouped into four subclasses (PFCAs, PFSAs, sulfonamides and sulfonamidoacetic acids) and within each subclass ordered according to chain length from shortest to longest. The total number of carbons, number of fluorinated carbons and functional groups are indicated in columns two to four. The total number of PFAS with positive (trophic magnification), negative (trophic dilution) and non-significant values are summarized at the bottom of the table.

PFAS list	# C total	# C-F	PFAS Functional groups	TER Absence	TER Presence	AQ Absence	AQ Presence
PFBS	4	4	SO3H	N.S.	N.S.		
PFPeS	5	5	SO3H	N.S.		N.S.	**0.59**
L-PFHxS	6	6	SO3H			N.S.	N.S.
Br-PFHxS	6	6	SO3H	N.S.	**1.53**	N.S.	N.S.
6:2FTS	8	6	SO3H			N.S.	
PFHpS	7	7	SO3H			N.S.	N.S.
L-PFOS	8	8	SO3H	**11.13**	**2.09**	N.S.	N.S.
Br-PFOS	8	8	SO3H	**6.27**	**2.09**		N.S.
PFECHS	8	8	SO3H; Cyclic			N.S.	
PFDS	10	10	SO3H			N.S.	N.S.
TFA	2	1	COOH	N.S.	**1.79**	N.S.	**4.79**
PFPrA	3	2	COOH	**0.21**		N.S.	**0.35**
PFBA	4	3	COOH	N.S.	N.S.	N.S.	**0.68**
PFPeA	5	4	COOH	N.S.	**1.46**	N.S.	N.S.
PFHxA	6	5	COOH			N.S.	**0.68**
PFHpA	7	6	COOH	N.S.	N.S.	N.S.	N.S.
PFOA	8	7	COOH	N.S.	**1.72**	N.S.	N.S.
PFNA	9	8	COOH	N.S.	N.S.		
PFDA	10	9	COOH			**2.48**	N.S.

PFAS list	# C total	# C-F	PFAS Functional groups	TER Absence	TER Presence	AQ Absence	AQ Presence
FBSA	4	4	O=S=O; NH2	**5.35**	**1.63**	N.S.	N.S.
FHxSA	6	6	O=S=O; NH2			**3.11**	N.S.
FOSA	8	8	O=S=O; NH2			N.S.	N.S.
L-MeFOSAA	11	8	O=S=O; N-CH3; COOH	N.S.	**2.06**	N.S.	**1.71**
L-EtFOSAA	12	8	O=S=O; N-C2H5; COOH	N.S.	**2.13**		N.S.
Br-EtFOSAA	12	8	O=S=O; N-C2H5; COOH	N.S.	N.S.		
			# TMF	**3**	**9**	**2**	**2**
			# TDF	**1**	**0**	**0**	**4**
			# N.S.	**12**	**5**	**18**	**14**

4.4 Discussion

4.4.1 PFAS profiles in the different trophic groups

Generally, very high total PFAS concentrations were measured in the organisms living in the studied contaminated ecosystem, with previously reported high bioaccumulation factors (Gkika et al., 2025). Among the terrestrial primary producers, despite some variation in the number of detected PFAS, levels and profiles were very similar, and by far dominated by short-chain PFCAs. This hints towards a common uptake mechanism in the plants, mainly via the soil pore water and the subsequent aqueous flow through the stems to the leaves (Adu et al., 2023; Felizeter et al., 2021; Groffen et al., 2023). Since plants were rinsed with ambient water upon collection, the contribution of particle-sorbed PFAS to the PFAS load of the plants can be considered negligible. For aquatic primary producers, a higher variation in the number of detected PFAS, levels and profiles was observed between taxa, with long-chain PFSAs and/or short-chain PFCAs being the dominant subclasses. Even in taxa with similar ∑PFAS concentrations, profiles could differ substantially (e.g. *Chara vulgaris* and *Potamogeton crispus*; or *Elodea canadensis* and Periphyton). Moreover, the occurrence of a higher number of PFAS did not always translate into higher PFAS concentrations (e.g. *Phragmites australis* and *E. canadensis*).

The PFAS profiles in phytoplankton and periphyton were similar, most likely due to the predominant uptake of PFAS from the water column. The deviating PFAS profile in the floating *Lemna minor* may be due to its position at the water-air interface. Although PFAS uptake from the sediment by the rooting macrophytes may explain the quite distinct profiles between pelagic and benthic primary producer taxa, the large variation in both ∑PFAS concentration and profiles between these sediment rooting taxa (*P. australis*, *E. canadensis*, *P. crispus*, and *C. vulgaris*) indicates a much more complex interplay of uptake mechanisms compared to terrestrial plants (Colomer-Vidal et al., 2022; Gkika et al., 2025).

Among the terrestrial consumers, the numbers of detected PFAS, levels and profiles were quite different. This aligns with earlier findings from terrestrial field studies, where species-specific differences in PFAS concentrations and bioaccumulation were found (Buytaert et al., 2025; Groffen et al., 2023; Hopkins et al., 2023; Koch et al., 2020). In our case, and despite including a gut depuration step, Lumbricidae had by far the highest ∑PFAS concentrations of all the terrestrial consumers, which aligns with previously reported observations (Buytaert et al., 2025; Ibor et al., 2025). This could relate to the extensive dermal contact of this organism with the soil. Compared to primary producers, higher variation was expected for animals, as they are able to move and adjust their diets according to the availability of resources (Szałkiewicz et al., 2022). Although clearly visible in the terrestrial ecosystem, this pattern was less clear in the aquatic ecosystem. In aquatic consumers, the PFAS profiles were quite consistent, but the number of detected PFAS and the total PFAS concentrations were not. The nymphs of *Cloeon dipterum* had by far the highest ∑PFAS concentration, which could relate to high exposure and uptake of PFAS through their extensive exterior gills. An additional explanation may be their distinct diet compared to the other aquatic organisms, as shown by their different $\delta^{13}C$ signature compared to the other aquatic invertebrates. The Trichoptera *Mystacides longicornis* had the lowest ∑PFAS concentration, and, interestingly, the highest trophic position compared to all other consumers, indicating omnivorous feeding behavior. *M. longicornis* is a case-building species (Morse, 2009), reducing its

interaction with the surrounding water and probably contributing to its lower PFAS concentrations. Besides varying exposure to the different environmental compartments, the differences in the number of detected PFAS, levels and profiles observed between terrestrial and aquatic consumers could also relate to their body composition as well as to their species-specific feeding strategies, which in turn may depend on their taxonomy and life-stage.

Although the predatory spiders as a group contained higher ∑PFAS concentrations compared to the consumers, the ∑PFAS concentrations in *C. dipterum* and Lumbricidae exceeded that of some individual spider taxa. This may be due to the lack of or limited exposure of spiders to PFAS via the water and soil, since their main exposure pathway is likely through their diet (Buytaert et al., 2025). PFAS profiles were rather consistent among the different spider taxa, however, this was not the case for the number of detected PFAS and the total PFAS concentrations. The two surface hunter taxa (*Clubiona sp.* and Lycosidae) had similar and much higher ∑PFAS concentrations than the web building species. In the latter more PFAS were quantified, which however, did not translate into higher ∑PFAS concentrations. The most obvious difference is that the web building spiders are exposed via air and food and the hunting species also to soil. Additionally, the observed difference in PFAS concentrations within the spider group could also relate to taxon-specific differences in diet reliance on emergent aquatic insects and trophic position (Chumchal et al., 2022).

4.4.2 Trophic transfer of PFAS

Examining the trophic transfer of PFAS at the base of the terrestrial food web, i.e. plants and primary consumers only, revealed that a higher trophic position did not always translate into higher concentrations of individual PFAS. Some compounds did show statistically significant trophic magnification, with high TMFs especially in the terrestrial food web, highlighting that some bioaccumulative PFAS also have the potential to biomagnify across the food chain. We were, however, not able to identify a common characteristic shared by all compounds with significant positive,

significant negative or non-significant regressions between PFAS concentrations and trophic positions. The high variation of the individual PFAS concentrations in primary producers, all sharing the same trophic position, could have played a role in rendering the regressions of many compounds not significant. This variation was more prominent for aquatic primary producers as previously reported (Chu et al., 2022; Griffin et al., 2023; Huang et al., 2022b; Wang et al., 2025). Nonetheless, the PFAS incorporation at the base of aquatic food webs still remains poorly understood (Farrell et al., 2025). Additionally, for organisms from lower trophic levels environmental exposure is expected to play a substantial role, due to their high surface area-to-volume ratio, high skin permeability and the short diffusion distances that the contaminants need to cross. Although distinguishing between environmental and dietary exposure was not possible with the current study design, previous studies suggested that for higher trophic levels dietary uptake seems to play a bigger role than environmental assimilation (Arnot et al., 2006; Chumchal et al., 2022; Koch et al., 2020; Mackay et al., 2016; Politi et al., 2021; Rosenberg et al., 1984).

When introducing the spiders as joint predators in the combined terrestrial and aquatic food web, the trophic transfer patterns changed substantially. There was, however, still little to no overlap between the compounds that showed significant regressions between PFAS concentration and trophic position in the two parts of the food web, nor between the absence or presence of the spiders). Similar to the "absence of spiders" scenario, we were not able to identify a common characteristic shared by all compounds with significant positive, significant negative or non-significant regressions between PFAS concentrations and trophic position. The compounds that biodiluted in the aquatic food web, however, were all short-chain compounds (mostly PFCAs), something that has previously been reported for Arctic benthic biota (Dai et al., 2025).

TFA concentrations were highest in the spiders, in which it contributed more than 80% to the total concentration in three out of the four taxa (*Tetragnatha sp.*, *A.*

diademantus, *Clubiona sp.*) and 56% in Lycosidae. Moreover, TFA was the only short-chain compound that biomagnified in both food webs in the presence of spiders, accompanied by a very high TMF in the aquatic food web. Hence, our results contradict the common opinion that the small, mobile and polar TFA is unlikely to bioaccumulate or biomagnify in biota (Hanson et al., 2024; Herzke et al., 2023). However, there is no way to distinguish if the TFA in the spiders (and other biota) was formed due to the (bio)transformation of other PFAS, or if it originated from dietary or environmental exposure, although the latter was expected to be limited for spiders. The significant trophic transfer of the supposedly non-bioaccumulating TFA in both parts of the food web together with the unpredictability of which other PFAS do biomagnify or biodilute, show that we still lack insight into the exact drivers of PFAS bioaccumulation and trophic transfer (Arp et al., 2024; Herzke et al., 2023).

In the presence of the spiders, more PFAS exhibited significant trophic magnification in the terrestrial food web compared to the scenario without spiders, but the obtained TMF values were lower. Adding the spiders to the aquatic food web showed more trophic dilution of PFAS in the aquatic food web. A possible reason behind these observations could relate to way spiders consume their prey, which is first pierced or crushed using their fangs and/or maxillae and digested externally to a liquid form, which is then sucked, discarding the hard parts (Foelix 1996; Forster and Forster, 1999; Nyffeler et al., 1994). Some PFAS compounds could remain in these parts of the prey that are not consumed. Taking up the prey fluids in liquid form may also have contributed to the short chain PFAS dominated profile in the spiders. The observed trophic dilution may also be due to the emerging insects that comprise a considerable part of the diet of the spiders (Burdon and Harding, 2008; Collier et al., 2002; Paetzold et al., 2006). Compared to PFAS concentrations in aquatic larvae, concentrations in adult emerged insects tend to be lower, since they may leave part of the contaminants behind in the pupal or nymphal skin (Koch et al., 2021). These potential PFAS losses, in combination with the consumption of terrestrial organisms that on average contained lower PFAS

concentrations, could partly explain why the PFAS concentrations in the spiders appeared to be diluted compared to the lower trophic levels in the aquatic food web.

Studying the aquatic and terrestrial part of contaminated ecosystems in isolation does not do justice to the life cycle of the many organisms that inhabit the aquatic environment as larvae or nymphs and the terrestrial environment as flying adults (Kraus et al., 2023), characteristic for a wide range of aquatic insect orders. Our findings further support this, since spiders were found to feed on prey originating from both the terrestrial and the aquatic compartments of the ecosystem, which clearly affected PFAS bioaccumulation and biomagnification in a compartment-specific, rather than a compound-specific way. The few studies that to the best of our knowledge have jointly examined PFAS biomagnification in more than one ecosystem type acknowledge the challenge to understand PFAS exposure routes for the multiple species comprising these food webs (Dai et al., 2025; Hopkins et al., 2023; Ibor et al., 2025) but also showed the added value of such studies. Hence, the current combined aquatic-terrestrial study on a variety of organisms and PFAS proved to be essential to unravel the complex environmental distribution and trophic transfer of these compounds. It should be noted though that the predators in the present study were invertebrate spiders and it is therefore expected that top predators would be even more impacted by the trophic transfer of PFAS, since adverse effects associated with PFAS exposure have been reported in terrestrial (Custer et al., 2021; Sebastiano et al., 2023) and aquatic vertebrates (Ma et al., 2023).

4.5. Conclusions

The present study revealed the omnipresence and variable distribution of PFAS in terrestrial and aquatic organisms of a contaminated ecosystem. For some PFAS-organism combinations very high concentrations were observed, and moreover, evidence that some PFAS also have the potential to biomagnify was revealed. The apparent arbitrariness of which substances biomagnify and which biodilute, in combination with trophic transfer of TFA, the smallest and most polar PFAS,

emphasizes that we still lack the insights into the drivers of environmental PFAS distribution, uptake and transfer.

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Supplementary Information

Section S1. Field sampling

Table S1: Overview of organisms sampled from Lake Blokkersdijk and the number of field replicates per species or genus.

Common name	Species/taxa	Organism category	Site 1	Site 2	Site 3	Site 4	Site 5
Terrestrial Biota							
Common Bramble (N_{field}=5)	*Rubus plicatus*	Primary Producer	X	X	X	X	X
Nettle (N_{field}=5)	*Urtica dioica*	Primary Producer	X	X	X	X	X
Hawthorn (N_{field}=3)	*Crataegus monogyna*	Primary Producer	X		X	X	
Black Alder (N_{field}=3)	*Alnus glutinosa*	Primary Producer	X	X	X		
Earthworm (N_{field}=5)	Lumbricidae	Consumer	X	X	X	X	X
Diplopoda (N_{field}=3)[1]	*Schizophyllum sabulosum*	Consumer	X	X		X	X
Isopods (N_{field}=5)	Oniscidea	Consumer	X	X	X	X	X
Slugs (N_{field}=3)	*Arion rufus*	Primary Consumer	X	X			X
Terrestrial snails (N_{field}=3)	*Cepaea nemoralis, Cepaea hortensis* (pooled)	Primary Consumer	X			X	X

Common name	Species/taxa	Organism category	Site 1	Site 2	Site 3	Site 4	Site 5
Aquatic Biota							
Phytoplankton (N_{field}=5)		Primary Producer	X	X	X	X	X
Duckweed (N_{field}=3)	*Lemna minor*	Primary Producer		X		X	X
Stonewort (N_{field}=2)	*Chara vulgaris*	Primary Producer	X			X	
Common Reed (N_{field}=4)	*Phragmites australis*	Primary Producer	X	X	X		X
Water weed (N_{field}=2)	*Elodea canadensis*	Primary Producer	X				X
Pondweed (N_{field}=4)	*Potamogeton crispus*	Primary Producer	X	X	X		X
Periphyton (N_{field}=5)		Biofilm	X	X	X	X	X
Zooplankton (N_{field}=5)		Primary Consumer	X	X	X	X	X
Sludge worms (N_{field}=4)	*Lumbricus tubifex*	Primary Consumer	X	X		X	X
Lesser water boatmen (N_{field}=5)	Corixidae	Primary Consumer	X	X	X	X	X
Chironomid larvae (N_{field}=5)	*Chironomus riparius*	Consumer	X	X	X	X	X
Mayfly nymph (N_{field}=5)	*Cloeon dipterum*	Consumer	X	X	X	X	X
Caddisfly larvae (N_{field}=1)	*Mystacides longicornis*	Consumer					X
Spiders							
Stretch spiders (N_{field}=3)	*Tetragnatha sp.*	Spider	X		X	X	
Garden spider (N_{field}=3)	*Araneus diadematus*	Spider	X		X	X	
Leaf-curling spiders (N_{field}=2)	*Clubiona sp.*	Spider	X	X			
Wolf spider (N_{field}=1)	Lycosidae	Spider		X			

[1] Diplopoda from sites 4 and 5 were pooled together to yield enough biomass for PFAS analysis and stable isotope analysis.

Section S2. Trophic position calculation

Text S1: Calculating trophic positions for terrestrial and aquatic taxa

The Gastropod *Arion rufus* and pooled *Cepaea nemoralis*/*Cepaea hortensis* were set as the baseline of the terrestrial food web (except for *Lumbricidae*) and the average of their $\delta^{15}N$ values determined the terrestrial $\delta^{15}N_{base}$ at 0.43. Similarly, the primary consumer taxa Zooplankton, *Lumbricus tubifex,* and Corixidae were set as the baseline of the aquatic food web and the average of their $\delta^{15}N$ values was set as the aquatic $\delta^{15}N_{base}$ at 9.41. The trophic position (TP) of all terrestrial and aquatic primary producers (excluding the periphyton) was set to one. For periphyton, the trophic position value from sampling site one was excluded as an outlier (Table S2). Data

from sampling site five were excluded from the trophic position calculations of the terrestrial taxa, because the location was considered too remote and resulted in many outliers. Similarly, data from sampling site four were excluded from the TP calculations of the aquatic taxa, because the sampled water body was independent (although still connected) from the main Blokkersdijk lake. For this reason, the pooled *Schizophyllum sabulosum*, consisting of samples from sites four and five, was also excluded and only data from sites one and two were considered for this species.

Section S3: PFAS analysis

Text S2: PFAS extraction and quantification protocols

The protocols used for PFAS extraction, analysis and quality assurance/quality control (QA/QC) assessment have been described previously (Gkika et al., 2024; 2025; Sadia et al., 2020; 2023). A detailed description of the PFAS extraction for all matrices, the quantification method, and the quality assurance/quality control criteria can be found in (Gkika et al., 2025), since results from the same samples were used in this study. The following biota samples did not fulfill the QA/QC criteria and were therefore excluded from further analysis: Zooplankton; Oniscidea; *Arion rufus, Lumbricus tubifex,* Lumbricidae replicates 2 and 4; *Rubus plicatus* replicate 1; *Araneus diadematus* replicate 1.

Section S4: Results from stable Isotope analysis

Table S2: Stable isotope data and calculated trophic positions for all organisms collected from Lake Blokkersdijk; number indicates sampled site.

Terrestrial Biota						
Sample	**Group**	**$\delta^{13}C$ (VPDB)**	**$\delta^{15}N$ (Air)**	**$d^{15}N_{base}$**	**$d^{15}N$**	**Trophic Position**
Soil organic matter 1	Organic matter	-28.46	1.32	0.43	0.89	N/A
Soil organic matter 2	Organic matter	-26.68	2.78	0.43	2.35	N/A
Soil organic matter 3	Organic matter	-28.20	2.74	0.43	2.31	N/A
Soil organic matter 4	Organic matter	-29.72	3.71	0.43	3.27	N/A
Alnus glutinosa 1	Primary producer	-28.57	-2.89	0.43	-3.32	1.00
Alnus glutinosa 2	Primary producer	-27.72	-2.53	0.43	-2.96	1.00
Alnus glutinosa 3	Primary producer	-29.72	3.27	0.43	2.84	1.00
Crataegus monogyna 1	Primary producer	-30.68	-2.51	0.43	-2.95	1.00
Crataegus monogyna 2	Primary producer	-31.14	-3.57	0.43	-4.01	1.00
Crataegus monogyna 4	Primary producer	-31.59	-4.31	0.43	-4.75	1.00
Rubus plicatus 2	Primary producer	-29.28	-0.17	0.43	-0.60	1.00
Rubus plicatus 3	Primary producer	-28.46	-0.55	0.43	-0.98	1.00
Rubus plicatus 4	Primary producer	-30.03	-0.14	0.43	-0.57	1.00
Urtica dioica 1	Primary producer	-32.33	-1.09	0.43	-1.52	1.00
Urtica dioica 2	Primary producer	-30.75	-1.48	0.43	-1.91	1.00
Urtica dioica 4	Primary producer	-32.34	0.57	0.43	0.14	1.00
Arion rufus 1	Herbivore	-29.09	-0.75	0.43	-1.18	1.54
Arion rufus 2	Herbivore	-27.14	-1.34	0.43	-1.77	1.31
Cepaea nemoralis/ Cepaea hortensis 1	Herbivore	-27.42	1.94	0.43	1.50	2.59
Cepaea nemoralis/ Cepaea hortensis 4	Herbivore	-27.53	1.88	0.43	1.44	2.57
Lumbricidae 1	Detritivore	-28.63	10.51	0.43	10.08	2.71
Lumbricidae 2	Detritivore	-27.64	8.34	0.43	7.90	2.57
Lumbricidae 3	Detritivore	-26.40	14.60	0.43	14.17	2.98
Lumbricidae 4	Detritivore	-28.09	9.21	0.43	8.77	2.63
Oniscidea 1	Detritivore	-26.49	1.28	0.43	0.85	2.33
Oniscidea 2	Detritivore	-26.29	2.28	0.43	1.84	2.72
Oniscidea 3	Detritivore	-24.80	3.41	0.43	2.98	3.17
Oniscidea 4	Detritivore	-25.98	3.92	0.43	3.49	3.37
Schizophyllum sabulosum 1	Detritivore	-25.82	-1.92	0.43	-2.36	1.08

Sample	Group	$\delta^{13}C$ (VPDB)	$\delta^{15}N$ (Air)	$d^{15}N_{base}$	$d^{15}N$	Trophic Position
Schizophyllum sabulosum 2	Detritivore	-24.97	-1.90	0.43	-2.33	1.09
Aquatic Biota						
Sample	**Group**	**$\delta^{13}C$ (VPDB)**	**$\delta^{15}N$ (Air)**	**$d^{15}N_{base}$**	**$d^{15}N$**	**Trophic Position**
Sediment 1	Organic matter	-22.13	5.96	9.41	-3.45	N/A
Sediment 2	Organic matter	-24.43	4.97	9.41	-4.43	N/A
Sediment 3	Organic matter	-25.91	5.18	9.41	-4.23	N/A
Sediment 5	Organic matter	-33.21	5.19	9.41	-4.21	N/A
Chara vulgaris 1	Primary producer	-20.69	4.82	9.41	-4.59	1.00
Elodea canadensis 1	Primary producer	-20.30	10.13	9.41	0.73	1.00
Elodea canadensis 5	Primary producer	-19.71	10.05	9.41	0.64	1.00
Potamogeton crispus 1	Primary producer	-19.92	7.77	9.41	-1.64	1.00
Potamogeton crispus 2	Primary producer	-19.67	7.25	9.41	-2.16	1.00
Potamogeton crispus 3	Primary producer	-21.60	6.69	9.41	-2.71	1.00
Potamogeton crispus 5	Primary producer	-18.72	6.00	9.41	-3.40	1.00
Lemna minor 1	Primary producer	-31.33	7.78	9.41	-1.63	1.00
Lemna minor 2	Primary producer	-31.98	5.83	9.41	-3.58	1.00
Lemna minor 5	Primary producer	-29.86	5.46	9.41	-3.95	1.00
Phragmites australis 1	Primary producer	-28.43	4.17	9.41	-5.24	1.00
Phragmites australis 2	Primary producer	-27.16	4.16	9.41	-5.25	1.00
Phragmites australis 3	Primary producer	-27.11	2.38	9.41	-7.02	1.00
Phragmites australis 5	Primary producer	-28.08	3.13	9.41	-6.28	1.00
Phytoplankton 1	Primary producer	-18.28	6.50	9.41	-2.91	1.00
Phytoplankton 2	Primary producer	-20.04	5.74	9.41	-3.66	1.00
Phytoplankton 3	Primary producer	-22.40	6.46	9.41	-2.95	1.00
Phytoplankton 5	Primary producer	-20.42	6.21	9.41	-3.19	1.00
Periphyton 1	Biofilm	-21.87	6.99	9.41	-2.42	5.69
Periphyton 2	Biofilm	-20.90	8.38	9.41	-1.02	1.60
Periphyton 3	Biofilm	-22.35	8.68	9.41	-0.73	1.72
Periphyton 5	Biofilm	-21.02	9.29	9.41	-0.12	1.95
Corixidae 1	Herbivore	-28.03	6.99	9.41	-2.42	1.05
Corixidae 2	Herbivore	-20.29	8.28	9.41	-1.13	1.56
Corixidae 3	Herbivore	-19.04	8.85	9.41	-0.56	1.78
Corixidae 5	Herbivore	-19.79	8.01	9.41	-1.40	1.45
Tubificidae 1	Herbivore	-18.54	9.17	9.41	-0.24	1.91

