Mutual impacts of wheat (*Triticum aestivum L.*) and earthworms (*Eisenia fetida*) on the bioavailability of perfluoroalkyl substances (PFASs) in soil

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**ABSTRACT**

Wheat and earthworms were exposed individually and together to soils contaminated with 11 perfluoroalkyl substances (PFASs). Wheat accumulated PFASs from soil with root concentration factors and bioconcentration factors that decreased as the number of perfluorinated carbons in the molecule increased. Earthworms accumulated PFASs from soil with biota-to-soil accumulation factors that increased with the number of carbons. Translocation factors (TF) of perfluorinated carboxylates (PFCAs) in wheat peaked at perfluorohexanoic acid and decreased significantly as the number of carbons increased or decreased. Perfluorohexane sulfonate produced the greatest TF of the three perfluorinated sulfonates (PFSAs) examined. Wheat increased the bioaccumulation of all 11 PFASs in earthworms and earthworms increased the bioaccumulation in wheat of PFCAs containing seven or less perfluorinated carbons, decreased bioaccumulation of PFCAs with more than seven carbons, and decreased bioaccumulation of PFSAs. In general, the co-presence of wheat and earthworms enhanced the bioavailability of PFASs in soil.

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1. Introduction

Perfluoroalkyl substances (PFASs) are widely used in various commercial products due to their hydrophobic and lipophobic properties and the strong C–F bond. PFASs are released into the environment during the production, application, transport and disposal of PFAS containing products or by degradation of their precursors, and they are ubiquitous in environmental matrices such as water, soil and sewage sludge (Buck et al., 2011; Paul et al., 2009). Many studies have demonstrated that PFASs can be bioaccumulated and biomagnified throughout the food chain due to their strong binding potential to proteins (Buck et al., 2011; D’eon and Mabury, 2011; Lasier et al., 2011; Liu et al., 2011; Loi et al., 2011). Lau et al. (2007) reported that PFASs could have adverse effects to wildlife and humans, such as developmental toxicity, immunotoxicity and hepatotoxicity. Land application of sewage sludge can introduce large amounts of PFASs to soils (Lindstrom et al., 2011; Sinclair and Kannan, 2006). Washington et al. (2010) found that the concentrations of PFASs in sludge-applied soils near Decatur, Alabama, USA were in the range of 16–986 ng g⁻¹ dry weight. Thus, PFASs in soil could be taken up by plants and terrestrial animals living in soil. The retention and mobility of PFASs in soil are the primary determinants of their environmental fate and behaviors including bioavailability (Yoo et al., 2011). Bioaccumulation of PFASs from soil may adversely affect individual organisms, food webs and human health (Prevedouros et al., 2006). There are a few studies which reported the bioaccumulation of PFASs from soil. According to our previous study (Zhao et al., 2013), PFASs can be effectively bioaccumulated in earthworms and the biota-to-soil accumulation factors (BSAFs) increased with perfluorinated carbon chain length. Several studies investigated plant uptake of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), which are the two most common PFASs in the environment, and demonstrated that they could be taken up by several crops from soil particularly in the vegetative compartments (Fischer et al., 2008; Lechner and Knapp, 2011; Stahl et al., 2008). Lechner and Knapp (2011) investigated the carryover of PFOA and PFOS from soil mixed with contaminated sewage sludge to potato (*Solanum tuberosum*), carrots (*Daucus carota ssp. Sativus*) and cucumbers (*Cucumis sativus*) and also found that they were mostly...
accumulated in the vegetative compartments. Yoo et al. (2011) found that the vegetation of PFASs in grass decreased from PFHpA to perfluoro-o-n-tetradecanoic acid (PFtDEA). However, little research has been conducted to investigate the uptake of other PFASs in plants and sparse information is available to understand the impacts of PFAS properties on the bioaccumulation potential in plants.