Sample	Group	$\delta^{13}C$ (VPDB)	$\delta^{15}N$ (Air)	$d^{15}N_{base}$	$d^{15}N$	Trophic Position
Tubificidae 2	Herbivore	-17.19	10.48	9.41	1.07	2.42
Tubificidae 5	Herbivore	-19.54	9.08	9.41	-0.32	1.87
Zooplankton 1	Herbivore	-20.45	10.30	9.41	0.89	2.35
Zooplankton 2	Herbivore	-20.41	10.43	9.41	1.03	2.40
Zooplankton 3	Herbivore	-20.48	10.49	9.41	1.09	2.43
Zooplankton 5	Herbivore	-20.27	10.22	9.41	0.81	2.32
Chironomus riparius 1	Detritivore	-18.79	11.15	9.41	1.75	2.68
Chironomus riparius 2	Detritivore	-20.55	10.59	9.41	1.18	2.46
Chironomus riparius 3	Detritivore	-24.71	11.20	9.41	1.79	2.70
Chironomus riparius 5	Detritivore	-22.19	10.87	9.41	1.47	2.57
Cloeon dipterum 1	Detritivore	-23.32	9.30	9.41	-0.11	1.96
Cloeon dipterum 2	Detritivore	-28.09	10.93	9.41	1.53	2.60
Cloeon dipterum 3	Detritivore	-26.53	12.50	9.41	3.09	3.21
Cloeon dipterum 5	Detritivore	-27.49	12.66	9.41	3.25	3.28
Mystacides longicornis 5	Detritivore	-21.30	12.39	9.41	2.98	3.17
Predatory Spiders						
Sample	**Group**	**$\delta^{13}C$ (VPDB)**	**$\delta^{15}N$ (Air)**	**$d^{15}N_{base}$**	**$d^{15}N$**	**Trophic Position**
Araneus diadematus 1	Top Predator	-20.19	8.59	0.43	8.15	5.20
Araneus diadematus 3	Top Predator	-23.73	9.07	0.43	8.64	5.39
Araneus diadematus 4	Top Predator	-23.54	9.29	0.43	8.85	5.47
Clubiona 1	Top Predator	-21.74	6.94	0.43	6.51	4.55
Clubiona 2	Top Predator	-24.61	6.73	0.43	6.29	4.47
Lycosidae 2	Top Predator	-26.63	4.46	0.43	4.03	3.58
Tetragnatha sp. 1	Top Predator	-19.94	9.80	0.43	9.36	5.67
Tetragnatha sp. 4	Top Predator	-24.10	9.34	0.43	8.91	5.49

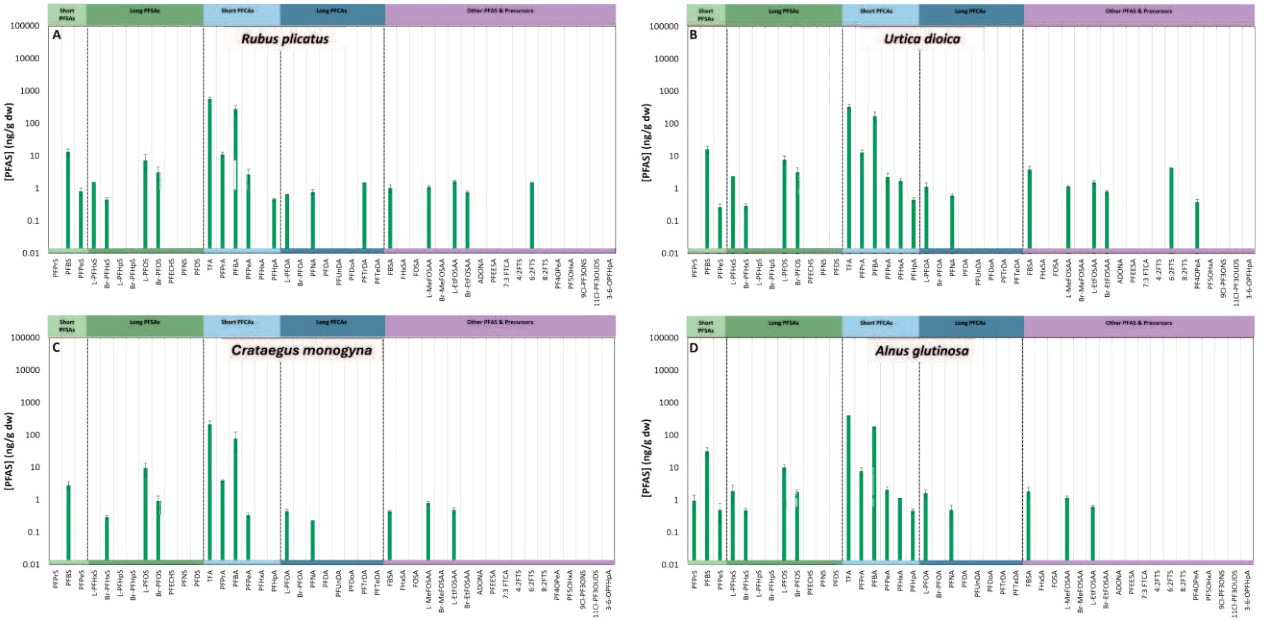
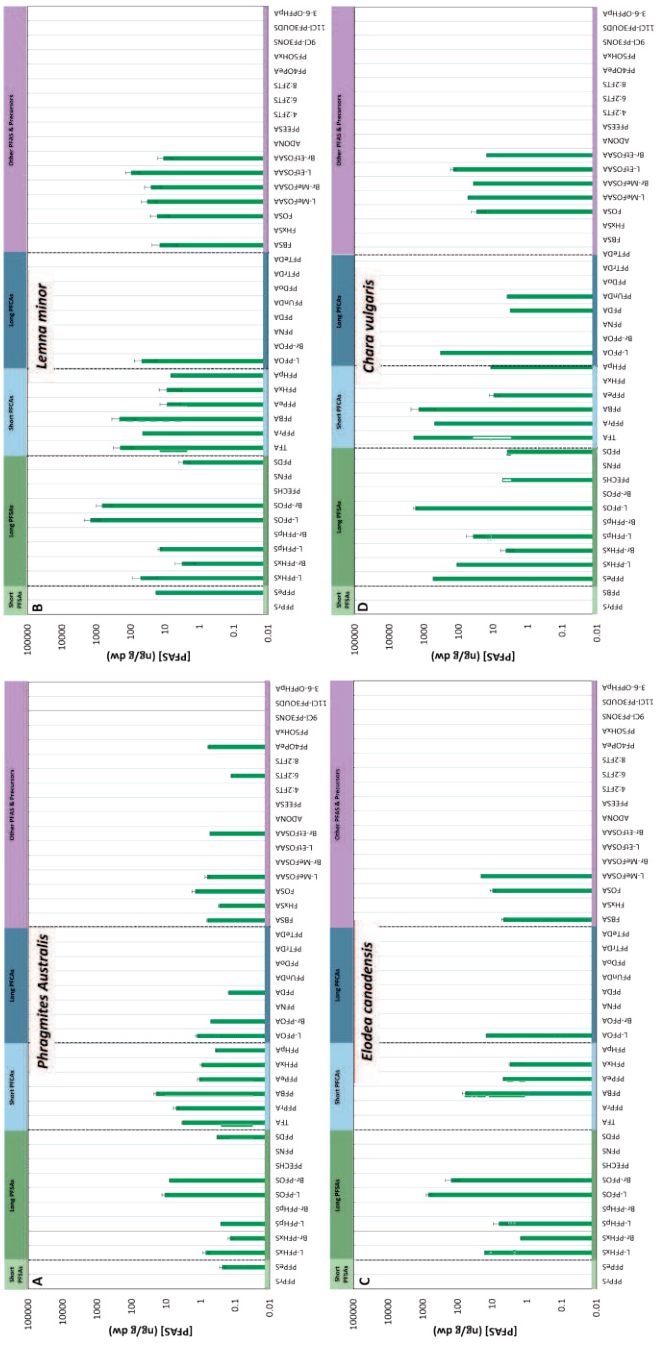


Figure S1: PFAS concentrations in terrestrial primary producers (A: *Rubus plicatus*; B: *Urtica dioica*; C: *Crataegus monogyna*; D: *Alnus glutinosa*) sampled near Lake Blokkersdijk plotted with increasing number of fluorinated carbons within subclasses. Error bars represent the Standard Error of the Mean (SEM) of the concentrations measured at the five sampling sites within the area. For visualization purposes, the PFAS concentrations on the Y axes 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 have been previously published (Gkika et al., 2025).


A
Phragmites Australis
B
Lemna minor
C
Elodea canadensis
D
Chara vulgaris
[PFAS] (ng/g dw)
Short PFASs
Long PFSAs
Short PFCAs
Long PFCAs
Other PFOS & Precursors

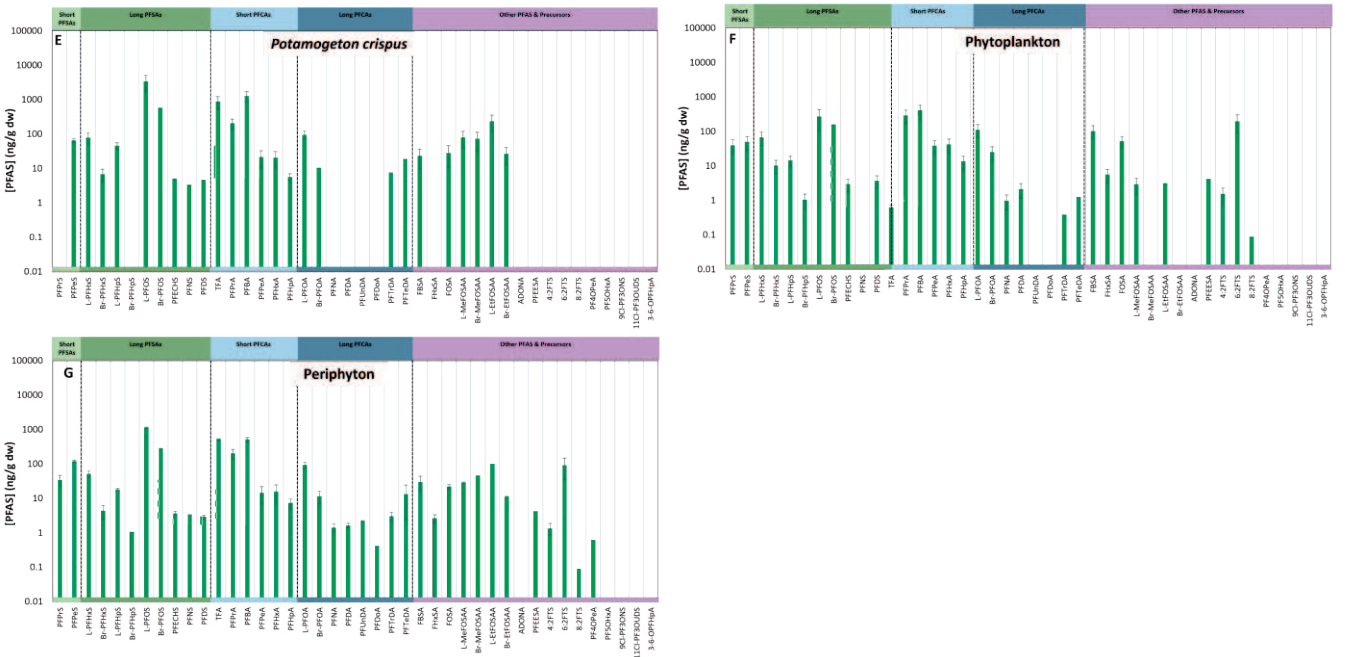


Figure S2: PFAS concentrations in aquatic primary producers (A: *Phragmites australis*; B: *Lemna minor*; C: *Elodea canadensis*; D: *Chara vulgaris*; E: *Potamogeton crispus*; F: Phytoplankton; G: Periphyton) from Lake Blokkersdijk plotted with increasing number of fluorinated carbons within subclasses. 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 PFAS concentrations on the Y-axes 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 have been previously published (Gkika et al., 2025).

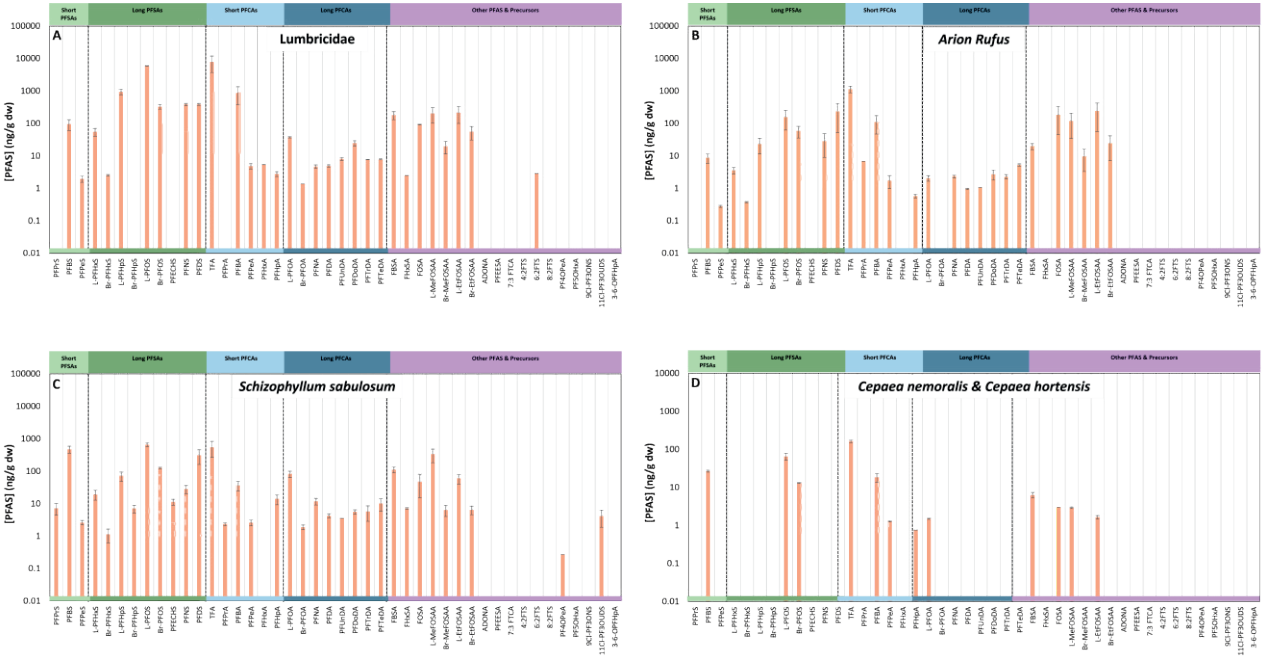


Figure S3: PFAS concentrations in terrestrial herbivores and detritivores (A: Lumbricidae; B: *Arion Rufus*; C: *Schizophyllum sabulosum*; D: *Cepaea nemoralis* & *Cepaea hortensis*) sampled near Lake Blokkersdijk plotted with increasing number of fluorinated carbons within subclasses. Error bars represent the Standard Error of the Mean (SEM) of the concentrations measured at the five sampling sites within the area. For visualization purposes, the PFAS concentrations on the Y-axes 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 have been previously published (Gkika et al., 2025).

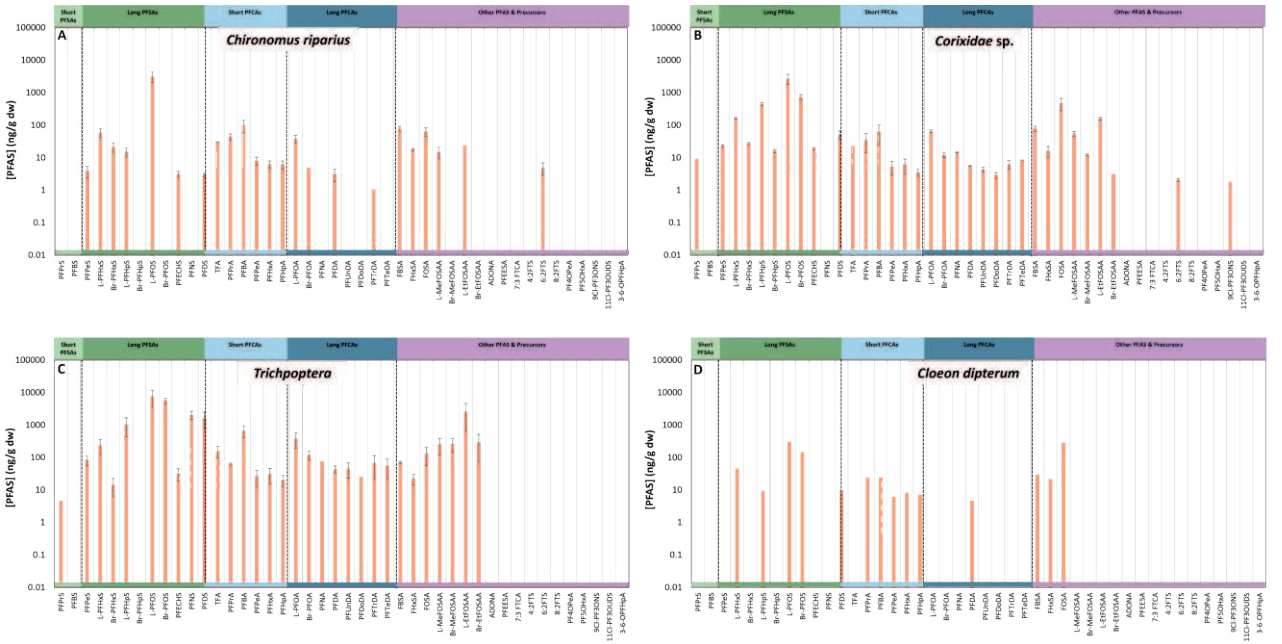


Figure S4: PFAS concentrations in aquatic herbivores and detritivores (A: *Chironomus riparius*; B: Corixidae; C: *Mystacides longicornis*; D: *Cloeon dipterum*) from Lake Blokkersdijk plotted with increasing number of fluorinated carbons within subclasses. Error bars represent the Standard Error of the Mean (SEM) of the concentrations measured at the five sampling sites within the area. For visualization purposes, the PFAS concentrations on the Y-axes 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 have been previously published (Gkika et al., 2025).

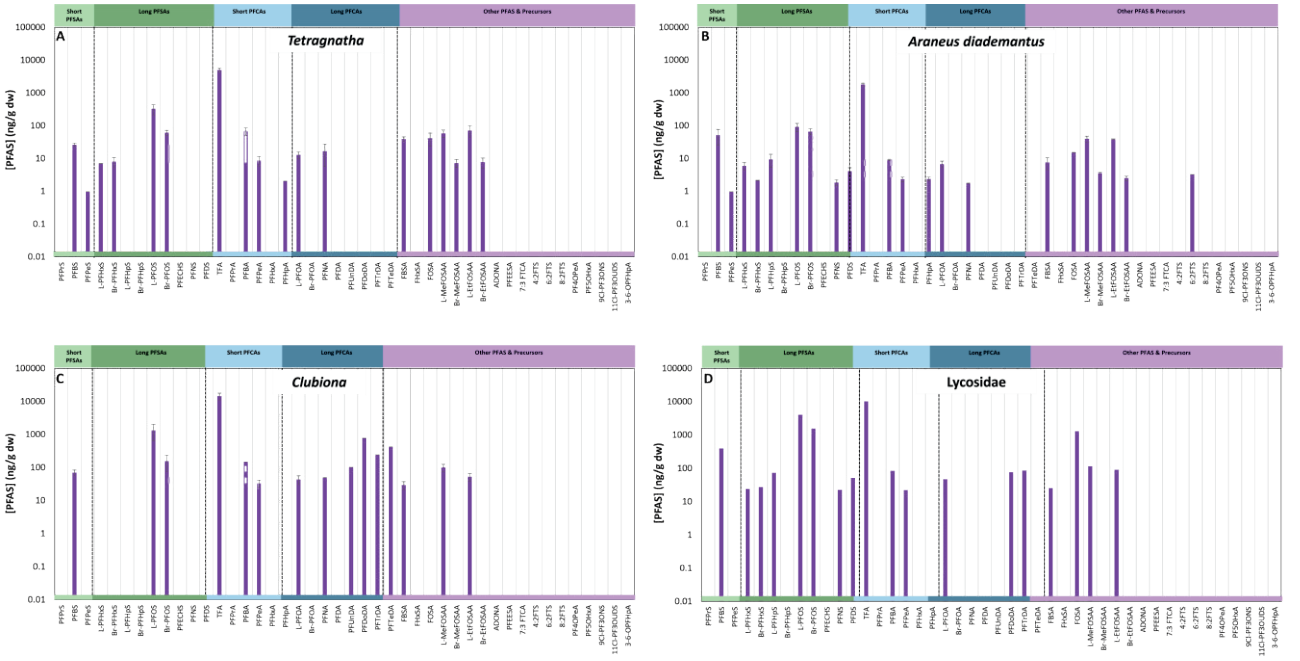


Figure S5: PFAS concentrations in spiders (A: *Tetragnatha sp.*; B: *Araneus diadematus*; C: *Clubiona sp.*; D: Lycosidae) sampled near Lake Blokkersdijk plotted with increasing number of fluorinated carbons within subclasses. Error bars represent the Standard Error of the Mean (SEM) of the concentrations measured at the five sampling sites within the area. For visualization purposes, the PFAS concentrations on the Y-axes 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 have been previously published (Gkika et al., 2025).

Table S3: Average and standard error of the mean (with Standard Error of the mean (SEM) and # of replicates >LOQ) individual PFAS concentrations in spiders sampled near Lake Blokkersdijk ($1 \leq N_{field} \leq 5$). The last column shows the calculated average PFAS concentrations for the group of terrestrial predatory spiders, the SEM corresponds to the variation between the different species and the number of replicates ($1 \leq n \leq 4$).

Analyte	*Tetragnatha* (ng/g dw) $N_{field} = 4$	*Araneus diadematus* (ng/g dw) $N_{field} = 3$	*Clubiona* (ng/g dw) $N_{field} = 2$	Lycosidae (ng/g dw) $N_{field} = 1$	Predator group (ng/g dw)
10:2FTS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11Cl-PF3OUDS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
3-6-OPFHpA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4:2FTS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
6:2diPAP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
6:2FTS	<LOQ	3.24 (n=1)	<LOQ	<LOQ	3.24 (n=1)
7:3 FTCA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
8:2FTS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
9Cl-PF3ONS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
ADONA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Br-EtFOSAA	7.78 (2.62; n=2)	2.53 (0.436; n=2)	<LOQ	<LOQ	5.16 (1.86; n=2)
Br-MeFOSAA	7.32 (1.98; n=3)	3.58 (0.314; n=2)	<LOQ	<LOQ	5.45 (1.32; n=2)
Br-PFHpS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Br-PFHxS	8.02 (2.79; n=4)	2.16 (0.023; n=2)	<LOQ	27.3	12.5 (6.21; n=3)
Br-PFOA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Br-PFOS	61.6 (10.5; n=4)	66.6 (13.2; n=2)	156 (82.6; n=2)	1553	459 (316; n=4)
FBSA	39.3 (6.54; n=4)	7.55 (3.04; n=2)	29.4 (8.10; n=2)	25.4	25.4 (5.74; n=4)
FHxSA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
FOSA	41.8 (17.6; n=2)	15.3 (0.128; n=2)	<LOQ	1299	452 (346; n=3)
L-EtFOSAA	71.6 (28.8; n=4)	38.2 (1.17; n=2)	52.3 (13.4; n=2)	90.2	63.1 (9.82; n=4)
L-MeFOSAA	58.8 (14.9; n=4)	39.9 (7.90; n=2)	101 (25.2; n=2)	115	78.7 (15.2; n=4)
L-PFHxS	7.10 (0.015; n=2)	5.92 (1.64; n=2)	<LOQ	24.1	12.4 (4.80; n=3)
L-PFOS	326 (112; 3)	92.3 (26.8; n=2)	1324 (679; n=2)	4110	1463 (798; n=4)
PF4OPeA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PF5OHxA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFBA	68.35 (18.07; n=4)	8.98 (0.521; n=2)	149.6 (n=1)	84.4	77.8 (25.0; n=4)
PFBS	26.4 (2.84; n=3)	51.3 (25.7; n=2)	69.9 (14.1; n=2)	395	136 (75.2; n=4)
PFDA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFDoA	<LOQ	<LOQ	779 (n=1)	76.6	428 (248; n=2)
PFDS	<LOQ	4.103 (n=1)	<LOQ	51.5	27.8 (16.7; n=2)
PFECHS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFEESA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFHpA	2.06 (n=1)	2.38 (0.343; n=2)	<LOQ	<LOQ	2.22 (0.114; n=2)
PFHpS	<LOQ	9.44 (4.12; n=2)	<LOQ	72.8	41.1 (22.4; n=2)
PFHxA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFNA	16.8 (11.1; n=3)	1.75 (0.025; n=2)	49.2 (n=1)	<LOQ	22.6 (11.4; n=3)
PFNS	<LOQ	1.86 (0.377; n=2)	<LOQ	22.6	12.2 (7.34; n=2)
PFOA	13.0 (3.03; n=4)	6.76 (1.57; n=2)	43.2 (12.9; 2)	47.0	27.5 (8.90; n=4)
PFPeA	8.62 (2.73; n=4)	2.36 (0.401; n=2)	33.0 (7.81; 2)	22.3	16.6 (5.95; n=4)
PFPeS	0.965 (n=1)	0.961 (n=1)	<LOQ	<LOQ	0.963 (0.001; n =2)
PFPrA	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFPrS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFTeDA	<LOQ	<LOQ	422 (n=1)	<LOQ	422 (n=1)
PFTrDA	<LOQ	<LOQ	241 (n=1)	85.9	163 (54.6; n=2)
PFUdA	<LOQ	<LOQ	102 (n=1)	<LOQ	102 (N/A; 1)
TFA	5,054 (730; n=4)	1,810 (186; n=2)	14,453 (3,414; n=2)	10,170	7,872 (2,415; n=4)

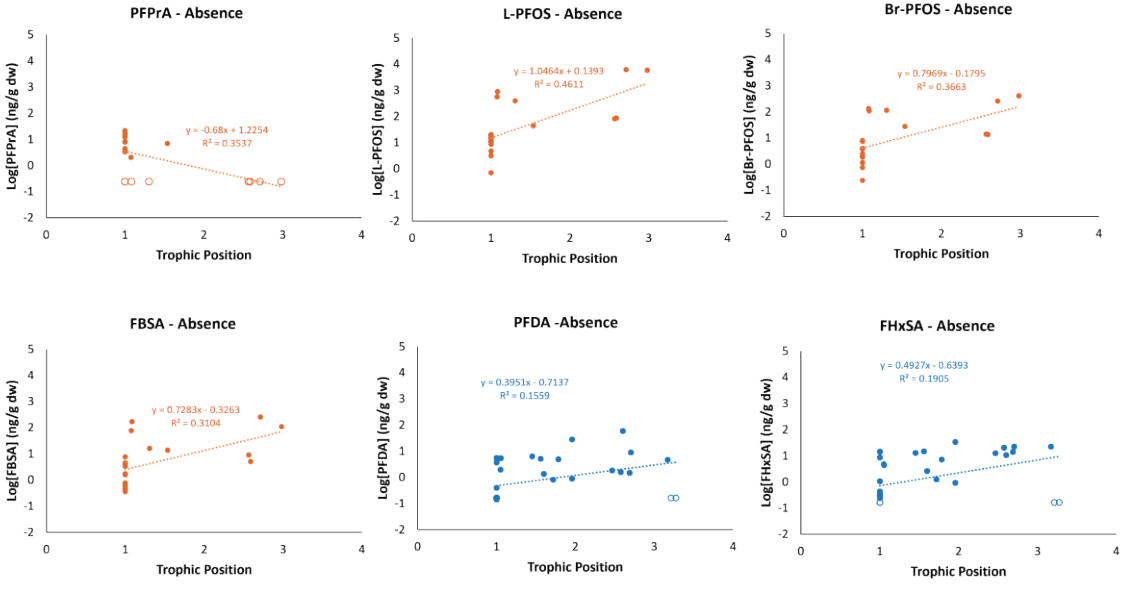
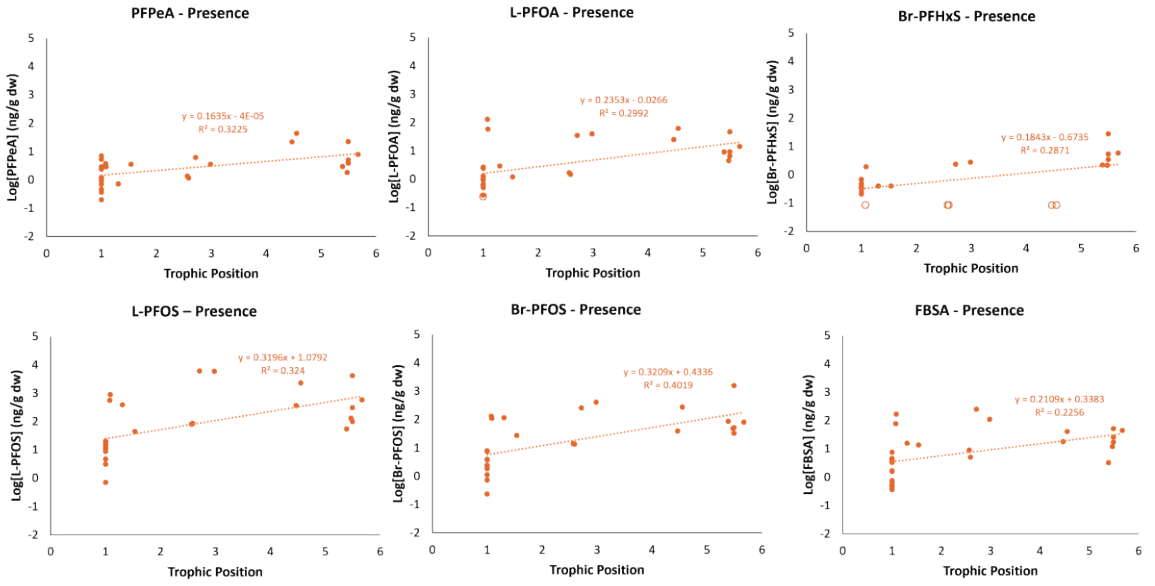
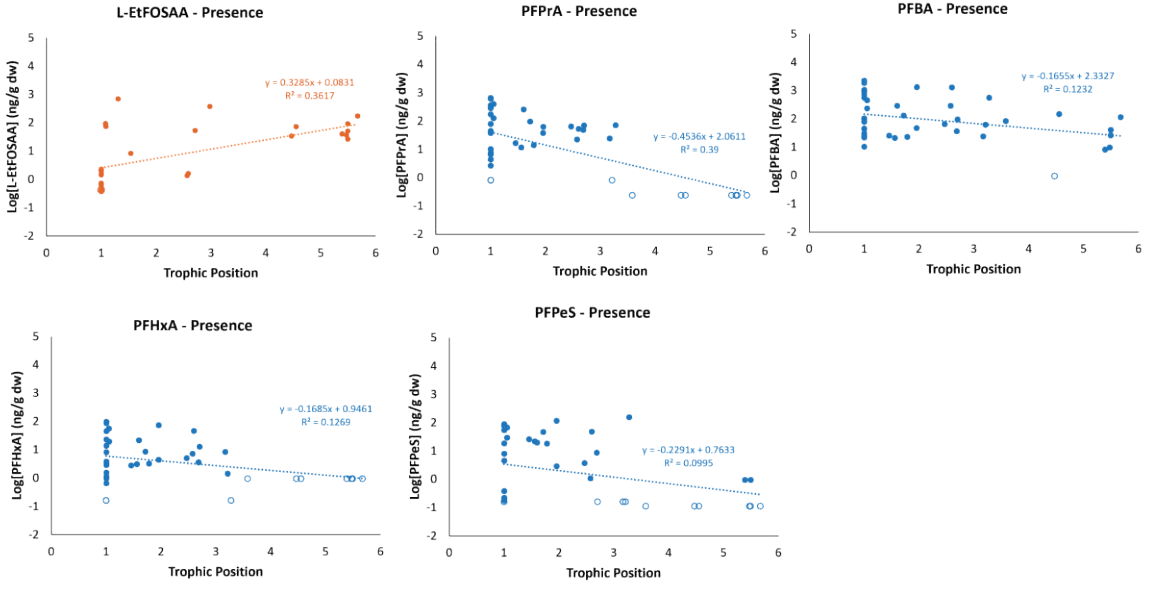


Figure S6: Regression plots for the terrestrial (brown) and aquatic (blue) food webs sampled from the Lake Blokkersdijk ecosystem in the absence of spiders for the PFAS that fulfilled the LOQ criterion (>50% of their data points are > LOQ) and the significance criterion (significant regressions according to the Pearson correlation test; α=0.05). The logarithmically transformed PFAS concentrations are plotted against the trophic positions for all individual organisms. Hollow symbols indicate cases where PFAS concentrations were <LOQ and substituted with 0.5 * LOQ, while closed symbols are actual measured PFAS concentrations.


PFPeA - Presence
y = 0.1635x - 4E-05
R² = 0.3225
Log[PFPeA] (ng/g dw)
Trophic Position
L-PFOA - Presence
y = 0.2353x - 0.0266
R² = 0.2992
Log[L-PFOA] (ng/g dw)
Trophic Position
Br-PFHxS - Presence
y = 0.1843x - 0.6735
R² = 0.2871
Log[Br-PFHxS] (ng/g dw)
Trophic Position
L-PFOS – Presence
y = 0.3196x + 1.0792
R² = 0.324
Log[L-PFOS] (ng/g dw)
Trophic Position
Br-PFOS - Presence
y = 0.3209x + 0.4336
R² = 0.4019
Log[Br-PFOS] (ng/g dw)
Trophic Position
FBSA - Presence
y = 0.2109x + 0.3383
R² = 0.2256
Log[FBSA] (ng/g dw)
Trophic Position


L-EtFOSAA - Presence
y = 0.3285x + 0.0831
R² = 0.3617
Log[L-EtFOSAA] (ng/g dw)
Trophic Position
PFPrA - Presence
y = -0.4536x + 2.0611
R² = 0.39
Log[PFPrA] (ng/g dw)
Trophic Position
PFBA - Presence
y = -0.1655x + 2.3327
R² = 0.1232
Log[PFBA] (ng/g dw)
Trophic Position
PFHxA - Presence
y = -0.1685x + 0.9461
R² = 0.1269
Log[PFHxA] (ng/g dw)
Trophic Position
PFPeS - Presence
y = -0.2291x + 0.7633
R² = 0.0995
Log[PFPeS] (ng/g dw)
Trophic Position

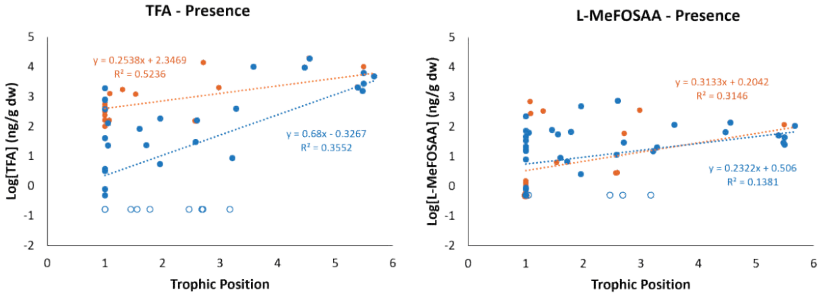


Figure S7: Regression plots for the terrestrial (brown) and aquatic (blue) food webs sampled from the Lake Blokkersdijk ecosystem in the presence of spiders for the PFAS that fulfilled the LOQ criterion (>50% of their data points are > LOQ) and the significance criterion (significant regressions according to the Pearson correlation test; α=0.05). The logarithmically transformed PFAS concentrations are plotted against the trophic positions for all individual organisms. Hollow symbols indicate cases where PFAS concentrations were <LOQ and were substituted with 0.5 * LOQ, while closed symbols are actual measured PFAS concentrations.

References

Gkika, I.S., Kraak, M.H.S., van Gestel, C.A.M., ter Laak, T.L., van Wezel, A.P., Hardy, R., Sadia, M., & Vonk, J.A. (2024). Bioturbation affects bioaccumulation: PFAS uptake from sediments by a rooting macrophyte and a benthic invertebrate. *Environmental Science and Technology, 58(46)*, 20607–18. https://doi.org/10.1021/acs.est.4c03868

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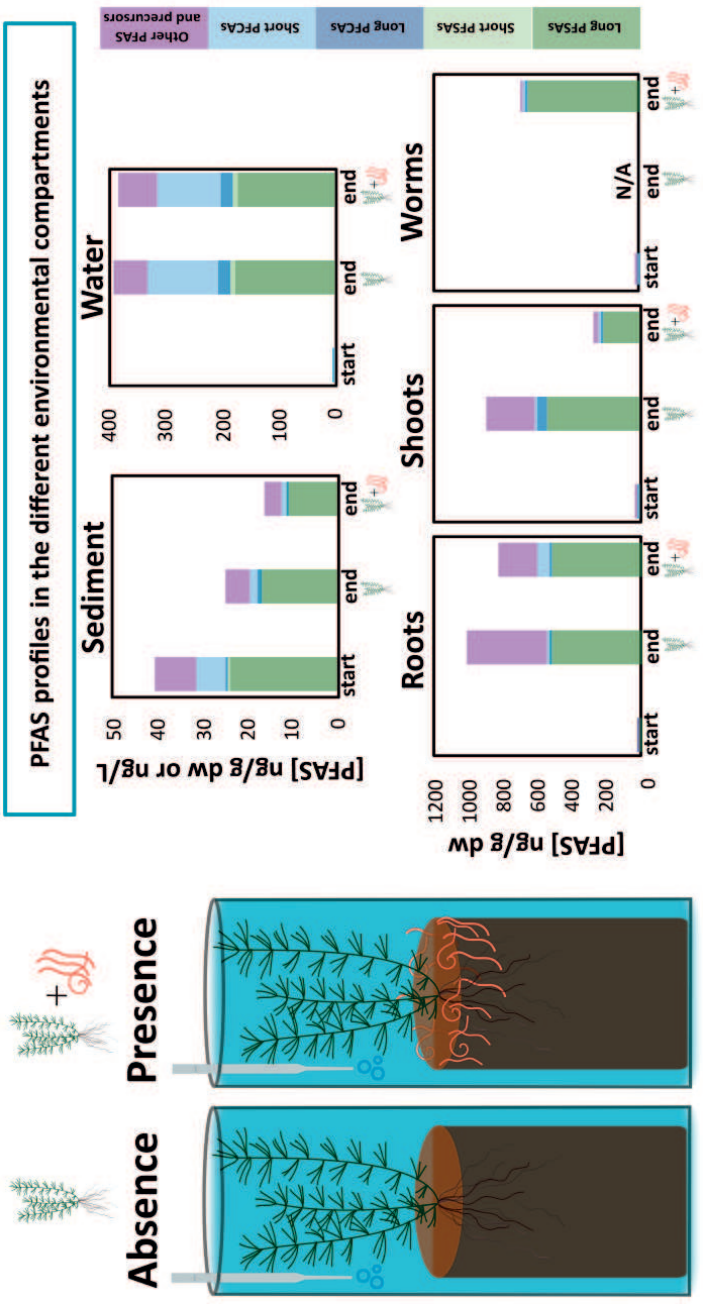
Chapter Five

Bioturbation Affects Bioaccumulation: PFAS Uptake from Sediments by a Rooting Macrophyte and a Benthic Invertebrate

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Ioanna S. Gkika, Michiel H.S. Kraak, Cornelis A.M. van Gestel, Thomas L. Ter Laak, Annemarie P. van Wezel, Robert Hardy, Mohammad Sadia, J. Arie Vonk

Graphical Abstract



Abstract

Despite the widespread presence of Per- and polyfluoroalkyl substances (PFAS) in freshwater environments, only few studies addressed their bioaccumulation in macrophytes and benthic invertebrates. This study therefore aimed at investigating the presence of 40 PFAS in sediments, assessing their bioaccumulation in a rooting macrophyte (*Myriophyllum spicatum)* and a benthic invertebrate (*Lumbriculus variegatus*), and examining the effects of the presence and bioturbation activity of the invertebrate on PFAS bioaccumulation in the plants. The macrophytes were exposed to sediments originating from a reference and a PFAS contaminated site. The worms were introduced in half of the replicates and at the end of the experiment PFAS were quantified in all environmental compartments. Numerous targeted PFAS were detected in both sediments and taken up by both organisms, with summed PFAS concentrations in organisms largely exceeding concentrations in the original sediments. Bioaccumulation differed between organisms and the two sediments. The presence of the worms significantly reduced the PFAS concentrations in the plant tissues, but for some compounds, the root bioaccumulation increased in the presence of the worms. This effect was most prominent for degradable PFAS precursors. It is concluded that organisms affect the environmental fate of PFAS, emphasizing that contaminant-macroinvertebrate interactions are two-sided.

Keywords: environmental occurrence, environmental fate, plant uptake, macrophyte, oligochaetes, bioaccumulation factors, field-contaminated sediment

Synopsis: This study reports the ubiquitous presence of various PFAS, their bioaccumulation by a macrophyte and a benthic invertebrate and the influence of this invertebrate on their bioaccumulation, with a view to enhancing our understanding on PFAS behavior and eventually improve their environmental risk assessment.

5.1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals, designed to be water, oil and stain repellent, as well as persistent (Glüge et al., 2020). These properties make PFAS suitable for a wide range of industrial applications, like surfactants, coatings, aqueous film–forming foams as well as consumer products (Glüge et al., 2020). Their persistency, however, is a chemical feature of environmental concern, since it can lead to prolonged exposure, bioaccumulation, trophic transfer and consequent adverse effects on human and environmental health (Fiedler et al., 2019; Guo et al., 2019).



Despite these worrisome characteristics, PFAS continue to be released into the environment on a large scale and have been detected in numerous abiotic (Brusseau et al., 2020; Kurwadkar et al., 2022) and biotic (Houde et al., 2011; Murakami et al., 2911) matrices at considerable concentrations. A substantial number of studies have documented the occurrence of PFAS in sediments (Ahmed et al., 2020; Ahrens et al., 2010). However, the uptake of these compounds by aquatic plants from sediment remains understudied. The great majority of mechanistic mesocosm studies have focused on agricultural plants (Bao et al., 2020; Gredelj et al., 2020a; Krippner et al., 2014; Liu et al., 2022; Liu et al., 2019b; Wen et al., 2014; Zhou et al., 2020) and soil-plant systems (Lee et al., 2014a; Zhang et al., 2021b), since these have a higher relevance for human health. Based on these studies, root uptake is considered to be the primary pathway for PFAS to enter plants (Wen et al., 2014; Lee et al., 2014b). PFAS can then reach the root vascular cylinder and from there migrate upward to the shoots (Blaine et al., 2013; 2014b). The few studies that have investigated PFAS uptake by rooting macrophytes from sediments have reported a high bioaccumulation potential of PFAS in plants, as well as different bioaccumulation patterns between roots and shoots, which were largely affected by the intrinsic properties of the compounds (Li et al., 2021; Pi et al., 2017; Zhang et al., 2019a; 2020a). It was also suggested that plant uptake favors linear PFAS isomers over branched ones (Felizeter et al., 2012; 2014; Gobelius et al., 2017). To determine how

molecular properties affect PFAS uptake in rooting macrophytes, further research into the interactions between PFAS contaminated sediments and aquatic plants is required, especially for a broader spectrum of PFAS than those studied so far (Gkika et al., 2023).

Sediment-bound PFAS can also be an important source of contamination to benthic invertebrates (Armitage et al., 2006; Bertin et al., 2014; Lasier et al., 2011), which are at the basis of the aquatic food web. However, there are still only a few mechanistic studies available on the bioaccumulation of PFAS from sediments into aquatic or benthic invertebrates (Bertin et al., 2014; Lasier et al., 2011; Higgins et al., 2007; Martín et al., 2019; Prosser et al., 2016). Some studies have indicated the ability of sediment dwellers to affect the bioavailability of compounds through bioturbation (Josefsson et al., 2010; Mustajärvi et al., 2019; Sobek et al., 2014). For PFAS, however, such research remains limited and to the best of our knowledge only one study has been performed, which suggested that the aquatic invertebrates *Hyalella azteca* and *Limnodrilus hoffmeisteri* enhanced the release of PFOS from the sediment (Wu et al., 2021). In addition to the bioturbation activity of benthic invertebrates, sediment properties such as organic carbon content may also affect the sorption capacity and consequently the environmental distribution of PFAS (Wang et al., 2022a). Recent studies on PFAS adsorption to soil and bioaccumulation by plants suggest that these processes are concentration dependent (Ghisi et al., 2019; Zhang et al., 2020a). This concentration-dependent sorption may affect PFAS bioavailability in sediment-water systems, and thereby the distribution of compounds between sediment, water and organisms. The limited information available on the bioaccumulation of PFAS by rooting macrophytes and benthic invertebrates from contaminated sediments and on how bioturbation can affect PFAS uptake kinetics and bioavailability, emphasizes the importance to study these processes for the wide variety of PFAS present in the environment (Gkika et al., 2023).