Earthworms are considered to be soil ecosystem engineer which may affect soil functions (Lavelle et al., 1997). They play a role in the incorporation, fragmentation, and decomposition of organic matter (OM) as well as in humus formation, soil porosity, and distribution and bioavailability of major nutrient elements such as nitrogen and potassium (Devliegher and Verstraete, 1997). Earthworms have often been shown to change the availability of inorganic and organic pollutants to plants. For instance, Wen et al. (2004) reported that earthworms (Eisenia fetida) increases the mobility and bioavailability of heavy metals to wheat (Triticum aestivum). The presence of earthworms led to an increase in the quantity of 2, 2-bis (p-chlorophenyl)-1, 1-dichloroethane (p.p-DDE) in zucchini (Cucurbita pepo) roots but a decrease in the roots of squash (Cucurbita maxima) (Kelsey and White, 2005; Kelsey et al., 2011). On the other hand, plants may influence the bioaccumulation of contaminants in earthworms as well. The total amount of p.p-DDE accumulated by earthworms decreased dramatically in the presence of plants (Kelsey and White, 2005). Earthworms and plants are important to soil characteristics and functions. It is vital to understand their individual and mutual impacts on the bioaccumulation of PFASs.

The present study measured the uptake of 11 PFASs from soil by plants and their translocation from plant root to plant shoot. Wheat (Triticum aestivum L.), an important monocotyledonous crop species, was selected as the plant species to be evaluated. Bioaccumulation of PFASs by earthworms (Eisenia fetida) exposed to the same soil treatments was also determined, and the effects of interaction between plants and earthworms on the efficiency of PFAS translocation in plants and bioaccumulation in plants and worms were investigated.

2. Materials and methods

2.1. Chemicals

The standard of perfluorobutane sulfonate (PFBS, 98%) was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Perfluorohexane sulfonate (PFHxS, 98%) was bought from Sigma–Aldrich (St. Louis, MO, USA). Perfluorooctane sulfonate (PFOS, 98%) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Japan). Perfluorooctanoic acid (PFPeA, 97%) and perfluorooctanoic acid (PFHxA, 96%) and perfluorododecanoic acid (PFDoA, 97%) were obtained from Geel, Belgium (New Jersey, USA). Perfluoroundecanoic acid (PFUnDA) was purchased from FluoroChem Ltd. (United Kingdom). Perfluorohexanoic acid (PFHxA, 98%), perfluoroctoanoic acid (PFCA, 95%) and perfluorooctanoic acid (PFNA, 97%) were purchased from Shanghai Adam Beta Reagent Co., Ltd. (China). Methanol of high-performance liquid chromatography (HPLC) grade was purchased from Tedia Chemical Company (Tianjin, China). Methyl tert-butyl ether (MTBE), tetraethyl ammonium hydrogen sulfate (TBASH), dichloromethane (DCM) and methanol for extraction, which was of pesticide grade, were purchased from Concord Science and Technology. Other chemicals were bought from Weida Chemical Commercial Ltd. (Tianjin, China). Milli-Q water was used throughout the study.

2.2. Soil properties and treatment

A loamy surface soil (0–10 cm) without detectable PFASs was collected from a farm about 20 km southwest of Tianjin China. The typical physicochemical properties of the soil were characterized as follows: pH 7.67; organic matter 4.11%; cation exchange capacity 38.47 cmol kg⁻¹, moisture content 1.03% for air-dried soil; clay 24%, silt 64%, and sand 12%. The soil was air-dried for 2 weeks, ground, and sifted through a 2-mm mesh and then received mineral nutrients at a rate of 100 mg P (KH₂PO₄), 300 mg N (NH₄NO₃), and 200 mg K (K₂SO₄) kg⁻¹ soil as basal fertilizers. A small portion of pre-weighed soil was spiked with 1 mL of a methanol solution containing 11 PFASs including eight perfluorinated carboxylates (PFCA: PFPeA, PFHxA, PFHpA, PFDA, PFNA, PFPeA, PFPD, PFDA) and three perfluorinated sulfonates (PFASs: PFBS, PFHxS, PFOS) and mixed thoroughly. The spiked soil was placed in a fume hood to allow the solvent to evaporate for 24 h. An aliquot of the untreated soil was added to the spiked soil and then mixed thoroughly. This step was repeated until all the pre-weighed soil was mixed. The soil was spiked with each PFAS compound at three levels: 200, 500 and 1000 ng g⁻¹ respectively. They were shaken by hand for 5 times (30 min/each time) each day, continuing for 6 days, and incubated in the dark for 14 d at room temperature (Huang et al., 2010).

2.3. Experimental design

The experiment was conducted in plastic pots (volume was 0.9 L) and were packed with 650 g of test soil. Three group tests were conducted: wheat (Triticum aestivum L.) and earthworms (Eisenia fetida) were cultured individually in two series of pots containing three levels of PFASs (namely Group I and II respectively), and they were co-cultured in another group of pots (Group III). Each group test was conducted in three replicates and the three test groups were accompanied with control experiments using non-spiked soil, in which the soil was clean without spiking PFASs. All the pots were covered with nylon net of 0.2 mm mesh to prevent the worms from escaping during exposures while plants and earthworms grew.

2.4. Earthworms

Mature earthworms (Eisenia fetida) were obtained from a local earthworm farming culturing farm, Tianjin, China. Earthworms were allowed to acclimatize to laboratory conditions for at least 14 days before use. The soil was brought to 30% moisture (by weight) prior to the addition of the earthworms. In each pot, 10 to 13 earthworms (weighting approximately 4 g (wet weight)) were added to the top of the soil where they proceeded to burrow. After 30 days, the worms were removed from the soil, washed with water and allowed to purge on moist filter paper for 24 h. The earthworms were pooled, washed with water, wiped with paper towel and weighed immediately. The worms were frozen at −20 °C for 24 h, freeze-dried for 48 h and weighed again. The dried earthworms were ground using a methanol-washed mortar and pestle in liquid nitrogen atmosphere. Homogenized samples were stored at −20 °C before chemical analysis.

2.5. Plants

Wheat seeds (Triticum aestivum L., obtained from the Chinese Academy of Agricultural Sciences, Beijing, China) were firstly surface sterilized in 3% H₂O₂, soaked in 2.8 mmol L⁻¹ Ca(NO₃)₂ for 4 h in darkness and germinated in a dish on moist filter paper at 22–27 °C. After 3 days of germination, uniform seedlings were selected and transferred to plastic pots (10 plants pot⁻¹). The seedlings were grown under plant growth chamber conditions for 14 h at 27 °C (day cycle) and for 10 h at 22 °C (night cycle). Plants were irrigated daily with water to maintain soil moisture at 30% by weight. The roots were positioned randomly and randomized every two days. Plants and earthworms were harvested after cultivation for 30 days. Roots and shoots were separated after harvest. They were washed thoroughly with water, wiped with filter paper and weighed immediately. Samples were weighed after freeze-drying for 48 h and weighed again. The dried root and shoot samples were homogenized by grinding in a methanol-washed mortar and pestle while adding ultrapure nitrogen. Homogenized samples were stored at −20 °C before chemical analysis.