The aim of this study was therefore to investigate the effects of the presence of benthic invertebrates and their bioturbation activity on the bioaccumulation of various PFAS from field-collected sediments by a rooting macrophyte and the invertebrates themselves. To this end we investigated **i)** the presence of different PFAS classes in sediments collected from both reference and contaminated sites; **ii)** the bioaccumulation of these PFAS by a rooting macrophyte (*Myriophyllum spicatum*) and a benthic invertebrate (*Lumbriculus variegatus*); and **iii)** the impact of the presence of and bioturbation by the benthic invertebrate on PFAS bioaccumulation and redistribution in a sediment-plant system. Rooting macrophytes were exposed to sediments collected from the sites of interest under laboratory conditions. After 28 days of exposure, sediment inhabiting worms were introduced to half of the replicates, and the experiment continued for another 28 days. PFAS concentrations were quantified in all compartments, including sediment, water, roots, shoots (in the presence and absence of worms) and worms. To the best of our knowledge, this is the first study to assess the effects of bioturbation on PFAS bioaccumulation and redistribution in a sediment-water system, using a combination of a rooting macrophyte and a benthic invertebrate.

5.2 Materials and Methods

5.2.1 Sediment sampling and characterization

Sediment cores were collected from two lowland lakes (Figure S1). Details on the exact locations and sampling methods are described in Section S1 (Text S1). A total of eight cores per location were used for setting up the mesocosms and another four cores per location for the sediment characterization (Text S2; Tables S1-S2) and the determination of the initial PFAS load of the sediment. The sediment analyzed initially, before the start of the experiment, did not come into contact with water, other than the water that was sampled together with it in the original sediment core taken from the field. This original overlaying water was then removed once the sediments were transferred to shorter cores (Text S1), which were then directly analyzed for PFAS.

5.2.2 Test organisms and experimental setup

The rooting submerged plant *Myriophyllum spicatum* (Eurasian watermilfoil or spiked watermilfoil) grows in shallow, stagnant or slow-moving water (Arts et al., 2021). Its wide geographical distribution and high ecological relevance for freshwater ecosystems has led to its broad use in environmental research (Arts et al., 2021). The specimens used in this experiment were cultured at the University of Amsterdam in aerated aquaria with artificial sediment (Text S3) and Dutch Standard Water (DSW; Text S4).

The oligochaete *Lumbriculus variegatus* (blackworm) is recommended by the U.S. EPA (2000) for assessing bioaccumulation of contaminants from freshwater sediments. *L. variegatus* lives in shallow marshes, ponds, and swamps, where it feeds on microorganisms and organic material. Worms were supplied by Romberg Aquariumhuis (NL) in 90 mL plastic bags filled with water. Prior to the experiment, worms were cultured in aerated aquaria containing artificial sediment and DSW.

To establish baseline PFAS concentrations, in addition to the four sediment cores per sampling site, four replicates of water, whole plants and worms were collected separately, before the start of the experiment and frozen for subsequent PFAS analysis. At the start of the experiment (2nd March 2022), two *M. spicatum* shoots (~7 cm length) were left to root in each of the eight sediment cores per lake, resulting in a total of 16 test cores. Each core (6 cm Ø) was immerged into a 4 L high-density polyethylene (HDPE) bottle that was filled with 3.5 L of DSW until the plants were covered (Figure S2). The bottles were placed in a climate room (20 °C, light:dark 16h:8h) and continuous aeration using a glass Pasteur pipette was installed in each bottle.

During the first 28 days of the experiment, the plants were allowed to grow, after which the bioturbating worms *L. variegatus* were introduced into half of the replicates from each location and the experiment was run for another 28 days (Figure S2). It should be noted that the four replicates per site and per condition were not technical replicates, but rather field replicates, since each core was individually sampled in the

field and brought into the laboratory intact, transferring local variability in sediment composition.

To obtain enough biomass for chemical analysis, a higher worm density than recommended by the OECD guidelines for bioaccumulation testing with sediment or soil inhabiting worms (OECD 2010, Test No. 225; OECD 2010, Test No. 317) was chosen. To this end, 2.7 and 1.7 g (wet weight) of *L. variegatus* were added to the reference and contaminated sediments, respectively, corresponding to a density of approximately one worm per g dry sediment.

At the end of the 56-d experiment, from each individual core, the plants were harvested from the sediment and gently rinsed with Mili-Q water to remove sediment particles. The shoots were separated from the roots using scissors that were previously cleaned with methanol, and the dry weights after freeze drying of both plant parts were recorded. Worms were harvested using a plastic Pasteur pipette and left to depurate for 48 h on wet filter paper in a petri dish. This method of gut depuration has been described previously and is commonly used for oligochaetes (Arnold et al., 2007; Nahmani e al., 2007; OECD 2010, Test No. 317). Sediment and water were subsampled, and all samples were stored at -20 °C until PFAS extraction.

5.2.3 PFAS extraction and analysis

All matrices were analyzed for 42 PFAS covering a wide range of structures, including six isomer pairs (Table S3). Individual branched isomers were not differentiated in the analysis and the term "branched isomers" here refers therefore to the sum of all detected branched isomers per compound. From each of the four replicate cores, one water, sediment, root, shoot and worm sample was analyzed per location for the initial PFAS concentrations at the start and at the end of the experiment for both the plant-only and plant-with-worm treatments. However, for the contaminated sediment exposures, worms from only one replicate could be extracted due to sample loss. PFAS concentrations in sediment and biota were expressed per dry weight.

The protocols used for PFAS extraction, analysis and quality control assessment have been described previously (Sadia et al., 2020; 2023). Briefly, for the water samples a weak anion exchange solid phase extraction was applied. For the sediment and biota samples, solid-liquid extraction, followed by a weak anion exchange solid phase extraction and a clean-up step were performed. A detailed description of the extraction protocols for all matrices, the quantification method, and the quality assurance/quality control criteria can be found in Section S3 (Text S5-S6; Tables S4-S6). It should be noted that sediment concentrations here reflect total concentrations, consisting of both the solid-sorbed and porewater-dissolved PFAS.

5.2.4 Bioaccumulation

The biota to sediment accumulation factor (BSAF) was used to describe the enrichment of PFAS concentrations in biota from the sediment, while the bioconcentration factor (BCF) was used to describe the ratio of PFAS concentrations between biota and water. All BSAFs and BCFs were calculated separately for each location and for all replicates (n = 4, unless stated otherwise). The BSAF was calculated from equation (7) for the macrophyte roots and the worms and the BCF for the shoots from equation (8). In these equations $[\mathrm{PFAS}]_{\mathrm{root}}$, $[\mathrm{PFAS}]_{\mathrm{worm}}$, $[\mathrm{PFAS}]_{\mathrm{shoot}}$ correspond to the PFAS concentrations in each of these respective compartments at the end of the experiment and $[\mathrm{PFAS}]_{\mathrm{sediment}}^{\mathrm{t=56}}$, $[\mathrm{PFAS}]_{\mathrm{water}}^{\mathrm{t=56}}$ correspond to the PFAS concentrations in the sediment and water at the end of the experiment.

$$\mathrm{BSAF}_{\mathrm{root\ or\ worm}} = \frac{[\mathrm{PFAS}]_{\mathrm{root\ or\ worm}}}{[\mathrm{PFAS}]_{\mathrm{sediment}}^{\mathrm{t=56}}} \left[\frac{\mathrm{kg}_{\mathrm{sediment\ dw}}}{\mathrm{kg}_{\mathrm{root\ or\ worm\ dw}}}\right] (7)$$

$$\mathrm{BCF}_{\mathrm{shoot}} = \frac{[\mathrm{PFAS}]_{\mathrm{shoot}}}{[\mathrm{PFAS}]_{\mathrm{water}}^{\mathrm{t=56}}} \left[\frac{\mathrm{L}_{\mathrm{water}}}{\mathrm{kg}_{\mathrm{shoot\ dw}}}\right] (8)$$

5.3. Results

5.3.1 Sediment characterization

The two sediments had comparable nitrogen contents (Table S1) but differed in the organic carbon content and the concentration of organically bound phosphorus, with the latter being more than six times higher in the contaminated compared to the reference sediment (9.39 and 1.48 mg/kg respectively). Grain size analysis revealed that the reference sediment mainly consisted of particles of size classes 125-250 μm and 250-500 μm, with almost equal parts, adding up to ~ 87 %, while the contaminated sediment was finer, containing more than 63 % of size class 125-250 μm particles, followed by 32 % of size class 63-125 μm particles (Table S2).

5.3.2. Occurrence of PFAS in environmental compartments and organisms

Data for 40 out of 42 target PFAS (Text S6) are reported for the collected sediments from the two locations, the used overlying water source and both test organisms. All matrices contained PFAS both at the start and the end of the experiment, with variable shifts in the PFAS profiles and the individual PFAS concentrations in all matrices (Figure 18).

PFAS concentrations in sediment and water

In the reference field collected sediment, PFAS profiles were dominated by long-chained perfluorocarboxylic acids PFCAs, followed by precursors and long-chained PFSAs (Table S7; Figure 18A). A total of 18 compounds were detected with a ∑PFAS concentration of 4.06 ng/g dw, and individual concentrations ranging from 0.023 (Br-PFOA) to 0.86 ng/g dw (PFTeDA). At the end of the 56-day experiment in the presence of the macrophytes, the ∑PFAS decreased to 32% (1.31 ng/g dw) of the initial concentration. The number of detectable PFAS decreased to eight, with PFAS profiles now being dominated by precursors (Table S8; Figure 18A). The presence of both macrophytes and benthic invertebrates resulted in an even lower ∑PFAS concentration (1.03 ng/g dw; 25% of the initial concentration), also dominated by precursors (Table S9; Figure 18A).

The contaminated sediment contained 10 times more PFAS than the reference sediment (∑PFAS 40.9 ng/g dw). More substances were detected (24 versus 18) in higher individual concentrations (up to 9.23 ng/g dw for L-PFHpS) and with a different composition, since the profile was dominated by long-chained PFSAs, followed by precursors and short-chained PFCAs (Table S7; Figure 18B). The ∑PFAS concentrations decreased to 60% of the initial concentration (∑PFAS 24.8 ng/g dw) in the presence of the plants and to 40% of the initial concentration (16.3 ng/g dw) when both organisms were present (Tables S8-S9). The PFAS profiles remained similar in the contaminated sediment (Figure 18B), in contrast to those in the reference sediment.

At the start of the experiment, four PFAS (∑PFAS concentration 3.66 ng/L) were detected in the DSW that was added on top of the sediments (Table S7; Figure 18C). Upon contact with the sediment from the reference location in the presence of the plants, the number of compounds increased to 12, and the ∑PFAS was more than six times higher (21.0 ng/L) than at the start of the experiment (3.66 ng/L), while the profile became more varied, including short and long-chained PFCAs, followed by long-chained PFSAs. The joint presence of macrophytes and worms resulted in a lower increase in ∑PFAS (10.3 ng/L) in the overlying water, with a comparable number of compounds (10) and a slightly changed profile, dominated by short-chained PFCAs and with less precursors and PFSAs compared to the macrophyte-only treatment (Tables S8-S9; Figure 18C).

In the replicates containing the PFAS contaminated sediment, the ∑PFAS concentration in the water increased more than 100 times after 56 days, both in the presence of only plants and in the presence of both organisms (392 and 382 ng/L, respectively). Over this period, the number of compounds in the water increased from four to 25 and 27, respectively (Tables S8-S9; Figure 18D). The PFAS profiles were similar in both treatments at the end of the experiment, consisting mostly of long-chained PFSAs and short-chained PFCAs.

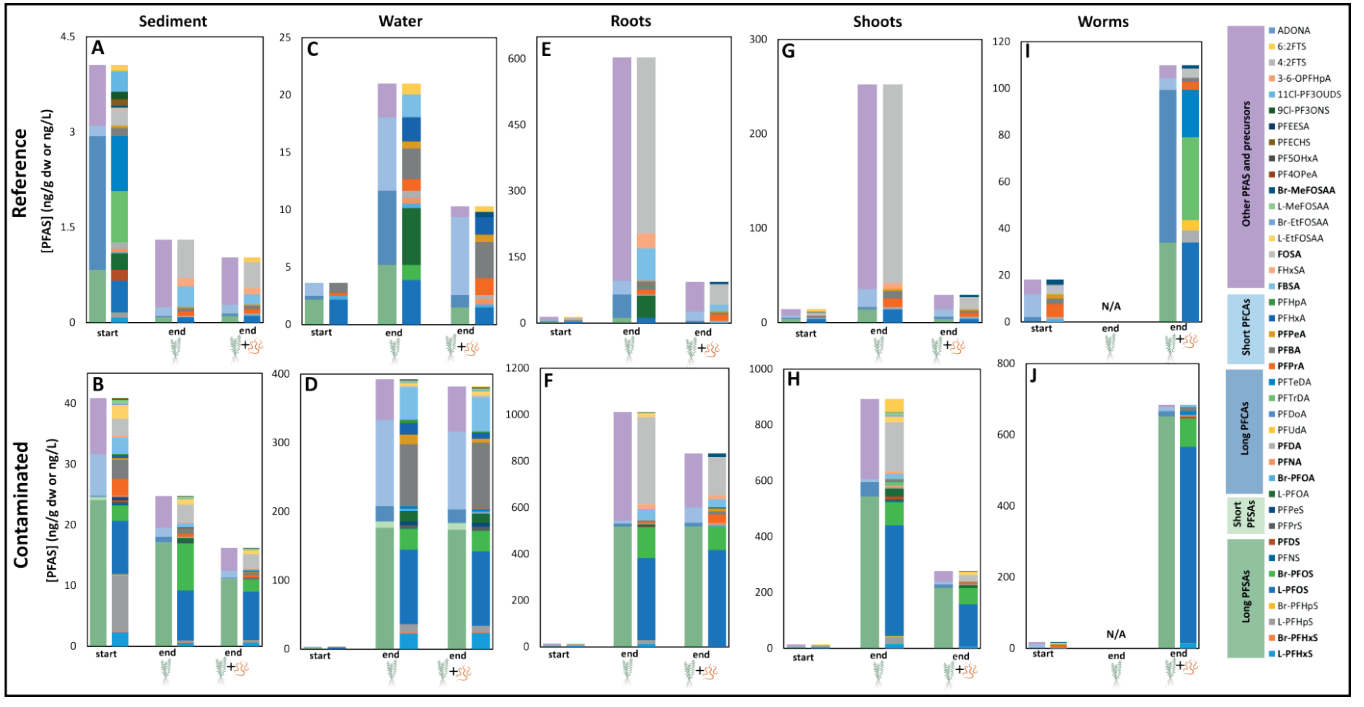

Sediment
Water
Roots
Shoots
Worms
Reference
[PFAS] (ng/g dw or ng/L)
Contaminated
[PFAS] (ng/g dw or ng/L)
A
B
C
D
E
F
G
H
I
J
start
end
N/A
Other PFAS and precursors
ADONA
6:2FTS
4:2FTS
3-6-OPFHpA
11Cl-PF3OUDS
9Cl-PF3ONS
PFEESA
PFECHS
PFSOHxA
PF4OPeA
Br-MeFOSAA
L-MeFOSAA
Br-EtFOSAA
L-EtFOSAA
FOSA
FHxSA
FBSA
Short PFCAs
PFHpA
PFHxA
PFPeA
PFBA
PFPrA
Long PFCAs
PFTeDA
PFTrDA
PFDoA
PFUdA
PFDA
PFNA
Br-PFOA
L-PFOA
Short PFSAs
PFPeS
PFPrS
Long PFSAs
PFDS
PFNS
Br-PFOS
L-PFOS
Br-PFHpS
L-PFHpS
Br-PFHxS
L-PFHxS

Figure 18: PFAS profiles and average (n = 4) individual PFAS concentrations in the different compartments (sediment, water, roots, shoots, and worms) at the start and the end of the 56 d mesocosm experiment in which plants (*Myriophyllum spicatum*) and worms (*Lumbriculus variegatus*) were exposed to reference (Gaasperplas) and contaminated (Blokkersdijk) sediments. For visualization purposes error bars are not plotted in the graphs, but the raw data behind the bar plots are included in Tables S7 – S9. In each pair of bars, the left one shows the different PFAS sub-classes, while the right one depicts the individual compounds that belong to each sub-class. The different treatments (absence or presence of worms) are indicated below the stacked bars with respective icons. In the legend, the compounds that were detected in all compartments (sediment, water, plant and worms) in at least one of the locations and for at least one time point are highlighted in bold. Results for worms shown in plot J are based on only 1 replicate. Please note that the scales of the y axes differ between the panels, to enable visualization.



PFAS concentrations in macrophytes and worms

At the end of the exposure, macrophytes were in good condition, showing no signs of necrosis. The average dry weight of the roots was 0.083 and 0.12 g and of the shoots 2.0 and 3.5 g for the reference and contaminated sediments respectively. Worms were in good condition, and comparable amounts were recovered from both types of sediments. In the original *M. spicatum* plants eight out of the 40 PFAS were detected (Table S7; Figure 18E-18F), with a low ∑PFAS concentration (14.5 ng/g dw). Roots grown in the reference sediment in the absence of the worms contained a 41-times higher ∑PFAS concentration (603 ng/g dw) than the original plant tissue. The profile consisted of 10 detectable PFAS, mainly precursors, and differed from the PFAS composition of the original sediment. The PFAS concentrations in the roots that were exposed to the reference sediment in the presence of worms had similar profiles as in the plant-only treatment, but the ∑PFAS was more than six times lower (94.2 ng/g dw) (Tables S8-S9; Figure 18E). In the shoots, the ∑PFAS concentration after 56 days was over two times lower than in the roots (252 ng/g dw), but with similar profiles and number of detected compounds (eight) (Table S8; Figure 18G). In the presence of the worms, the ∑PFAS concentration in the shoots was almost nine times lower (29.5 ng/g dw; eight compounds) compared to the plant-only treatment and was three times lower than in the roots, nonetheless with similar profiles, dominated by precursors (Table S9; Figure 18G).

Roots exposed to the contaminated sediment in the absence of the worms accumulated almost exclusively long-chained PFSAs and precursors, consisting of 23 compounds, more than twice as many as in the roots of the plants grown on the reference sediment (10) (Table S8; Figure 18F). The ∑PFAS concentration was 1.01 µg/g dw, 72 times higher compared to the initial concentrations in the plants, and almost twice as high as in the roots grown on the reference sediment. In the presence of both organisms, PFAS profiles were similar, but the number of detected PFAS decreased to almost half (12) of the number in the plant-only treatment (23), while

the ∑PFAS concentration was only slightly lower (834 ng/g dw) (Table S9; Figure 18F).

In the shoots of the plants exposed to the contaminated sediment in the absence of worms, the PFAS profiles and the number of detected compounds (22) were similar to those in the roots (Table S8; Figure 18H). The ∑PFAS (893 ng/g dw) was 61 times higher than in the original plants, but lower than in the roots. When also the worms were present, fewer compounds were detected (14), the ∑PFAS was three times lower (278 ng/g dw) and the profile changed, consisting almost exclusively of long-chained PFSAs (Table S9; Figure 18H).

Prior to contact with the sediments, the worms contained seven PFAS, mostly short PFCAs, with a ∑PFAS concentration of 18.2 ng/g dw (Table S7; Figure 18I-18J). After exposure to the reference sediment, a similar number of compounds was detected in the worms (nine), but the ∑PFAS concentration increased more than six times (110 ng/g dw), while the PFAS profile was now dominated by long PFCAs and PFSAs (Table S9; Figure 18I). After exposure to the contaminated sediment, 11 PFAS were quantified in the worms, almost exclusively long-chained PFSAs (Table S9; Figure 18J), resulting in a ∑PFAS concentration of 684 ng/g dw, 38 times higher than at the start of the experiment and six times higher than in the worms exposed to the reference sediment.

Mass balance

To investigate how the compounds distributed between the sediments, plants, worms and overlying water, mass balances were calculated. The details behind the calculations are presented in the SI (section S5, Text S7). Overall, recoveries of most PFAS were below 100%, except for L-PFOS, Br-PFOS and FOSA (Table S10).

3.3 Bioaccumulation factors

PFAS Bioaccumulation factors for macrophytes and worms

Logarithmically transformed biota to sediment accumulation factors were obtained for roots in the absence ($BSAFs_{root-}$) or presence of worms ($BSAFs_{root+}$) and for worms ($BSAFs_{worm}$), all in kg sediment dw / kg root or worm dw (Tables S11-S13; Figure 19). For the reference sediments, average BSAF values could be calculated for six PFAS both in the absence and presence of worms (Figures 19A-19B). The compound that exhibited the highest average BSAF values was different between the two treatments with a higher value for the plant-only treatment (FOSA; 1053) compared to the joint presence (PFPrA; 199). For worms, BSAFs could be calculated for five PFAS (Figure 19C). In the worms generally lower values were observed compared to the macrophyte roots, with the maximum average value reaching 326 (L-PFOS). For the contaminated sediments in the plant-only treatment, BSAFs could be calculated for 23 PFAS (Figure 19D), while in the joint presence of both organisms for less than half of this number (11) (Figure 19E). In both cases the highest average BSAF values were found for precursors (FOSA; 150 in plant-only treatment; FHxSA; 233 in worms and plant treatment). Similar to the reference sediment, BSAFs for worms generally were lower: PFDA showed the highest average BSAF of 91 (Figure 19F). Overall, for both sediment types, precursors tended to bioaccumulate more in the macrophyte roots and much less in the worms. The BSAFs for linear and branched isomers did differ in most cases, but these differences were not consistent.

Comparison of locations

Comparing the bioaccumulation behavior of individual PFAS, as well as PFAS sub-classes between the two locations, revealed some differences. For plants grown in the reference sediment, $BSAFs_{root-}$ for PFCAs was not correlated with chain length (Figure 19A), while for the contaminated sediment BSAFs seemed to increase with increasing chain-length (Figure 19D). For the worms, no relationship between bioaccumulation and chain-length was observed for the reference nor for the

contaminated sediment (Figures 19C, 19F). For the organisms exposed to the highly contaminated sediment, the PFAS concentrations were higher than in the reference sediment, which enabled the quantification of BSAF values for more PFAS. However, the BSAFs were generally lower for the contaminated sediment compared to the reference sediment for both organisms. Comparing the difference in bioaccumulation between the two organisms was not possible as little overlap was found between the PFAS present in the two organisms and almost no overlap between the two locations (only one compound) (Figure S3).

5.3.4 Effect of bioturbation on PFAS bioaccumulation by macrophytes



To evaluate the effect of the presence of the benthic invertebrate and its bioturbation activity on the bioaccumulation of PFAS by the macrophytes, the bioaccumulation in the roots in the presence ($BSAF_{root+}$) and absence ($BSAF_{root-}$) of the worms in the sediments from both locations (Figure 20) was compared. Only compounds that were concurrently present in the roots of both treatments (absence or presence of worms) and for which BSAFs could be calculated are plotted. In the scatterplots, two categories were distinguished, based on the distance of the data points from the 1:1 line (X = Y) shown in orange. The first category included compounds that were on or close to the 1:1 line, indicating similar bioaccumulation factors between the two treatments. These data points were within a ± 0.2 log unit range from the 1:1 line. The second category concerned compounds that deviated more than 0.2 log units from the 1:1 line. For the reference site (Figure 20A), we could compare the BSAFs for five compounds, two of which (PFBA and FOSA) deviated strongly from the 1:1 line and were positioned below it, indicating a higher bioaccumulation in the plants in the absence of worms (Figure 20A). The BSAFs in the macrophytes for the remaining three compounds, consisting of two short- and one long-chained PFCAs, were similar in the presence or absence of the worms. In the contaminated sediment, the effect of the worm presence could be evaluated for 10 PFAS (Figure 20B), showing opposite patterns than for the reference sediment. Three out of these 10 PFAS (two precursors and one long-chained PFSA) were positioned on or close to the 1:1 line, indicating comparable bioaccumulation

between the two treatments. The remaining seven compounds were positioned above the 1:1 line, suggesting higher bioaccumulation in the presence of worms. Finally, there were only four compounds for which BSAFs could be compared in the presence and absence of worms for both the reference and contaminated sediment (Figure 20C), all of which exerted opposite behavior between the two types of sediments. More specifically, compounds for which the BSAFs of plants growing in the contaminated sediment did not seem to be affected by the presence of the worms were impacted in the reference sediments and *vice versa*. On top of that, BSAFs from the contaminated sediments that were influenced by the presence of worms exhibited higher values in the presence of worms, while the opposite was observed for bioaccumulation into the plants from the reference sediment. Finally, the shoot bioconcentration factors from water, expressed as BCF, in the absence and presence of worms, are presented in the SI (Text S8; Figure S4).

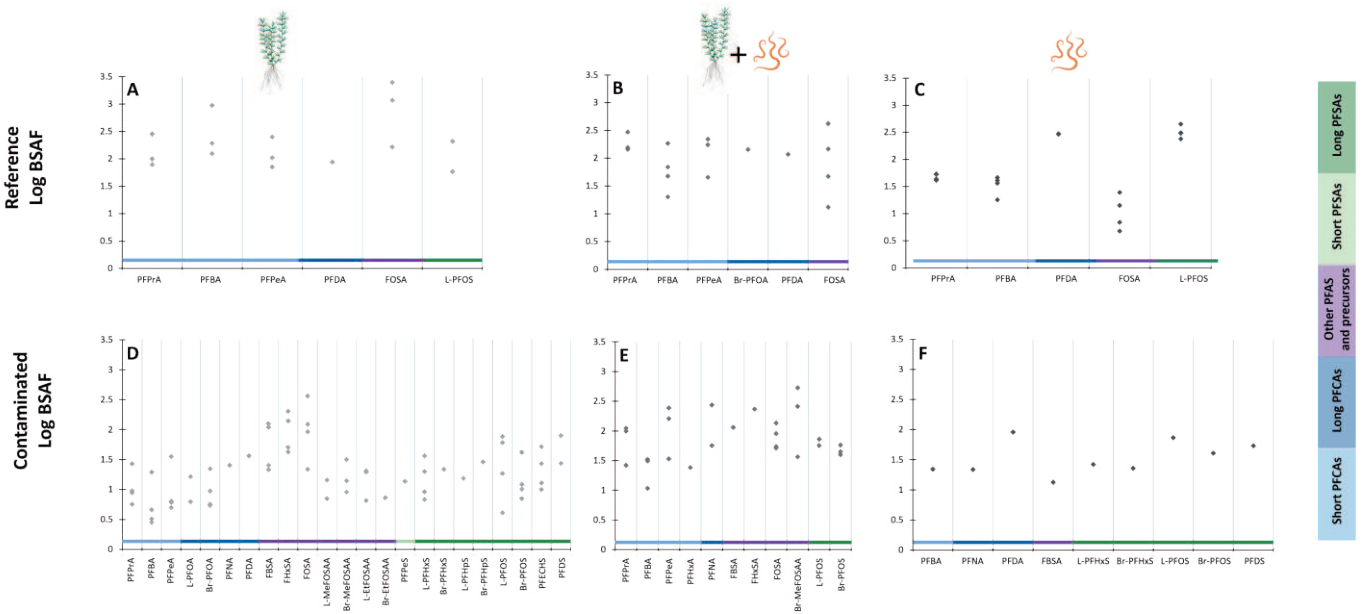


Figure 19: Logarithmically transformed biota to sediment bioaccumulation factors (BSAFs) for the uptake of PFAS from reference (Gaasperplas) and contaminated (Blokkersdijk) sediment into the roots of *Myriophyllum spicatum* [kg sediment dw/kg root dw] in the absence (A and D, respectively) and presence of worms *(Lumbriculus variegatus*) (B and E, respectively) and into the worms [kg sediment dw/kg worm dw] themselves (C and F, respectively).Each data point represents one replicate.. The horizontal colour bars indicate the different PFAS sub-classes, with the compounds per sub-class plotted with increasing number of fluorinated carbons. In general, n = 4, except for some compounds that were not detected in all replicates, as can be seen in Tables S11-13. BSAFs in worms shown in plot F are based on only 1 replicate.

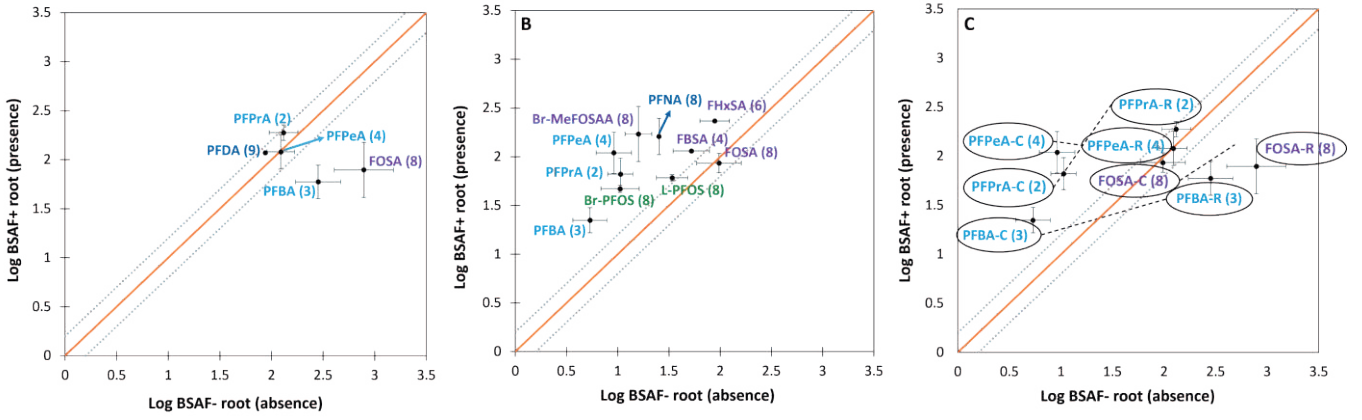


Figure 20: Effect of the presence of worms (*Lumbriculus variegatus*) on the bioaccumulation of PFAS in plant roots (*Myriophyllum spicatum*) following exposure to field sediments from two locations. Average (± SEM) Log BSAFs for PFAS bioaccumulation in roots in the presence and absence of worms are plotted for the reference (Gaasperplas) (A) and contaminated (Blokkersdijk) (B) sediments (n = 4, except with missing error bars n = 1). In panel C BSAFs for PFAS overlapping between the two locations are plotted together and for each pair the same color is used. The names of the compounds corresponding to each data point are presented and the number in parenthesis indicates the number of fluorinated carbons. The letters R (reference) and C (contaminated) in the last panel indicate the locations. All BSAF values are given in kg sediment dw / kg root dw. The solid orange line indicates the 1:1 line and the dotted grey lines the margin of ± 0.2 log units, which was used to evaluate the distance of the compounds from the 1:1 line.

5.4 Discussion

This study presented the occurrence and profiles of PFAS in reference and contaminated freshwater sediments and their bioaccumulation by a rooting macrophyte and a benthic invertebrate. The experimental design allowed to assess the effect of the presence and bioturbation activity of the benthic invertebrate on PFAS bioaccumulation and redistribution in the sediment-water-macrophyte system.

5.4.1 Presence of PFAS from different sub-classes in freshwater sediments

The need to broaden the spectrum of targeted PFAS has been underlined by earlier research (Ateia et al., 2019; Gkika et al., 2023; Hamid et al., 2024; Macorps et al., 2023). This study therefore examined PFAS structures which are more rarely screened for in PFAS monitoring and experimental studies, including ultra-short PFAS, branched isomers, chlorinated polyfluoroether sulfonic acids, as well as a cyclic sulfonic acid. Despite this extensive PFAS list, we acknowledge that the actual number of potentially present PFAS remains higher, being either unknown precursors, or simply unscreened structures. The presence and potential (bio)transformation of unknown precursors could also have contributed to the increased concentrations of PFAAs observed at the end of the experiment. In the reference sediments, more than 40 % of the targeted compounds were quantified, underlining that there may be hardly any reference site concerning PFAS. Comparing the presently measured PFAS concentrations in the reference sediments with those from earlier studies (Ahmed et al., 2020) revealed similar levels, although slightly different PFAS were screened for. The PFAS concentrations in contaminated sediments reported in the literature vary greatly, which is to be expected since different PFAS sources result in distinct PFAS fingerprints (Joseph et al., 2023). Nevertheless, qualitative comparisons showed that in one case PFAS concentrations were similar to those currently measured (Boiteux et al., 2016), but in other sediments, the concentrations reported were orders of magnitude higher compared to those detected in the present study (Langberg et al., 2020; 2024; Song

et al., 2023). It is concluded that broadening the spectrum of targeted PFAS indeed revealed the presence of many of these, which may also imply that many more unmeasured PFAS could be present in the environment, where profiles and concentrations are location and source specific. Acknowledging the fact that screening for numerous PFAS in various environmental matrices is expensive and labor intensive, a monitoring list that includes at least some representatives from the different sub-classes is proposed.

5.4.2 Bioaccumulation of PFAS by a rooting macrophyte and a benthic invertebrate



The findings of the present study suggest that many PFAS do have a high bioaccumulation potential. From the 18 PFAS, for which BCFs for shoots were calculated for both sampling sites in the absence of worms, eight had values > 5,000 L/kg in at least one or both locations, reaching up to 97,695 L/kg shoot dw for FOSA. Based on the PBT/vPvB assessment criteria set by ECHA (2023), these compounds, which in our case included mostly PFSAs and precursors, are categorized as "very bioaccumulative" and therefore require stricter regulations compared to compounds not categorized as PBT/vPvB. Although a comparison for the BSAF values was not possible, due to their absence in the available literature, based on the calculated values, reaching up to 1053 kg sediment dw/kg root dw for FOSA, it may be argued that at least some PFAS classes have a high bioaccumulation potential from sediments to macrophytes.

Although PFAS uptake by terrestrial plants has previously been reported (Lesmeister et al., 2021), information about PFAS bioaccumulation in aquatic macrophytes remains limited (Evich et al., 2022; Gkika et al., 2023; Griffin et al., 2023). In a recent review gathering BSAFs for PFAS, almost no data on macrophytes were reported (Burkhard et al., 2023), since the few studies that did investigate PFAS uptake by rooting macrophytes from sediments reported only BCFs from water (Blaine et al., 2014b; Pi et al., 2017; Zhang et al., 2019a; Zhang et al., 2020a). Concerning the benthic invertebrates, there are some studies on bioaccumulation from field

collected and PFAS spiked sediments (Bertin et al., 2014; Lasier et al., 2011; Higgins et al., 2007; Martín et al., 2019; Prosser et al., 2016; Yun et al., 2023). We compared our BSAF data for *L. variegatus* with previously reported BSAFs for the same organism after harmonizing all units (Table S15). Our data were on the lower end of the BSAF calculated in another study using field contaminated sediment (Lasier et al., 2011), while they were much lower compared to the other two studies (Higgins et al., 2007; Yun et al., 2023). A major distinction, which could have led to these discrepancies, is the presence of the macrophytes, which may have competed with the worms for PFAS uptake. Moreover, one of the studies was performed with spiked, artificial sediment (Yun et al., 2023), where bioavailability of organic compounds is expected to be higher compared to field-contaminated sediments that have been in contact with the contaminants for an extensive time period (Alexander 2000; Schuler et al., 2003). It should be realized that BSAFs can only be reliably calculated when the system is in steady state, which was only addressed by Higgins et al., (2007), observing a lack of equilibrium for several PFAS after 28 days. Our experimental setup did, however, not allow to evaluate whether equilibrium was reached. The water content of the worms could be another confounding factor, specifically for the BSAF calculation of the short-chained compounds, which might have contributed to the high BSAF observed for PFPrA. On top of that, because the worms were not rinsed prior to extraction, any PFAS present in the water attached to the worms, as well as the internal water content of the worms may have contributed to the body burden of the worms. Yet, assuming steady state between overlaying water, worms and pore water, this contribution was estimated to be low to negligible. However, this steady state assumption cannot be verified and therefore BSAF comparisons even between studies on the same species, like performed here (Table S15), should be interpreted with some caution. Additional differences in terms of the PFAS load and profiles, as well as the properties of the sediments could further explain the variation in the BSAFs. We chose not to compare BSAFs reported for other benthic invertebrates, since on top of the aforementioned factors, there is additional variation related to the taxonomy, physiology, feeding behavior

and exposure route of the organism based on its habitat, living either in or on top of the sediment.

The BSAF calculations were based on the final PFAS concentrations in the sediment measured at the end of the experiment. It should be noted, however, that these BSAFs may only be correct for compounds that reached steady state, while they may have been overestimated for the ones that did not. Yet, our experiment did not allow for assessing PFAS desorption kinetics from the sediment nor uptake kinetics in the plants and worms. Nonetheless, using the arithmetic mean of the sediment concentrations measured at the start and end of the experimental period, still resulted in high values, highlighting the high bioaccumulation potential of PFAS (Table S14).



The bioaccumulation of PFAS in the roots and the worms in relation to their chain length, were not consistent between the two locations nor for the two treatments in our study. In some cases, increased bioaccumulation in relation to the fluorinated chain length was observed for the carboxylic acids and some sulfonamide-based precursors (contaminated sediment roots in absence of worms), which aligns with earlier research (Pi et al., 2017; Lasier et al., 2011), but in other cases this relationship was absent. Absence or even negative relationships between bioaccumulation and PFAS chain length have also been reported previously concerning bioaccumulation factors based on soil, instead of porewater concentrations (Blaine et al., 2014b; Navarro et al., 2017; Wen et al., 2014). Two main explanatory factors were proposed for the different relationships between bioaccumulation and chain-length that are often reported in literature. Although these explanations originate from terrestrial studies, they could also apply to aquatic systems, like the one in our study. The first explanation concerns the variation in root structures among plants. These different root structures could lead to distinct interactions with contaminants (Blaine et al., 2014a), as well as adsorption/absorption mechanisms of pollutants, which can involve, among others, root exudates, ion channels and/or aquaporins (Chen et al., 2023; Greger et al., 2024; Xiang et al., 2020; Yu et al., 2021). The second one relates

to the inability to differentiate between the strong external adsorption of long-chained and the absorption of shorter-chained PFAS (Gredelj et al., 2020a). Based on these findings, PFAS uptake by plants seems to be compound and species specific. However, our setup did not allow differentiation between external plant sorption or internal plant uptake, so the second explanatory factor could partly justify the inconsistency of the observed bioaccumulation patterns between the different compounds. Relationships between bioaccumulation and chain-length may be further obscured by differences between compounds in reaching steady state, as might have been the case in our study.



Similar PFAS profiles and trends were observed in roots and shoots, but we cannot unravel the processes resulting in these profiles and trends. Our setup did not allow to distinguish PFAS that bioconcentrated from the water to the shoots and compounds that could have translocated between roots and shoots. Earlier research reported positive correlations between root and shoot concentrations (Greger et al., 2024; Müller et al., 2016) with submerged species having higher BCFs than free floating ones (Pi et al., 2017). Merging these results with our observations, suggests that it was the combination of both mechanisms (i.e., uptake from the water phase and translocation from the roots) that contributed to the exposure of the shoots in this study and the resulting PFAS bioaccumulation profiles. This implies that the calculated BCFs may have overestimated the PFAS bioaccumulation potential from water to the shoots (Table S13).

The differences in the bioaccumulation patterns observed between the two locations could to some extent be attributed to the different sediment characteristics and to concentration dependent uptake. The organic carbon contents of the two sediments, the grain size distribution and other characteristics were quite distinct (Tables S1, S2), causing potential differences in PFAS bioavailability. The lower organic carbon content and the lower clay content of the reference sediment could result in a weaker adsorption of the PFAS to this sediment, compared to the contaminated one, resulting in higher bioavailable concentrations. In addition, the aqueous chemistry

(pH, ionic strength), composition and concentrations of PFAS differ between the field contaminated sediments, affecting the bioaccumulation. Moreover, the concentration of organically bound phosphorus (P_{org}) was more than six times higher in the contaminated than in the reference sediments (Table S1). The higher nutrient availability increased plant growth on the contaminated sediment compared to the reference sediment, as indicated by their higher (dry) weights. The difference in the trophic state between the two locations may have affected the distribution and bioaccumulation of PFAS due to biomass dilution, as previously reported for polycyclic aromatic hydrocarbons (PAHs) (Tao et al., 2019). All these factors possibly also played a role in the way the presence of the worms affected the PFAS bioaccumulation. However, the limited overlap between the compounds taken up in the presence and absence of worms in both sediments hampers firm conclusions.

Another factor that could have played a role in the different patterns observed between the two sediments is the initial PFAS load. The contaminated sediment contained approximately one order of magnitude higher ∑PFAS concentrations and the sub-class of long-PFSAs was much more dominant, compared to the reference sediment, where PFCAs were the most abundant, followed by PFSAs and precursors with almost equal contributions. Competing uptake mechanisms have previously been reported for PFAS bioaccumulation (Colomer-Vidal et al., 2022; Müller et al., 2016; Maimaiti et al., 2018), while various studies suggested that PFAS uptake by plants is concentration dependent (Ghisi et al., 2019; Zhang et al., 2020a). Concentration dependency of bioaccumulation factors has been proposed for metals (DeForest et al., 2007), as well as for PFAS (Liu et al., 2011). In the latter study, where the bioconcentration factors from water to green mussels were assessed at various PFAS concentrations, lower BCFs were observed for the organisms exposed to higher PFAS concentrations. This inverse relationship was supported by the non-linear adsorption mechanism underlining the uptake of the investigated compounds (Liu et al., 2011). Also, in this work generally lower BCFs were observed for the organisms exposed to the highly contaminated sediment, which further

supports the inverse relationship between exposure concentration and bioaccumulation.

In the present study, the wide variety of PFAS exhibited different bioaccumulation degrees between different organisms, locations and treatments. It is therefore challenging to assign a general bioaccumulation potential to each PFAS sub-class. Nonetheless, this work showed that a wide variety of PFAS present in the environment appear to have a high bioaccumulation potential. However, the model describing the bioaccumulation potential of this diverse group of compounds likely contains many variables, including, on top of their molecular properties, factors like sediment/soil properties, species interactions, total PFAS loads, as well as interactions with other chemicals.

5.4.3 The effect of the presence and bioturbation by the benthic invertebrate on PFAS redistribution and plant bioaccumulation

Even though the assessment of the bioaccumulation in the worms from the contaminated sediment was based on only one replicate, we were still able to assess the impact of the presence of the worms on the distribution and plant uptake of PFAS. Our findings revealed that the presence and bioturbation activity of the benthic invertebrate did affect the distribution of PFAS over the system, and in the presence of the worms the ∑PFAS concentration in the plant roots and shoots was lower. This was the case for both locations but was more prominent for the plants grown on the reference sediment, where the initial PFAS load of the sediment was much lower. This suggests that there was competition for PFAS uptake between the organisms, as proposed by other studies (Colomer-Vidal et al., 2022; Maimaiti et al., 2018; Müller et al., 2016) or that some compounds had higher affinity for the worms compared to the macrophyte. This is supported by the BSAF values for the plants at the reference sediment that for some compounds were lower in the presence of the worms. In our test system, worm densities were on the high end of abundances found in natural environments. This might had an effect on the relative distribution

and plant uptake of PFAS. However, examining how different worm densities could affect PFAS environmental fate was beyond the scope of our study.

For the organisms exposed to the contaminated sediment, the PFAS load seemed to be so high that even in the presence of both organisms, the plants, and especially the roots, were still able to take up considerable amounts of PFAS. In the contaminated sediment, the concentrations of FOSA and L-PFOS remained relatively constant over the 56-day period, in both treatments, while all other compounds seemed to be depleted by the uptake by the organisms or by the release into the overlaying water. This could imply that the sediment had a high accessible stock of these two PFAS and once the readily bioavailable concentration was taken up by the organisms, it was constantly being replenished. This replenishment of depleted bioavailable concentrations via diffusion processes has previously been reported (Reichenberg et al., 2006; Sijm et al., 2000).

The reduction in terms of the number of PFAS present in the plant roots in the presence of worms was more noticeable for the contaminated sediment. In the contaminated sediment, the root bioaccumulation of mainly PFCAs was affected by the presence of the worms, which could indicate that bioturbation made them more bioavailable to the macrophyte roots. This is supported by the BSAF values for the plants at the contaminated sediment that for some compounds were higher in the presence of the worms. The remaining non-precursor compounds that were not found in the roots when worms were present, were found either in the worm tissues or in the overlaying water. The presence of the worms notably reduced the concentrations of precursors in the roots as well as shoots, while very few to no precursors were detected in the worms themselves at both locations. These observations could relate to the increased oxidation conditions caused by the bioturbation activity of the worms, which could have enhanced the (bio)transformation of some precursors into PFSAs. Earlier research has shown that invertebrates are capable of biotransforming PFAS precursors, including N-EtFOSAA and FOSA to PFSAs (Higgins et al., 2007; Zhao et al., 2018). As

previously mentioned, unmonitored PFAS including precursors could also have contributed to the increased concentrations of "terminal" PFAS observed at the end of the experiment. This is also illustrated by the calculated mass balances, where the observed mass balances above 100% might be due to potential (bio)transformation of precursors into linear and branched PFOS in our systems, especially in the presence of the worms. Our setup did not allow to differentiate between external transformation or biotransformation within the organisms. Yet, we were able to demonstrate that the presence of the worms and their bioturbation had an impact on the PFAS profile in the plants.

Another way in which bioturbation could have affected PFAS bioaccumulation is due to alterations in the benthic microbial community and biogeochemistry.[82] Microbiota are able to accumulate PFAS, but they could also play role in PFAS degradation (Greger et al., 2024) and redistribution from the sediment to the different environmental compartments (Fitzgerald et al., 2018; Li et al., 2021). Contaminant-macroinvertebrate interactions, driven by the interplay between contaminant properties and macroinvertebrate traits have been reported for other compound groups (van der Meer et al., 2022) and the present study showed that there are also various ways through which benthic invertebrates can interfere with the environmental redistribution and bioaccumulation of sediment-associated PFAS. It is therefore concluded that organisms affect the environmental fate of PFAS, highlighting that contaminant-macroinvertebrate interactions should be viewed as a bilateral relationship.

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Supplementary Information

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Chapter Six

Synthesis

The aim of this thesis was to assess the environmental distribution, bioaccumulation and trophic transfer of PFAS in aquatic and terrestrial ecosystems. The exploration of the available literature (**chapter 2**) revealed significant knowledge gaps in all these aspects and helped us to define tangible research objectives in order to address these gaps in the experimental chapters of this thesis (**chapters 3-5**). Reflecting on the objectives formulated in the introduction, this synthesis highlights the main findings of the present thesis, further discusses the various factors that drive PFAS environmental fate and proposes ways to utilize our data to support PFAS environmental risk assessment.

6.1 The more you look for the more you find

The first challenge was to expand the spectrum of targeted PFAS, addressed in all experimental chapters of this thesis (**chapters 3-5**). We screened for up to 44 PFAS, and detection percentages were 67% (43%−84%) when averaging all abiotic environmental samples and 71% (50%−83%) when averaging all organisms. Yet, even this extended target list represents only a small fraction of the total number of PFAS that can be present in the environment (Baqar et al., 2025). Pinning down the sum of all PFAS around the globe in a single number is quite challenging, as PFAS counts differ depending on the applied definition, consulted regulatory inventory and whether the sum is restricted to commercially produced substances or also includes theoretical, patented, and rarely synthesized molecules. The Organization for Economic Co-operation and Development (OECD) identified approximately 4,700 PFAS that are (or have been) produced, used, or reported globally (Wang et al., 2021), while regulatory inventories like the U.S. EPA's CompTox database (2025) already list more than 14,000 unique PFAS structures.

From the 44 PFAS that were targeted in this thesis, 41 were overlapping between all chapters and from those, we considered only the ones that could be reliably quantified (> LOQ) in the abiotic samples. On average 66% (27 out of the 41) could be reliably quantified in the abiotic matrices. If we were to extrapolate our detection percentages to the more conservative one of the two total PFAS counts mentioned

above, we would end up with an average of 3,102 PFAS in the abiotic environment. Assuming that legacy PFAS have higher detection frequencies (> 80%) compared to emerging ones, we also made this calculation only including compounds with detection frequencies < 80% and came up with 1,645 emerging PFAS in the abiotic environment, in addition to the 1,457 most commonly detected ones. These calculations are obviously speculative and even if these compounds would occur in the environment, most of them would probably be present below the quantification limits of the currently available analytical methods. These calculations do, however, help to contextualize our observation that the more you look for the more you find.

The compounds that were commonly detected or were present in relatively high concentrations differed between environmental compartments and organisms. In many cases, PFOS, which is a legacy PFAS that has been phased out since the year 2000, was still the most prominent one, highlighting that even if we would stop PFAS production now, we would still find PFAS in the environment for decades yet to come. In some organisms, (ultra)short-chained PFAS, like TFA and PFBA, were prevalent, while sulfonamide- and ether-based PFAS alternatives were also commonly detected. With legacy PFAS persisting and additional PFAS structures being detected, the complexity of the environmental PFAS blend is constantly increasing. Expanding the spectrum of targeted PFAS is therefore vital to assess the current environmental PFAS contamination and distribution, which can subsequently help to define further research priorities and regulatory actions.

To further enlarge the coverage of the PFAS chemical space, suspect and non-target screening approaches come into play, since they can reveal novel structures that would otherwise be missed by targeted analysis alone (Helmus et al., 2021; Sadia et al., 2025; Schüßler et al., 2025). Employing such analysis can be especially useful for samples where fluorine mass-balance approaches, like total organic fluorine (TOF) and extractable organic fluorine (EOF), reveal that much of the fluorine present cannot be explained by targeted approaches alone (Aro et al., 2022; Baqar et al., 2025). Suspect and non-target screening, however, produce huge datasets, which as

such merely state the obvious, that many more PFAS are present in the environment. To make the most out of these exploratory approaches, repeatedly detected features could be flagged and grouped into structural families. The next step would be to employ quantitative structure–activity relationship (QSAR) models to assess the persistence, mobility, bioaccumulation and toxic potential of these emerging PFAS. In addition, non-target screening could also be combined with effect-directed analysis, in order to identify which fraction of (unidentified) chemicals are responsible for the biological activity determined in bioassays (Hong et al., 2023; Jonkers et al., 2022). Utilizing these complementary emerging methodologies into an integrative chemical risk assessment can help to prioritize regulatory actions, as well as research on the development of new analytical standards, PFAS environmental distribution and ecotoxicity.

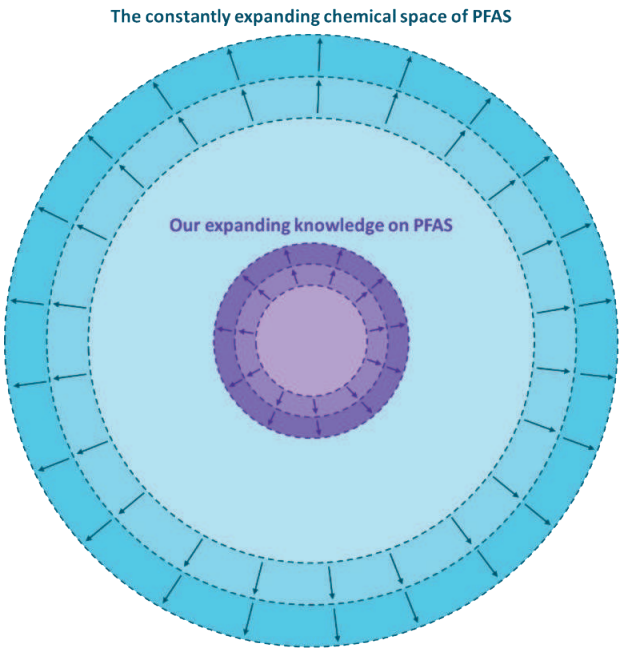


Figure 21: Schematic representation of our constantly expanding knowledge on PFAS (purple shades), within the broader constantly growing PFAS chemical space (blue shades). The ratio of the circles is not to scale.

Despite the potential of combining such methodologies in dealing with the chemodiversity of PFAS, it is important to realize that while our knowledge on PFAS keeps expanding, so does the PFAS chemical space, with ongoing research on alternative PFAS that will eventually be introduced on the market (Figure 21). For each (new) compound that is being manufactured and eventually may reach the environment, numerous additional transformation products are likely to be formed and new interaction pathways, for example with other pollutants (van Loon et al., 2025), are introduced, thereby adding new layers of complexity to an already poorly understood chemical landscape. In other words, the speed at which the PFAS chemical space expands outruns the capacity for a proper risk assessment and risk mitigation (Fenner & Scheringer, 2021). In order to catch up, it is vital to stop or at least limit the speed at which the outer circle is expanding (Figure 21). The importance of reducing the chemodiversity and the total chemical production volumes in order to mitigate the impact of chemicals on the environment has previously been highlighted (Fenner & Scheringer, 2021; Mueller et al., 2023; Persson et al., 2022), which obviously also applies to PFAS (Flerlage & Slootweg, 2023).

Although reducing the volume and identities of PFAS on the global market is easier said than done, there are ways to meet this challenge. The concept of essential use, although already launched in the 1970s in relation to ozone-depleting substances, has recently been revitalized as a useful tool towards the phase out of PFAS (Cousins et al., 2019a; 2021). According to the definition, the use of a chemical is deemed essential when it is "necessary for health, safety or is critical to the functioning of society" with "no available technically and economically feasible alternatives", while non-essential uses are primarily market driven (Cousins et al., 2019a; Ling, 2024). A recent survey among European citizens concluded that people are more likely to find common ground when it comes to defining non-essential uses, so maybe this should indeed be the starting point (Karinen et al., 2024). Next, for PFAS uses considered to be essential, a function-oriented, rather than a chemical-for-chemical substitution, in parallel with a safe and sustainable by design (SSbD) approach

should be adapted, which combines performance testing with toxicity and social sustainability metrics (Cousins et al., 2019a; Roy et al., 2022; Tickner et al., 2015; van Dijk et al., 2023). This way, the pitfall of regrettable substitutions can be largely avoided. According to a recent study using function-level information to identify replaceable PFAS uses, PFAS-free alternatives are available for 40 applications (Figuière et al., 2025). Several manufacturers have already recognized that using PFAS is not essential to their business models, although the situation is more complicated concerning fluoropolymer and semiconductor industries, where the technical requirements may be more demanding (Ateia & Scheringer, 2024; ESIA 2023). This highlights that the progress of PFAS phase-out inherently demands some trade-offs, especially in the first stages, as not all substitutes can be expected to meet the performance criteria reached by compounds that have been optimized over decades (Ateia & Scheringer, 2024; Bălan et al., 2023). Such temporary compromises, however, should not overshadow the urgency of the transition towards a PFAS free environment.

6.2 PFAS strongly bioaccumulate in a wide variety of organisms

According to the OECD, bioaccumulation is the net result of the competing rates of chemical uptake into, and elimination from an organism (OECD 2024). In other words, it describes the tendency of a chemical to bioconcentrate in an organism, in relation to its concentration in the environment. However, a bioaccumulation factor alone does not provide any information on the actual exposure of the organism, nor whether adverse effects can be expected. In the case of PFAS, their persistency amplifies the problem, because it translates into chronic, continuous and accumulating exposure over long time scales (Brunn et al., 2023; Cousins et al., 2020b). Persistency by itself is a major cause of concern, as it implies that the quantities of a chemical present in the environment will consistently rise, with increasing potential for known and unknown adverse effects (Cousins et al., 2019b; Ling, 2024). Combined with the strong, yet unconventional bioaccumulation patterns observed for many PFAS (**chapters 3** and **5**) (Evich et al., 2022; Lesmeister

et al., 2021; Munoz et al., 2020; Xu et al., 2022) this creates a unique environmental threat. PFAS are either poorly degradable or will eventually transform into stable analogues (Hurley et al., 2004; Liou et al., 2010; Vaalgamaa et al., 2011), while our knowledge on bioaccumulation is still limited to only a small fraction of all PFAS. Their case-specific and atypical bioaccumulation behavior (**chapter 3**) prevent reliable extrapolations for unstudied PFAS (subclasses). In addition to the diversity of PFAS chemical structures, the diversity of the environmental compartments and organisms affected by them adds another layer of complexity (Evich et al., 2022), not to mention the potential interactions between PFAS and other pollutants (Blanc et al., 2017; Boltes et al., 2012; Valle et al., 2025; van Loon et al., 2025; Wang et al., 2022b). This highlights an important knowledge gap, since most of these compounds will persist and accumulate in the environment for decades yet to come.

Providing missing bioaccumulation data for understudied PFAS and organisms was one of the objectives of the present thesis. This research gap was addressed in **chapters 3** and **5**, employing two distinct experimental approaches: a mesocosm laboratory study (**chapter 5**) and a field study (**chapter 3**). The mesocosm study used "naturally spiked" sediments from a heavily contaminated field site, thereby incorporating realistic contaminant mixtures and sediment properties, while limiting field complexity. This approach also simplified exposure pathways, since the sediment was initially the only PFAS source in the system, from which they then distributed into the overlaying water, macrophytes and worms. Concentrations in the different compartments at the end of the experimental period were used to determine bioaccumulation factors. From the field study, *in situ* bioaccumulation factors were directly calculated by dividing the PFAS concentration in the organism by the concentration in the abiotic compartment that it inhabits (soil, sediment or water). The contaminated sediment used for both studies was collected from the same site and so, despite the difference in the study design, this allowed us to compare the bioaccumulation factors for the PFAS detected in both studies. To this end, the two organisms that were more similar in terms of their ecological function and exposure, *Lumbriculus variegatus* (laboratory) and *Chironomus riparius* (field), were

selected for this comparison. Both organisms are sediment-dwelling detritivores, although their sediment bioturbation and bio-irrigation intensities are different (van der Meer et al., 2017). Despite further differences in their life cycle and physiology (Brooks, 1995; Seeley et al., 2021), their environmental exposure to PFAS is mainly through the sediment and pore water, which facilitates the comparison.

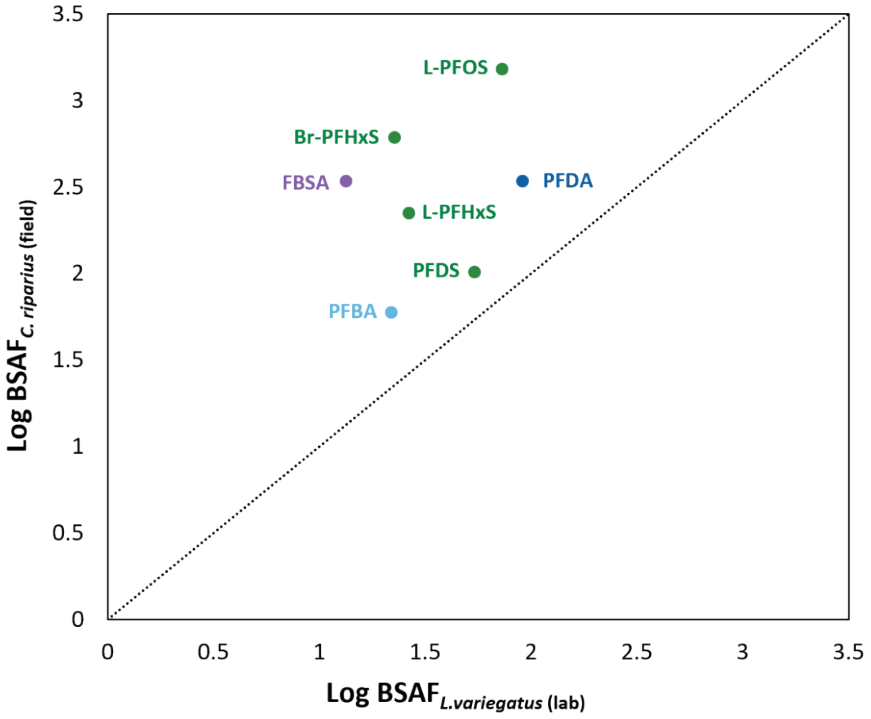


Figure 22: Comparison of logarithmically transformed biota-to-sediment accumulation factors (BSAFs) for PFAS between *Lumbriculus variegatus* (laboratory study) and *Chironomus riparius* (field study). For both organisms, BSAFs are expressed in kg sediment dw/kg organism dw. For the laboratory study, the comparison was done only for the bioaccumulation from the contaminated sediment. The names of the compounds corresponding to each data point are presented and the colours represent the subclass to which the compound belongs (green: long PFSAs; dark blue: long PFCAs; light blue: short PFCAs; purple: precursors). The dotted diagonal line indicates the 1:1 line.

Logarithmically transformed biota-to-sediment accumulation factors (BSAFs) could be compared for seven PFAS, including four long-chain PFSAs, one short-chain PFCA, one long-chain PFCA and one sulfonamide-based precursor (Figure 22). In both studies a very high bioaccumulation was found for some PFAS-organism combinations. The calculated BSAFs were higher in the field study than in the laboratory experiment, especially for most long-chain PFSAs and the short-chain sulfonamide-based precursor FBSA. There are several explanations for the higher BSAFs observed in the field study. Besides that, we compared different species, the *C. riparius* larvae were collected from the field where they had integrated PFAS exposure for a much longer period compared to *L. variegatus* that was obtained from a clean culture and then exposed in the laboratory experiment (Yun et al., 2023). Moreover, animals in the field are exposed to multiple contaminated environmental compartments as well as through their diet, while in our mesocosm study the sediment was initially the sole PFAS source in the system. Another important perquisite when studying bioaccumulation is that the system has reached steady state (Burkhard & Votava, 2023b; Kelly et al., 2024). This may not have been reached during the 28-day laboratory experiment, resulting in an underestimation of the PFAS bioaccumulation (Burkhard et al., 2012). Notwithstanding the differences between the experimental design of the studies reported in **chapters 3** and **5**, the high BSAFs found in many cases emphasize the high bioaccumulation potential of PFAS.

The comparison of results obtained from different published studies requires cautious interpretation of the findings. Notwithstanding the discrepancies among publicly available bioaccumulation data, the fact that some PFAS are highly bioaccumulative is widely accepted (Evich et al., 2025; Yun et al., 2023) and confirmed for understudied PFAS in the present thesis (**chapters 3** and **5**). Our findings highlight that apart from legacy compounds, like PFOS, that were already known to have a high bioaccumulation potential, other PFAS subclasses may exhibit comparable high bioaccumulation. The ultra-short PFCA TFA, a compound that has received much attention during recent years (Arp et al., 2024), contributed the

most to the PFAS load observed in predatory spiders and along with other short-chain PFAS showed evidence of high bioaccumulation in several organisms (**chapters 3** and **5**). This is quite remarkable and alarming, especially for TFA, since based on its small size and high polarity, a low bioaccumulation potential was expected (Hanson et al., 2024; Herzke et al., 2023). Despite their lower persistency compared to PFAAs, several precursors also exhibited high bioaccumulation in some organisms (**chapters 3** and **5**). It should be noted, however, that there was high variation in the bioaccumulation factors calculated for a single compound among different organisms, even among organisms sharing the same habitat and exposure routes. This underlines that the high PFAS bioaccumulation observed in many cases is not merely a compound-specific trait, but is driven by multiple environmental factors and species-specific characteristics, whose interplay and relative contribution are circumstantial and still poorly understood (Hu et al., 2024; Li et al., 2022).

6.3 Organisms transfer and redistribute PFAS

After documenting the strong bioaccumulation of PFAS in a wide variety of organisms (**chapters 3** and **5**), the subsequent objective was to quantify the food chain transfer of PFAS. To this end, we related PFAS concentrations in multiple taxa to their trophic position, which was calculated based on stable isotope analysis (**chapter 4**). Our results revealed the importance of dietary uptake for certain PFAS from different subclasses in some primary consumers and spiders and highlighted the differential trophic transfer of PFAS through the terrestrial and aquatic food webs to spiders that prey on both aquatic and terrestrial resources. Moreover, for some of the compounds that exhibited significant trophic transfer in our study, other studies reported a high bioaccumulation directly from the environment, emphasizing that both processes contribute to the PFAS concentrations in these organisms (Martin et al., 2003; Yun et al., 2023; Vogs et al., 2019). Our results indicated that environmental exposure seems to be more relevant than dietary uptake for short-chain PFAS, which is supported by (Xiong & Li, 2024). The very small, mobile and

polar TFA was, however, among the two compounds that exhibited significant trophic magnification along both the terrestrial and aquatic food chain, highlighting that the biomagnification potential of short chain compounds should not be overlooked (Du et al., 2021; Miranda et al., 2022). The presence of precursors can be another confounding factor that could result in amplifying the trophic magnification of some legacy compounds (PFOS), but also that of short-chain PFAS (PFBS, TFA) which can originate from the (bio)transformation of other PFAS (Miranda et al., 2022; Penland et al., 2020). Nonetheless, in **chapter 3** of this thesis and similar to most studies calculating *in situ* bioaccumulation factors (Burkhard & Votava, 2023a; Buytaert et al., 2025; Farrell et al., 2025), the reported values encompass the contribution of dietary uptake. Since the contribution of dietary exposure is hidden in the ratio of the concentration in the organism and the concentration in the inhabited abiotic matrix, this could to some extent also be responsible for the large variation observed between different studies. Even if we were able to explicitly determine the contribution of dietary versus environmental exposure, still variation would occur, since the characteristics of the food webs and the specific behavior of organisms would influence the trophic transfer and bioaccumulation of PFAS (**chapter 4**) (Miranda et al., 2022; Munoz et al., 2017; Zhang et al., 2022a).

In the general introduction (**chapter 1**) it was argued that organisms are not just passive receivers of pollution. Since they also actively shape their environment, organisms affect the environmental fate of chemicals (Gilroy et al., 2012; van der Meer et al., 2017). In **chapter 5**, the effect of the presence of a benthic invertebrate and its bioturbation activity on PFAS environmental distribution and bioaccumulation highlighted that contaminant-organism interactions are not unidirectional. For some PFAS, the bioaccumulation in the plants was higher in the presence of the bioturbator, but for other PFAS the opposite was observed. Moreover, the differences in the initial PFAS load between the two sediments (reference vs. contaminated) also played a role in the observed patterns. Combining these insights with the inherent complexity of the interactions between and within

food webs, implies that the arrow affecting the environmental distribution of PFAS has more than two heads. Organisms can shape their environment and adapt their behavior, thereby affecting the distribution of PFAS in ecosystems in multiple ways. In other words, PFAS chemical properties might set the baseline for their environmental fate, but ecological processes further modulate it.

The pronounced bioaccumulation of certain PFAS in specific primary producers and invertebrates raises the question whether these organisms could be used for bioremediation purposes. PFAS phytoremediation is an emerging research field with studies supplementary that phytoremediation offers a sustainable, low-cost *in situ* option (Itumoh et al., 2024; Kavusi et al., 2023). Yet, limitations of this approach include that the total PFAS mass removal is relatively low, with uptake kinetics getting slower over time and therefore, multiple harvesting cycles are required (Nason et al., 2024; Nassazzi et al., 2023). Plant uptake is also species-specific and highly compound-dependent (Nason et al., 2024), making this approach mainly suitable for specific contamination fingerprints. Moreover, the harvested PFAS containing biomass should be properly contained or treated to prevent PFAS from reentering the environment (Cornelissen et al., 2025; Nason et al., 2024). Although most of the challenges associated with PFAS phytoremediation also apply to bioremediation using invertebrates, research on these organisms is quite limited, despite their high PFAS bioaccumulation. Such bioremediation options could be effective for specific contamination scenarios and as part of integrated approaches employing more remediation techniques, however, further investigation on this matter is required, but was beyond the scope of the present thesis.

6.4 Towards a new equation to predict PFAS bioaccumulation

Aspiring to experimentally determine bioaccumulation metrics for the entire PFAS chemical space is not realistic and therefore ways to describe and possibly model these are needed. Traditionally, the octanol-water partition coefficient, K_{ow}, is used as a proxy for the lipophilicity and consequent bioaccumulation potential of neutral organic contaminants, but due to their unique properties this descriptor performs

poorly for PFAS (Hodges et al., 2019). Many PFAS are charged under the prevailing environmental conditions and their amphiphilic and surfactant like nature causes them to accumulate on interfaces and to form micelles at high concentrations, rendering results from commonly used K_{ow} measurements unreliable and irreproducible (ITRC 2023; Kim et al., 2015; Nendza et al., 2025). Experimental alternatives for such measurements, like HPLC retention time-based methods, are available, but are not regularly employed (ITRC 2023; Xiang et al., 2018). This results in K_{ow} estimates for most PFAS relying on predictive modeling, where outcomes heavily depend on model selection and parameterization, while PFAS also tend to fall outside the applicability domain of such predictive tools (Chelcea et al., 2020; Endo & Goss, 2014). Moreover, the weak and inconsistent relationships that have been observed between bioaccumulation and PFAS chain length (**chapter 3**) (Hopkins et al., 2023; Lesmeister et al., 2021; Wen et al., 2014), a property directly linked to lipophilicity and thus to K_{ow} (Jing et al., 2009; Xiang et al., 2018), further suggests that K_{ow} cannot adequately describe PFAS bioaccumulation. Hence, alternative molecular descriptors are required, that were explored in the present thesis (**chapter 3**) and re-evaluated below.

Two promising descriptors that could replace or complement K_{ow} in an attempt to better predict PFAS bioaccumulation are the membrane-water partition coefficient (K_{mw}) and the protein-water partition coefficient (K_{pw}). K_{mw} and K_{pw} are defined as the ratio between the concentration of the chemical in a membrane or protein and the concentration of the chemical dissolved in water, respectively. Systematic K_{mw} measurements have only been published recently (Chen et al., 2025; Droge, 2019), while for K_{pw} more data are available, however, mostly using mammalian albumin proteins (Alesio et al., 2022; Han et al., 2003; MacManus-Spencer et al., 2010). Despite having gained attention over recent years, the predictive power of these two metrics for PFAS bioaccumulation remains, to the best of our knowledge, unexplored for many organisms. In **chapter 3**, we investigated the relationships between PFAS bioaccumulation in various organisms and K_{mw} values and obtained significant correlations for primary producers in many cases. For terrestrial primary

producers, bioaccumulation seemed to decrease with increasing K_{mw} ($p < 0.05$; average $R^2 = 0.80$), while for pelagic organisms the opposite relationship was observed ($p < 0.05$; average $R^2 = 0.80$) (**chapter 3**). Here, we re-evaluated these relationships for a broader range of PFAS, using a more recently published K_{mw} database and also included K_{pw}. We further examined whether separating the results for the different PFAS subclasses would improve the observed relationships. Data were extracted from (Chen et al., 2025), who derived K_{mw} and equilibrium dissociation constants (K_D) as a proxy of K_{pw} from *in vitro* experiments for a broad range of PFAS. From their data we calculated the equilibrium association constants (K_A), which represent the binding affinity of the examined compounds to Human Serum Albumin (HSA), expressed as L_{HSA}/kg_{PFAS}. Potential relationships were examined by plotting the logarithmically transformed bioaccumulation factors against the logarithmically transformed K_{mw} and K_A. In line with **chapter 3**, relationships were examined separately for terrestrial, benthic and pelagic primary producers and animals (Figure 23).

This exercise showed that both K_{mw} and K_A were more effective in capturing the observed variation in PFAS bioaccumulation in primary producers than in animal species. Subclass separation improved the correlations for both primary producers and animals in some cases. In line with what was observed in **chapter 3**, BSAFs for terrestrial organisms were inversely correlated with both descriptors, which implies that for these organisms, uptake of PFAS dissolved in the (pore)water phase is more relevant. For pelagic organisms on the other hand, a higher K_{mw} or K_{pw} translated into higher bioaccumulation. It is concluded that this small exercise already resulted in better relationships between PFAS bioaccumulation and their molecular descriptors than when K_{ow} was used. This supports the potential of both K_{mw} and K_{pw} coefficients to predict PFAS bioaccumulation at least to some extent, in combination with additional descriptors related to for example PFAS intrinsic properties.

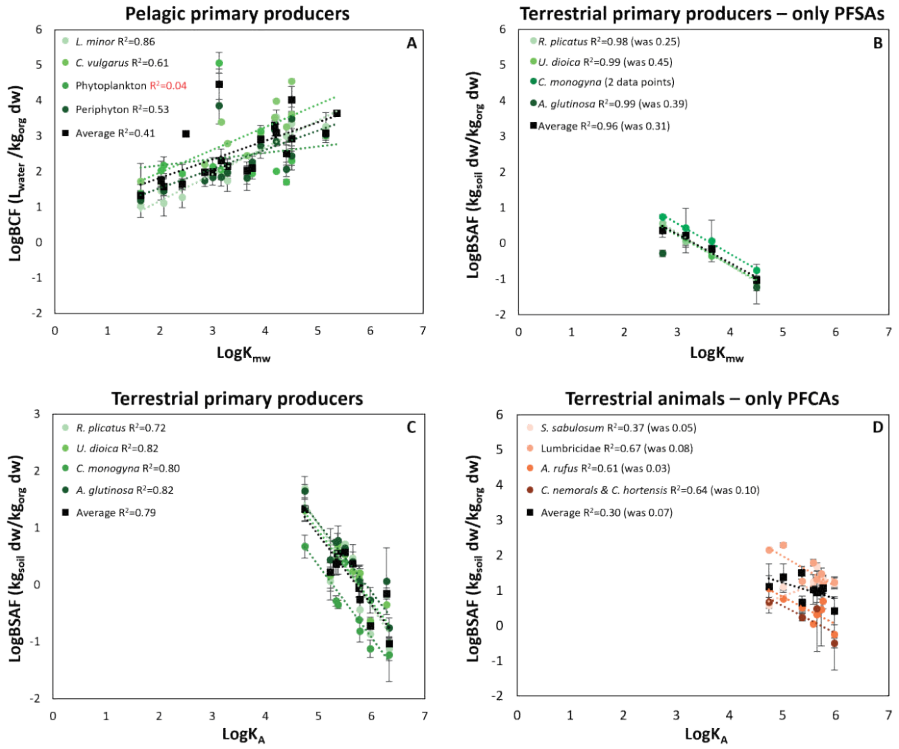


Figure 23: Examples of correlations between average bioaccumulation factors of PFAS and membrane-to-water (K_{mw}) and protein-to-water (K_A as proxy) partition coefficients. For the uptake of PFAS from water into pelagic primary producers (A) the logarithmically transformed bioconcentration factors (LogBCFs; L water/kg org dw) are plotted, while for the uptake of PFAS from soil into terrestrial organisms (B, C, D), the logarithmically transformed biota-to-soil bioaccumulation factors (LogBSAFs; kg soil dw/kg org dw) are plotted. The error bars represent the Standard Error of the Mean (SEM) of the BCFs and BSAFs per taxon at the five sampling sites from Lake Blokkersdijk (n = 1–5), while for the average the error bars represent the SEM of the BCFs or BSAFs of the taxa within each organism category. In panels B and D, the R^2 values of the regressions prior to the subclass separation are indicated in the parenthesis.

Concentration-dependent uptake is another factor that could affect and in some cases mask correlations between bioaccumulation and K_{mw} or K_{pw} (Chen et al., 2025). Furthermore, differences in the kinetics behind PFAS membrane and protein binding could also alter observed relationships between bioaccumulation and the examined coefficients. Membranes comprise a bigger, more continuous substrate,

where partitioning is primarily non-specific and mainly driven by the amphiphilicity, chain length and polar head chemistry of PFAS (Droge, 2019; Ebert et al., 2020; Fitzgerald et al., 2018).Competition effects can be expected at very high PFAS concentrations, leading to extended membrane surface coverage that could alter the membrane structure and indirectly affect the partitioning of other PFAS (Naumann et al., 2022; Nouhi et al., 2018). On the other hand, protein binding is limited to a finite number of (high affinity) binding sites, like albumin, fatty acid binding proteins and transport proteins, which can be saturated by various endogenous or exogenous ligands, making competition highly relevant (Maso et al., 2021; Moro et al., 2022; Zhao et al., 2023). This also aligns with the finding of (Chen et al., 2025) that at lower concentrations protein binding was more pronounced, but at higher concentrations both K_{mw} and K_{pw} seem to play an important role in PFAS uptake.

It should be noted that sorption to soil, sediment and suspended particles can affect PFAS bioavailability to terrestrial, benthic and pelagic organisms. The extent and direction of this effect relates to physicochemical properties of these systems, like organic matter content, clay content, presence of cations (Campos-Pereira et al., 2023; Yun et al., 2023), but also the variation in sorption affinities between the different PFAS subclasses (Evich et al., 2025; Yates et al., 2025). Our results did not allow us to specify the effect of sorption and environmental parameters on PFAS bioaccumulation. However, we acknowledge that these factors could have contributed to the variation observed in the bioaccumulation factors reported in this thesis, as well as in those reported in literature. This subject was, however, beyond the scope of the present thesis.

Besides abiotic drivers, the ecological characteristics of organisms and their species-specific traits bring in additional layers of complexity (Hedgespeth et al., 2023; Miranda et al., 2022). As shown here (**chapters 3** and **5**), but also in previous studies, PFAS concentrations can vary greatly among different organisms, so diet breadth and diet switching can result in different (lower or higher) contaminant loads between omnivores and more specialized predators (Hedgespeth et al., 2023;

Moorhouse-Gann et al., 2020). Apart from trophic links, specific organism traits can also affect bioaccumulation and thus biomagnification patterns. Feeding mode (e.g. detritivory, filter feeding, grazing) (Hedgespeth et al., 2023; Yun et al., 2023), as well as life cycle and life stages including molting (Bertin et al., 2014; Yun et al., 2023), play a major role in PFAS exposure, which will then define their transfer in the food web. Moreover, the mobility of an organism can further define its exposure. Sessile organisms (like vascular plants and bivalves) show limited to no movement, implying that they might be continuously exposed to rather constant PFAS concentrations. Mobile organisms on the other hand, may move in and out of contaminated hotspots, where the scale of movement depends on the size of the organisms and their dispersal capacity (Hedgespeth et al., 2023; Miranda et al., 2022). Adult insects emerging from aquatic ecosystems further contribute to the redistribution of resources including contaminants between food webs, thereby contributing to the exposure of terrestrial predators (**chapter 4**) (Collier et al., 2002; Koch, 2020; Koch et al., 2021).

Capturing the interplay between these factors would require an equation with multiple variables, each affecting bioaccumulation to a different extent and sometimes into different directions. Earlier attempts to build such predictive models have mostly focused on single groups of organisms. For example, models on PFAS uptake from soil to crops, taking into account factors like PFAS concentrations in the environment, PFAS molecular weight, chain length, plant concentration factors, organic matter (OM) content, plant transpiration rate, and soil-to-pore water partition coefficients (Felizeter et al., 2021; Gredelj et al., 2020b; Lasters et al., 2024; Liu et al., 2023; Wang et al., 2020b). Respective attempts for animals and especially invertebrates are scarcer, but the few available ones also tend to incorporate organism traits, like metabolism, elimination, distribution coefficients between tissues, lipid and protein content and diet. These ecological factors, in addition to the abiotic drivers discussed above, seem to greatly influence PFAS bioaccumulation, although they are not yet systematically included in current predictive models (Hedgespeth et al., 2023; Kelly et al., 2024; Sun et al., 2022; Yun

et al., 2023). Based on the progressive insight obtained from the present PhD project in combination with the expanded evaluation of the alternative descriptors reported above, we propose a general equation that would include a combination of physicochemical and intrinsic PFAS properties, environmental parameters and ecological drivers (Equation 9). In this equation, the different letters correspond to different constants (if the value would be fixed) or coefficients (if the value is expected to change depending on the examined system), $[PFAS]_{matrix1}$ and $[PFAS]_{matrix2}$ are the PFAS concentrations in relevant environmental matrices (e.g. pore water, soil, etc.,), %OM is the percentage of organic matter and $\%lipid_{org}$ and $\%prot_{org}$ correspond to the lipid and protein content of the organism, respectively. The equation presented here is just a conceptual example, which can be modified and extended depending on the application, although the relative contribution of each variable remains uncertain and will probably be case-specific depending on the examined system.

$$\begin{aligned}\textbf{Environmental fate metric} = {} & \pm \alpha * \textbf{chain length} \pm \beta * \textbf{charge distribution} \\ \text{(e.g. bioaccumulation)} \quad & \pm \gamma * \mathbf{K_{mw}} \pm \delta * [\mathbf{PFAS}]_{\mathbf{env}} \pm \varepsilon * \%OM \pm \zeta * \mathbf{\%lipid_{org}} \\ & \pm \eta * \mathbf{\%prot_{org}} \pm \ldots \end{aligned} \quad (9)$$

6.5 Perspectives for PFAS environmental risk assessment

The empirical multi-compartment and multi-organism dataset for a variety of PFAS generated in this thesis can be used to refine and strengthen the exposure pillar of PFAS environmental risk assessment. The reported PFAS concentrations in the various abiotic environmental compartments can be used as input for environmental exposure models. Used alongside concentrations in organisms inhabiting these compartments, these measurements can help to parameterize and validate such models, giving them a higher ecological relevance and credibility. Combining PFAS bioaccumulation factors and ecotoxicity effect concentrations with molecular descriptors, like K_{mw} and K_{pw}, can be used to train and validate existing QSAR models

and to improve the predictions of PFAS environmental distribution and ecotoxicity. Expanding the dataset of PFAS concentrations in the environment and corresponding bioaccumulation factors can also support read across for untested PFAS, although extrapolations should be done with caution, since a high variability in bioaccumulation is observed also between compounds that share structural and molecular features. Another aspect to take into account is the concentration dependent environmental partitioning and overall fate of PFAS documented in the present thesis, extending beyond traditional equilibrium-based kinetics and thereby complicating exposure predictions. Models like the conceptual equation presented here could be a first step towards integrating as many driving factors as possible, in an attempt to better predict PFAS bioaccumulation, which is a critical yet challenging component of PFAS environmental risk assessment (Ankley et al., 2021).

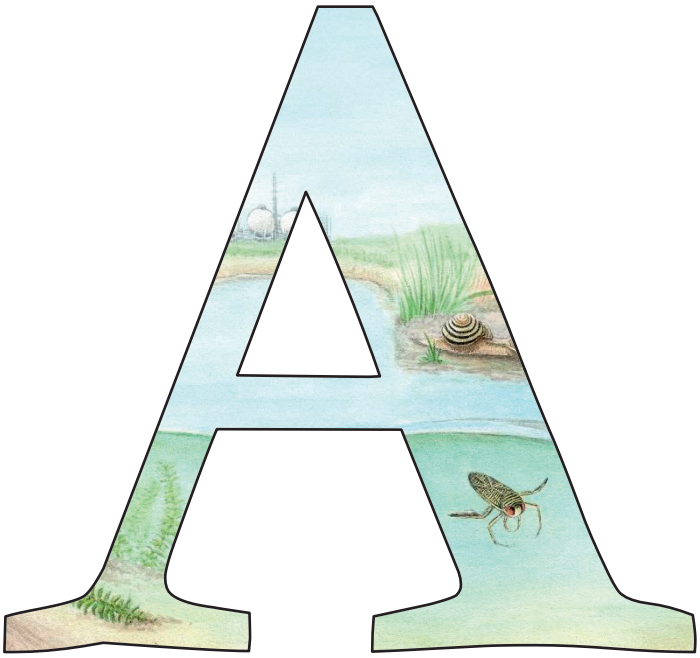


6.6 Conclusions

This thesis addressed some of the main research priorities related to the environmental distribution and bioaccumulation of PFAS. Starting by expanding the spectrum of targeted PFAS to include understudied structures, the widespread presence of many of these newly analyzed compounds across multiple abiotic and biotic compartments was confirmed. Various primary producers and invertebrates from terrestrial and aquatic ecosystems showed very high bioaccumulation factors for specific PFAS-organism combinations. To the best of our knowledge, the food-web transfer study presented here is the first one to combine various low trophic level organisms from a combined terrestrial and aquatic ecosystem, while targeting a broad spectrum of PFAS. Few studies have experimentally investigated how sediment reworking processes, like bioturbation, can affect PFAS bioaccumulation and environmental distribution, even less so for such a variety of PFAS structures. Our findings showed that the arrow affecting the environmental distribution of PFAS branches into several directions, since organisms can modify their environment and adjust their behavior, thereby affecting the distribution of PFAS in ecosystems in various ways. In order to capture this multidimensional nexus, we

proposed a conceptual equation, as a first step towards better describing and predicting the capricious environmental distribution of the persistent and pervasive PFAS.





Appendix

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Summary

Per- and polyfluoroalkyl substances (PFAS) comprise a large and diverse group of compounds with unique characteristics that have led to their extensive use and consequent release into the environment. Despite the constantly growing body of information on PFAS environmental occurrence and bioaccumulation, important knowledge gaps on these aspects still remain. The aim of this thesis was therefore to assess the environmental distribution, bioaccumulation and trophic transfer of PFAS in aquatic and terrestrial ecosystems. Employing a stepwise approach, objective 1 was to translate the general knowledge gaps concerning PFAS environmental risk assessment into specific, actionable research priorities to steer subsequent experimental work. This was achieved by consulting publicly available literature and databases and the resulting research priorities were shaped into the experimental objectives of this thesis:

2. To expand the spectrum of PFAS screened for in environmental and biotic matrices.

3. To expand the spectrum of organisms and taxa in which PFAS concentrations and bioaccumulation are measured.

4. To assess and compare the trophic transfer of a wide variety of PFAS in a terrestrial and an aquatic food web.

5. To investigate how the presence of benthic invertebrates and their bioturbation activity affect PFAS bioaccumulation.

6. To examine if PFAS molecular descriptors can describe their environmental distribution concerning a) bioaccumulation in primary producers and invertebrates, b) trophic transfer at the base of the food web, and c) redistribution in sediment-plant systems.

Chapter 2 synthesized the recent advancements in PFAS environmental research and identified the remaining knowledge gaps and the subsequent research priorities

to support a more comprehensive environmental risk assessment for PFAS. This was achieved by consulting the open literature and databases on the environmental occurrence, hazard, and risk of PFAS. Results revealed that the current knowledge on the environmental fate of PFAS is based on the analysis of less than 1% of the compounds categorized as PFAS. Moreover, soils and suspended particulate matter remain largely understudied. Bioavailability, bioaccumulation, and trophic transfer studies on PFAS also focus on only a very limited number of compounds and are biased toward aquatic biota, predominantly fish, and less frequently aquatic invertebrates and macrophytes. The available ecotoxicity data cover only a small number of PFAS and are also largely biased toward aquatic organisms. Therefore, ecotoxicity studies in the terrestrial environment, as well as chronic, multigenerational, and community ecotoxicity research are needed. Finally, there is an urgent need to unravel the relationships between sorption, bioaccumulation, and ecotoxicity on the one hand and molecular descriptors of PFAS chemical structures and physicochemical properties on the other, in order to allow predictions of exposure, bioaccumulation, and toxicity.



Addressing the research priority identified in chapter 2, chapter 3 investigated the distribution and bioaccumulation of various PFAS in a contaminated terrestrial and aquatic ecosystem. Subsequently, it was examined if the calculated bioaccumulation factors, relating concentrations in the organisms to concentrations in water, sediment or soil, were 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 found in all environmental compartments with varying profiles and concentrations. Generally, higher concentrations were found in aquatic than in terrestrial organisms, as well as in animals compared to plants. Biota-to-soil and biota-to-sediment accumulation factors (BSAFs) demonstrated a strong bioaccumulation of some PFAS, reaching 96,708 kg sediment/kg biota. Similarly, a high bioconcentration of PFAS 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 in some cases, but our limited understanding of the interplay between the various factors driving PFAS bioaccumulation calls for further mechanistic research. Nonetheless, it was concluded that many of the 44 analyzed PFAS strongly bioaccumulate in terrestrial and aquatic primary producers and animals, making these compounds of serious and long-term environmental concern.

Subsequently, chapter 4 aimed to unravel the transfer of PFAS through a terrestrial and aquatic food web, jointly ending up in predatory spiders as the top predators. To this end, predatory spiders were collected from the same PFAS-contaminated ecosystem, simultaneously with the organisms studied in chapter 3. The spiders were screened for 44 PFAS, combining the results with those of the primary producers and invertebrates from chapter 3. The trophic position of the organisms was investigated using stable isotopes. PFAS were present in organisms from all trophic levels (primary producers, herbivores, detritivores, predators), albeit with varying total concentrations and profiles. Several PFAS appeared to biomagnify in the spiders through the terrestrial food web but were biodiluted through the aquatic food web. Very high concentrations were observed for some PFAS-organism combinations, and on top of that, chapter 4 proved that some PFAS have the potential to biomagnify. The apparent arbitrariness of which substances biomagnify and which biodilute, in combination with the trophic transfer of TFA, the smallest and most polar PFAS, emphasized that we still lack insight into the drivers of PFAS distribution in the environment, their uptake and trophic transfer.

Chapter 5 examined how organisms can affect the environmental distribution of PFAS. To this end, we measured the concentrations of 40 PFAS in sediments, assessed their bioaccumulation in a rooting macrophyte (*Myriophyllum spicatum*) and a benthic invertebrate worm (*Lumbriculus variegatus*), and examined the effects of the presence and bioturbation activity of the invertebrate on PFAS bioaccumulation in the plants. The macrophytes were exposed to sediments originating from a reference and a PFAS-contaminated site. After 28 days, the worms were introduced into half of the replicates, and at the end of the experiment (56 days), PFAS were quantified

in all environmental compartments. Numerous targeted PFAS were detected in both the reference and the contaminated sediment and taken up by both organisms, with summed PFAS concentrations in organisms largely exceeding concentrations in the sediments. Bioaccumulation differed between organisms and sediment type. The presence of the worms significantly reduced the PFAS concentrations in the plant tissues, but for some compounds, root bioaccumulation increased in the presence of the worms. This effect was most prominent for the degradable PFAS precursors. It is concluded that organisms can shape their environment, thereby affecting the environmental distribution of PFAS, which emphasizes that contaminant-macroinvertebrate interactions are two-sided.

This thesis addressed some of the main research priorities related to the environmental distribution and bioaccumulation of PFAS. By expanding the spectrum of targeted PFAS to include understudied structures, the widespread presence of many of these newly analyzed compounds across multiple abiotic and biotic compartments was confirmed. The inclusion of various primary producers and invertebrates from terrestrial and aquatic ecosystems showed very high bioaccumulation factors for specific PFAS-organism combinations. To the best of our knowledge, the food-web transfer study presented here is the first one to combine various low trophic level organisms from a combined terrestrial and aquatic ecosystem, while targeting a broad spectrum of PFAS. Few studies have experimentally investigated how sediment reworking processes, like bioturbation, can affect PFAS bioaccumulation and environmental distribution, even less so for such a variety of PFAS structures. Our findings showed that the arrow affecting the environmental distribution of PFAS branches into several directions, since organisms can modify their environment and adjust their behavior, thereby affecting the distribution of PFAS in ecosystems in various ways. In order to capture this multidimensional nexus, we proposed a conceptual equation, as a first step towards better describing and predicting the capricious environmental distribution of the persistent and pervasive PFAS.

Samenvatting

Per- en polyfluoralkylstoffen (PFAS) zijn een grote en diverse groep van chemische stoffen met unieke eigenschappen, waardoor ze op allerlei manieren kunnen worden toegepast. Dit heeft geleid tot een wijdverbreid gebruik, maar ook tot een daaruit voortvloeiende belasting van het milieu. Ondanks dat er steeds meer informatie beschikbaar komt over het voorkomen van PFAS in het milieu en de opname van deze stoffen door organismen, zijn er nog steeds belangrijke kennisleemtes op dit gebied. Dit proefschrift had daarom tot doel om het voorkomen in het milieu, de opname door organismen en de doorgifte van PFAS in aquatische en terrestrische voedselketens vast te stellen. In een stapsgewijze benadering zijn eerst (doelstelling 1) algemeen geformuleerde kennisleemtes wat betreft de ecologische risicobeoordeling van PFAS vertaald naar meer specifieke en praktisch uitvoerbare onderzoeksdoelen om zo het daaropvolgende experimentele onderzoek beter te kunnen vormgeven. Hiertoe zijn de open literatuur en een aantal gegevensbestanden geraadpleegd. Dit resulteerde in de formulering van de meest prangende onderzoeksprioriteiten, die vervolgens zijn omgezet in de experimentele doelstellingen van dit proefschrift:

2. Het uitbreiden van het spectrum van PFAS die in milieumonsters en organismen worden gemeten.

3. Het uitbreiden van het spectrum van organismen en taxa waarin PFAS-concentraties en bioaccumulatie worden gemeten.

4. Het bepalen en vergelijken van de opname en doorgifte van een breed scala aan PFAS naar hogere trofische niveaus in de voedselketen van een terrestrisch en een aquatisch ecosysteem.

5. Het vaststellen van het effect van de aanwezigheid van sedimentbewonende ongewervelde dieren en hun activiteit op de bioaccumulatie van PFAS.

6. Te onderzoeken of parameters die de chemische structuur van PFAS beschrijven hun verdeling in het milieu kunnen voorspellen met betrekking tot a) bioaccumulatie

in planten en ongewervelde dieren, b) doorgifte door de basis van aquatische en terrestrische voedselketens, en c) herverdeling in een sediment-plantensysteem.

Hoofdstuk 2 geeft een overzicht van de recente ontwikkelingen in het milieuonderzoek aan PFAS. Dit hoofdstuk identificeerde de huidige kennishiaten en formuleerde prioriteiten voor verder onderzoek dat kan bijdragen aan een betere risicobeoordeling van deze stoffen in het milieu. Hiertoe is literatuur geraadpleegd, alsmede een aantal gegevensbestanden over het voorkomen van PFAS in het milieu, hun giftigheid en de risicobeoordeling hiervan. De resultaten lieten zien dat de huidige kennis over het gedrag van PFAS in het milieu gebaseerd is op de analyse van slechts minder dan 1% van de stoffen die als PFAS worden aangeduid. Ook blijkt dat het voorkomen van PFAS in bodems en zwevend stof nog nauwelijks is onderzocht. Studies naar de biobeschikbaarheid en bioaccumulatie van PFAS en hun doorgifte door voedselketens zijn gebaseerd op een beperkt aantal stoffen, en richten zich vooral op organismen in het oppervlaktewater, met name vissen, en veel minder op ongewervelde dieren en planten. De beschikbare ecotoxicologische gegevens betreffen de giftigheid van slechts een klein aantal PFAS en vooral voor waterorganismen. Er is daarom een grote behoefte aan ecotoxicologische studies in de bodem, en aan studies met langdurige blootstelling, over meerdere generaties, en naar effecten op levensgemeenschappen van organismen. Er is ook een grote behoefte aan het ontrafelen van de relaties tussen enerzijds de binding van PFAS aan bodem en sediment, hun bioaccumulatie, en hun giftigheid voor organismen in het milieu en anderzijds de moleculaire parameters die de chemische structuur en de fysisch-chemische eigenschappen van PFAS beschrijven. Dergelijke relaties kunnen helpen om de blootstelling, bioaccumulatie en giftigheid van PFAS in het milieu beter te voorspellen.

Uitgaande van de onderzoeksprioriteiten die zijn gedefinieerd in hoofdstuk 2, is in hoofdstuk 3 de verdeling van een breed scala aan PFAS over de milieucompartimenten bodem, water en sediment, en de bioaccumulatie in organismen in een vervuild terrestrisch en aquatisch ecosysteem onderzocht.

Vervolgens is onderzocht of de berekende bioaccumulatiefactoren zijn gerelateerd aan moleculaire parameters die de structuur en de eigenschappen van PFAS beschrijven. Milieumonsters en organismen zijn verzameld in de aquatische en terrestrische compartimenten van een PFAS verontreinigd ecosysteem en geanalyseerd op 44 stoffen. PFAS werden aangetroffen in alle milieucompartimenten, met verschillende profielen en in verschillende concentraties. De PFAS-concentraties waren over het algemeen hoger in waterorganismen dan in bodemorganismen, zowel voor planten als voor dieren. Biota-bodem en biota-sediment accumulatiefactoren (BSAFs) lieten een sterke bioaccumulatie zien voor sommige PFAS, tot waarden van 96,708 kg sediment/kg biota. In waterorganismen werd bovendien een hoge bioconcentratie van PFAS gemeten, met bioconcentratiefactoren (BCFs) tot 55,597 L water/kg biota. In sommige gevallen was de membraan-water verdelingscoëfficiënt (K_{mw}) redelijk in staat om de PFAS bioaccumulatie te verklaren. Maar onze beperkte kennis van de interactie tussen de verschillende factoren die de bioaccumulatie van PFAS beïnvloeden vraagt om meer mechanistisch onderzoek. Desalniettemin kan worden geconcludeerd dat veel van de 44 geanalyseerde PFAS sterk bioaccumuleren in terrestrische en aquatische planten en dieren. Daarom kunnen PFAS op de lange termijn een grote bedreiging vormen voor het milieu.

Hoofdstuk 4 had tot doel om de doorgifte van PFAS naar hogere trofische niveaus te bepalen in een terrestrische en aquatische voedselketen, die samenkomen in spinnen die prooien vangen die afkomstig zijn uit zowel het terrestrische als het aquatische ecosysteem. De spinnen werden verzameld in hetzelfde PFAS vervuilde ecosysteem dat ook al werd onderzocht in hoofdstuk 3, en werden net als de andere organismen geanalyseerd op 44 PFAS. De trofische positie in de voedselketen werd voor iedere soort bepaald met behulp van de analyse van stabiele isotopen. PFAS waren aanwezig in organismen van alle trofische niveaus (primaire producenten, herbivoren, detritivoren en predatoren), hoewel het profiel en de concentraties verschilden. Sommige PFAS vertoonden een afname door de aquatische voedselketen naar de spinnen, maar een sterke toename door de terrestrische

voedselketen (biomagnificatie). Hoofdstuk 4 liet dus zien dat sommige PFAS de potentie hebben tot biomagnificatie, maar ook dat in sommige organismen zeer hoge concentraties van specifieke PFAS werden gemeten. Er was echter geen patroon te zien in welke PFAS ophopen en welke verdunnen in de voedselketen. Bovendien vertoonde ook TFA, de kleinste en best wateroplosbare PFAS, een sterke doorgifte in de voedselketen, terwijl dit op basis van de fysisch-chemische eigenschappen niet werd verwacht. Dit betekent dat er nog steeds een groot gebrek aan kennis is van de factoren die de verdeling van PFAS over de verschillende milieucompartimenten en hun bioaccumulatie en doorgifte in voedselketens beïnvloeden.

In hoofdstuk 5 is onderzocht hoe organismen de verdeling van PFAS over de verschillende milieucompartimenten beïnvloeden. Hiertoe is een laboratoriumexperiment uitgevoerd waarin de concentraties van 40 PFAS zijn gemeten in het sediment en in de daaraan blootgestelde waterplanten (*Myriophyllum spicatum*) en sedimentbewonende wormen (*Lumbriculus variegatus*). Daarnaast werd bepaald of de aanwezigheid en het graafgedrag van de wormen effect hadden op de bioaccumulatie van PFAS in de planten. De planten werden blootgesteld aan sediment van een referentielocatie en van een met PFAS vervuilde locatie. Na 28 dagen werden wormen geïntroduceerd in de helft van de testsystemen. Het experiment werd beëindigd na 56 dagen, waarna de PFAS-concentraties werden gemeten in alle compartimenten van het laboratoriumexperiment. Veel van de gemeten PFAS werden aangetroffen in zowel het referentiesediment als het vervuilde sediment, en werden opgenomen in beide organismen. De PFAS-concentraties in de organismen waren veel hoger dan de concentraties in het sediment. De mate van bioaccumulatie verschilde tussen de twee organismen en het sediment type. De aanwezigheid van de wormen leidde tot significant lagere PFAS-concentraties in de planten, maar voor sommige stoffen nam de ophoping in de plantenwortels juist toe in de aanwezigheid van de wormen. Dit effect was het sterkst voor de afbreekbare voorlopers van bepaalde PFAS. Geconcludeerd werd dat organismen hun directe leefomgeving kunnen veranderen en daarmee de verdeling van PFAS over de verschillende milieucompartimenten kunnen beïnvloeden. Dit

benadrukt dat interacties tussen verontreinigende stoffen zoals PFAS en organismen niet éénzijdig maar tweezijdig zijn.

Dit proefschrift onderzocht enkele van de belangrijkste onderzoeksvragen met betrekking tot de verdeling van PFAS in het milieu en hun bioaccumulatie. Door het spectrum van de geanalyseerde PFAS uit te breiden met minder vaak onderzochte stofgroepen, werd de brede verspreiding van deze nieuw-geanalyseerde PFAS-verbindingen in verschillende abiotische en biologische milieucompartimenten bevestigd. Onderzoek aan diverse primaire producenten en ongewervelde dieren uit terrestrische en aquatische ecosystemen liet heel hoge bioaccumulatiefactoren zien voor specifieke PFAS-organisme combinaties. Voor zover wij weten is onze voedselwebstudie de eerste die zo'n breed scala aan PFAS heeft gemeten in zoveel verschillende organismen van de lagere trofische niveaus van een gecombineerd terrestrisch en aquatisch ecosysteem. Er is ook nog maar weinig onderzoek gedaan naar de invloed van het omwoelen van het sediment door organismen op de bioaccumulatie en de verdeling van PFAS in het milieu. Nog minder studies hebben dit gedaan voor zo'n groot scala aan PFAS als onderzocht in onze studie. De resultaten van dit proefschrift laten zien dat de pijl van factoren die de verdeling van PFAS in het milieu beschrijven zich vertakt in diverse richtingen. Dit komt doordat organismen hun eigen milieu kunnen beïnvloeden, en daarmee op verschillende manieren effect kunnen hebben op het transport van PFAS in ecosystemen. Dit proefschrift stelt daarom een wiskundige formule voor, waarmee de multidimensionale invloed van deze factoren op de grillige verdeling van deze zeer persistente en wijdverspreid voorkomende PFAS in het milieu beschreven kan worden.

Σύνοψη

Οι περ- και πολυφθοροαλκυλικές ουσίες (PFAS) αποτελούν μια εκτενή και ποικιλόμορφη ομάδα χημικών ενώσεων με μοναδικά χαρακηριστικά, τα οποία έχουν οδηγήσει στην εκτεταμένη χρήση τους και στη συνεπακόλουθη απελευθέρωσή τους στο περιβάλλον. Παρά τον συνεχώς αυξανόμενο όγκο πληροφοριών σχετικά με την παρουσία των PFAS στο περιβάλλον και τη βιοσυσσώρευσή τους, εξακολουθούν να υπάρχουν σημαντικά γνωσιακά κενά γύρω από αυτούς τους τομείς. Επομένως, ο κεντρικός σκοπός αυτής της διατριβής ήταν να αξιολογήσει την περιβαλλοντική κατανομή, τη βιοσυσσώρευση και την τροφική μεταφορά των PFAS σε υδάτινα και χερσαία οικοσυστήματα. Υιοθετώντας μια σταδιακή προσέγγιση, ο νούμερο 1. επιμέρους στόχος ήταν η αποτύπωση των γνωσιακών κενών που σχετίζονται με την περιβαλλοντική αξιολόγηση κινδύνου των PFAS σε σαφείς, ερευνητικούς στόχους, οι οποίοι θα κατευθύνουν τον μετέπειτα πειραματικό σχεδιασμό. Μέσω ανασκόπησης της διαθέσιμης βιβλιογραφίας και δημοσίων βάσεων δεδομένων, οι συναχθείσες ερευνητικές προτεραιότητες ενσωματώθηκαν στους επιμέρους πειραματικούς στόχους της παρούσας διατριβής:

2. Να διευρυνθεί το φάσμα των PFAS που περιλαμβάνονται στις αναλύσεις περιβαλλοντικών και βιοτικών δειγμάτων.

3. Να διευρυνθεί το φάσμα των οργανισμών και των ταξινομικών ομάδων για τις οποίες προσδιορίζονται οι συγκεντρώσεις και η βιοσυσσώρευση των PFAS.

4. Να αξιολογηθεί και να συγκριθεί η τροφική μεταφορά ενός ευρέος φάσματος PFAS σε χερσαία και υδάτινα τροφικά πλέγματα.

5. Να εξεταστεί πώς η παρουσία βενθικών ασπόνδυλων και η ανατάραξη των ιζημάτων από τη δραστηριότητά τους επηρεάζουν τη βιοσυσσώρεση των PFAS.

6. Να εξεταστεί αν οι μοριακοί δείκτες των PFAS μπορούν να περιγράψουν την περιβαλλοντική τους συμπεριφορά όσον αφορά α) τη βιοσυσσώρευση σε πρωτογενείς παραγωγούς και ασπόνδυλα β) την τροφική μεταφορά στη βάση των τροφικών

πλεγμάτων και γ) την ανακατανομή σε συστήματα που περιλαμβάνουν ίζηματα και φυτά.

Το κεφάλαιο 2 συνέθεσε τα πιο πρόσφατα επιστημονικά ευρήματα στην περιβαλλονική έρευνα των PFAS και προσδιόρισε τα υφιστάμενα κενά γνώσης, καθώς και τις επακόλουθες ερευνητικές προτεραιότητες προς μια πιο ολοκληρωμένη αξιολόγηση περιβαλλοντικού κινδύνου για αυτές τις ουσίες. Αυτό επιτεύχθηκε μέσω της αξιολόγησης διαθέσιμης βιβλιογραφίας και δημοσίων βάσεων δεδομένων σχετικά με την περιβαλλοντική παρουσία και τις επιπτώσεις των PFAS. Βάση των αποτελεσμάτων, η υπάρχουσα γνώση σχετικά με την περιβαλλοντική συμπεριφορά των PFAS βασίζεται σε δεδομένα που αφορούν λιγότερο από το 1 % του συνόλου που υπάγονται σε αυτή την ομάδα χημικών ενώσεων. Επιπλέον, δείγματα εδαφών και αιωρούμενης σωματιδιακής ύλης παραμένουν ανεπαρκώς μελετημένα. Μελέτες σχετικά με τη βιοδιαθεσιμότητα, τη βιοσυσσώρευση και την τροφική μεταφορά των PFAS συνήθως αφορούν περιορισμένο αριθμό ενώσεων και τείνουν να επικεντρώνονται σε υδρόβιους οργανισμούς, κυρίως ψάρια και σπανιότερα σε ασπόνδυλα ή μακροφύκη. Αντίστοιχα, τα διαθέσιμα δεδομένα οικοτοξικότητας καλύπτουν έναν πολύ μικρό ποσοστό των PFAS και είναι επίσης προσανατολισμένα κυρίως σε υδρόβιους οργανισμούς. Συνεπώς, κρίνεται αναγκαία η διεξαγωγή μελετών σε χερσαία οικοσυστήματα, μακροχρόνιες, πολυγενεακές οικοτοξικολογικές έρευνες, καθώς και μελέτες οικοτοξικότητας σε επίπεδο οικολογικών κοινοτήτων. Τέλος, κρίθηκε σκόπιμο να αποσαφηνιστούν οι σχέσεις μεταξύ προσρόφησης, βιοσυσσώρευσης και οικοτοξικότητας αφενός, και μοριακών δεικτών και φυσικοχημικών χαρακτηριστικών των PFAS αφετέρου, ώστε να καταστεί δυνατή η πρόβλεψη της βιοσυσσώρεσης και τοξικότητας αυτών των ουσιών.

Εστιάζοντας στην ερευνητική προτεραιότητα που επισημάνθηκε στο κεφάλαιο 2, στο κεφάλαιο 3 εξετάστηκε η κατανομή και η βιοσυσσώρεση διαφόρων PFAS σε ένα ρυπασμένο χερσαίο και υδάτινο οικοσύστημα. Εν συνεχεία, διερευνήθηκε η σχέση των υπολογισμένων συντελεστών βιοσυσσώρεσης, οι οποίοι συσχετίζουν τις συγκεντρώσεις οργανισμών με τις αντίστοιχες συγκεντρώσεις σε νερό, ίζημα ή έδαφος, με κάποιους μοριακούς δείκτες των PFAS. Αβιοτικά και βιοτικά δείγματα συλλέχθηκαν από τα

υδάτινα και χερσαία διαμερίσματα ενός οικοσυστήματος με αυξημένα επίπεδα PFAS, τα οποία υποβλήθηκαν σε χημικό έλεγχο για 44 ουσίες. Όλα τα δείγματα, περιείχαν PFAS με ποικίλα χημικά προφίλ και συγκεντρώσεις. Γενικά, υψηλότερες συγκεντρώσεις παρατηρήθηκαν στους υδρόβιους οργανισμούς, παρά στους χερσαίους και στα ασπόνδυλα ζώα σε σχέση με τα φυτά. Οι συντελεστές βιοσυσσώρευσης στους οργανισμούς σε σχέση με το έδαφος ή το ίζημα (BSAFs) έδειξαν έντονη βιοσυσσώρευση, με τιμές ως και 96,708 kg ίζημα/kg οργανισμού. Ομοίως, παρατηρήθηκε υψηλή βιοσυγκέντρωση των PFAS από το νερό στους οργανισμούς, με αντίστοιχους συντελεστές να φτάνουν τα 55,597 L νερού/kg οργανισμού. Οι συντελεστές κατανομής μεμβράνης–νερού μπορούν να αποδώσουν ως ένα βαθμό τη βιοσυσσώρευση των PFAS, ωστόσο η περιορισμένη κατανόησή μας όσον αφορά την αλληλεπίδραση των διαφόρων παραγόντων που επηρεάζουν τη βιοσυσσώρευση αυτών των ουσιών απαιτεί περαιτέρω έρευνα σε μηχανιστικό επίπεδο. Παρά τις γνωσιακές ελλείψεις, καταλήξαμε στο ότι πολλά από τα 44 PFAS που εξετάστηκαν παρουσιάζουν έντονη βιοσυσσώρεση σε χερσαίους και υδρόβιους πρωτογενείς παραγωγούς και ζώα, καθιστώντας αυτές τις ενώσεις ένα σοβαρό και μακροπρόθεσμο περιβαλλοντικό πρόβλημα.

Εν συνεχεία, το κεφάλαιο 4 επικεντρώθηκε στη διερεύνηση της μεταφοράς των PFAS διαμέσου ενός χερσαίου και υδάτινου τροφικού πλέγματος, το οποίο καταλήγει σε αράχνες ως κορυφαίους θηρευτές. Για τον σκοπό αυτό, από το ίδιο οικοσύστημα που εξετάστηκε στο κεφάλαιο 3 συλλέχθηκαν αράχνες, παράλληλα με τους υπόλοιπους οργανισμούς που μελετήθηκαν στο ίδιο κεφάλαιο. Οι αράχνες αναλύθηκαν για 44 PFAS και τα αποτελέσματα συνδυάστηκαν με εκείνα των πρωτογενών παραγωγών και ασπόνδυλων του κεφαλαίου 3. Η τροφική θέση των οργανισμών καθορίστηκε μέσω της ανάλυσης σταθερών ισοτόπων. PFAS ανιχνεύθηκαν σε οργανισμούς από όλα τα τροφικά επίπεδα (πρωτογενείς παραγωγοί, φυτοφάγοι, αποικοδομητές, θηρευτές) σε ποικίλες συγκεντρώσεις και με διαφορετικά χημικά προφίλ. Ορισμένα PFAS παρουσίασαν βιομεγέθυνση στις αράχνες μέσω του χερσαίου τροφικού πλέγματος, ενώ παρατηρήθηκε βιοδιάλυση μέσω του υδάτινου τροφικού πλέγματος. Εκτός από τις πολύ υψηλές συγκεντρώσεις που παρατηρήθηκαν για ορισμένους συνδυασμούς PFAS-

οργανισμών, το κεφάλαιο 4 απέδειξε ότι κάποια PFAS βιομεγενθύνονται κατά μήκος τροφικών πλεγμάτων. Η απροσδιόριστη κατανομή των PFAS που βιομεγενθύνονται ή βιοδιαλύονται, σε συνδυασμό με την τροφική μεταφορά του TFA, της μικρότερη και πιο πολικής ουσίας PFAS, υπογραμμίζει την περιορισμένη κατανόησή μας για τους παράγοντες που καθορίζουν την περιβαλλοντική κατανομή, πρόσληψη και τροφική μεταφορά αυτών των ενώσεων.

Στο κεφάλαιο 5 διερευνήθηκε ο ρόλος των οργανισμών στη διαμόρφωση της περιβαλλοντικής κατανομής των PFAS. Στο πλαίσιο αυτό, προσδιορίστηκαν οι συγκεντρώσεις 40 PFAS σε ιζήματα, η βιοσυσσώρευσή τους σε ένα ριζοφόρο μακρόφυτο (*Myriophyllum spicatum*) και έναν βενθικό σκώληκα (*Lumbriculus variegatus*) και αξιολογήθηκε η επίδραση της παρουσίας και της βιοδιαταρακτικής δραστηριότητας του ασπόνδυλου στη βιοσυσσώρευση των PFAS στο συγκεκριμένο φυτό. Τα μακρόφυτα εκτέθηκαν σε δυο διαφορετικά είδη ιζημάτων. Το πρώτο συλλέχθηκε από μια μη-ρυπασμένη περιοχή (περιοχή αναφοράς), ενώ το άλλο από μια περιοχή επιβαρυμένη με PFAS (ρυπασμένη περιοχή). Μετά από 28 ημέρες, οι σκώληκες προστέθηκαν στα μισά δείγματα και στο τέλος του πειράματος (56 ημέρες) προσδιορίστηκαν οι συγκεντρώσεις των PFAS σε όλες τις περιβαλλοντικές μήτρες του συστήματος. Πολλές από τις προεπιλεγμένες ενώσεις PFAS ανιχνεύθηκαν τόσο στα ιζήματα της ρυπασμένης περιοχής, όσο και στα ιζήματα της περιοχής αναφοράς και προσλήφθηκαν από αμφότερους τους οργανισμούς, με τις συνολικές συγκεντρώσεις στους οργανισμούς να υπερβαίνουν κατά πολύ τις αρχικές συγκεντρώσεις των ιζημάτων. Η βιοσυσσώρευση διέφερε μεταξύ των δυο οργανισμών και των δυο ιζημάτων. Η παρουσία των σκωλήκων οδήγησε σε σημαντική μείωση των συγκεντρώσεων PFAS στους φυτικούς ιστούς, ωστόσο για ορισμένες ενώσεις παρατηρήθηκε αυξημένη βιοσυσσώρευση στις ρίζες των φυτών, παρουσία των σκωλήκων. Το φαινόμενο αυτό ήταν εντονότερο για τους αποικοδομήσιμους PFAS precursors. Βάση αυτών των αποτελεσμάτων, συμπεραίνεται ότι οι οργανισμοί μπορούν να διαμορφώσουν το περιβάλλον τους, επηρεάζοντας κατ' αυτόν τον τρόπο την περιβαλλοντική μοίρα των PFAS, γεγονός που υπογραμμίζει ότι οι αλληλεπιδράσεις μεταξύ ρυπαντών και μακροασπόνδυλων είναι αμφίπλευρες.

Η παρούσα διατριβή ασχολήθηκε με ορισμένες από τις κύριες ερευνητικές προτεραιότητες που σχετίζονται με την περιβαλλοντική κατανομή και βιοσυσσώρευση των PFAS. Διευρύνοντας το φάσμα των PFAS που συνήθως αναλύονται σε περιβαλλοντικά δείγματα ώστε να συμπεριλάβει λιγότερο μελετημένες δομές, επιβεβαιώθηκε η παρουσία πολλών από αυτές τις νεοαναλυθείσες χημικές ενώσεις σε πολλαπλές αβιοτικές και βιοτικές μήτρες. Η μελέτη διαφόρων πρωτογενών παραγωγών και ασπόνδυλων από χερσαία και υδατικά οικοσυστήματα ανέδειξε πολύ υψηλούς συντελεστές βιοσυσσώρευσης για συγκεκριμένους συνδυασμούς PFAS–οργανισμών. Εξ όσων γνωρίζουμε, η μελέτη τροφικής μεταφοράς που παρουσιάζεται εδώ είναι η πρώτη που συνδυάζει μια ποικιλία οργανισμών χαμηλών τροφικών επιπέδων από ένα ενιαίο χερσαίο και υδατικό οικοσύστημα, εξετάζοντας ταυτόχρονα ένα ευρύ φάσμα PFAS. Λίγες μελέτες έχουν διερευνήσει πειραματικά πώς διεργασίες αναμόχλευσης των ιζημάτων, όπως η βιοδιαταρακτική δραστηριότητα ασπόνδυλων, επηρεάζουν τη βιοσυσσώρευση και περιβαλλοντική κατανομή των PFAS, ιδίως όσον αφορά μεγάλο εύρος χημικών δομών. Τα ευρήματα της παρούσας μελέτης υποδηλώνουν ότι η ροή επίδρασης των διεργασιών που επηρεάζουν την περιβαλλοντική κατανομή των PFAS διακλαδώνεται σε πολλαπλές κατευθύνσεις, καθώς οι οργανισμοί μπορούν να τροποποιούν το περιβάλλον και τη συμπεριφορά τους, επηρεάζοντας με πολλαπλούς τρόπους τη μεταφορά των PFAS στα οικοσυστήματα. Προκειμένου να αποτυπωθεί αυτή η πολυδιάστατη διασύνδεση προτάθηκε μια εννοιολογική εξίσωση ως πρώτο βήμα προς τη βελτιωμένη περιγραφή και πρόβλεψη της σύνθετης και ακανόνιστης περιβαλλοντικής κατανομής των επίμονων και πανταχού παρόντων PFAS.

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