2.6. Chemical extraction and analysis

Extraction of plant samples were subjected to change on the basis of the original method (Yoo et al., 2011). Homogenized shoot samples (0.5 g) or root samples (0.1 g) were added to a 50-mL polypropylene (PP) centrifuge tube containing 5 mL of DCM and extracted in an ultrasonic bath for 30 min. Methanol (5 mL) was added and the sample was shaken for 1 h in an oscillator (Shanghai Bocai Co., Ltd., China, TZB-03MZ). The extraction solvent was separated by centrifugation at 10,000 g for 30 min and transferred to a new 15 mL PP tube. This two-step extraction was repeated once and the extracts were allowed to evaporate to almost dryness under a gentle stream of N₂. The extracts were cleaned up using an ion-pairing extraction (IPM) method and similar to that described by Zhang et al. (2011). One mL of 0.5 M TBASH (adjusted to pH 10) and 2 mL of 0.25 M sodium carbonate were added to the PP tube and mixed, and then 5 mL of MTBE was added. After vortex, the mixture was shaken at 200 rpm for 40 min. The organic layer was separated from the aqueous layer by centrifugation at 3500 rpm for 5 min and then frozen at −20 °C for 2 h. The MTBE fraction was collected in a new PP tube. The extraction procedure was repeated with another 5 mL of MTBE and the organic layer was combined and evaporated to almost dryness under a gentle stream of N₂ and then reconstituted with 5.0 mL (wheat shoots) or 1 mL (wheat roots) of methanol respectively. For shoot samples, C14-labeled Pesticarb-SPE cartridge (500 mg/6 mL, Agela Technologies) was applied to remove pigment. The cartridge was conditioned with 5 mL of 0.1 M NH₄OH in methanol, 5 mL of water and 5 mL of methanol. Then 5 mL of extract was passed through the preconditioned cartridge at a rate of 1 drop per second, and was collected in a PP tube. The cartridge was eluted with 5 mL of methanol, which was also collected in the same tube. The collected solution was evaporated under a gentle stream of N₂ to dryness and reconstituted in 1 mL of methanol.
The extraction and cleanup of earthworms followed the procedure provided by Taniyasu et al. (2005) with modifications. Approximately 0.1 g of dried earthworm sample was extracted with 5 mL of 10 mM NaOH methanol solution (NaOH 0.01 mol L⁻¹ in methanol) in 50 mL PP tubes. The mixture was vortexed, shaken at 250 rpm for 12 h, and centrifuged at 3500 rpm for 15 min. The supernatant was transferred to a new 15 mL PP tube. This extraction process was repeated 3 times and the extracts were combined and then evaporated under a gentle stream of N₂ to 2 mL. The extract was diluted with 100 mL of water, which was adjusted to pH 4 with 2% aqueous acetic acid and purified using C18 PWAX (weak anion exchange) SPE cartridge (500 mg/6 mL, Agela Technologies).

The PWAX cartridges were preconditioned by passage of 10 mL of 0.1% NH₄OH in methanol, followed by 10 mL of methanol and 10 mL of water at 1 drop s⁻¹. The sample was then loaded on the cartridge, which was then washed with 10 mL of 2% aqueous acetic acid, followed by 10 mL of water. The target analytes were eluted with 10 mL of methanol and then eluted with 10 mL of 0.1% NH₄OH in methanol. The eluent was collected and combined and evaporated under a gentle stream of N₂ to dryness and reconstituted in 1 mL of methanol. Prior to liquid chromatography-mass spectrometry (LC-MS) analysis, the extract was centrifuged at 12,000 rpm for 30 min, 0.5 mL of the supernatant was transferred to an auto sampler vial and stored at −20 °C before analysis.

2.7. LC-MS analysis and quantitation

PFASs in the extracts were analyzed by an Agilent 1200 LC equipped with an Agilent 6310 ion trap mass spectrometer (MS) operated in negative electrospray ionization (ESI) mode. PFASs separation was performed using 2.5 mmol L⁻¹ ammonium acetate and methanol as mobile phase starting at 10% methanol at a flow rate of 0.25 mL min⁻¹. The gradient increased to 80% methanol at 250 rpm for 2 min, then increased to 100% methanol at 12.8 min before reversioning to original conditions at 17.8 min, and was then maintained until 19 min. 20 μL of the extract was automatically injected and the oven temperature of LC was 40 °C. The analytes were separated on an Agilent Eclipse Plus C18 (3.5 μm, 2.1 mm × 50 mm). Chromatograms were recorded using multiple reaction monitoring (MRM) mode, and when possible at least two transitions per analyte were monitored. Mass transitions used are given in Table S1 of the SI. The ion source working parameters were as follows: capillary voltage, 3500 V; capillary exit voltage, 200 V; nebulizer was 20.0 psi; dry gas temperature was 350 °C; dry gas flow rate of 0.25 mL min⁻¹; and scan range was from 78 to 628 m/z; compound stability was 100%; trap dive level 100% and optimize was normal.

2.8. Quality assurance and statistical analysis

The method detection limit (MDL) was determined with a signal-to-noise ratio of 3:1 based on the matrix samples which were collected in the control tests. The recoveries were determined by spiking a certain amount of PFAS standards in clean earthworms and wheat. The recoveries of PFASs were 74–100% for shoots and 73–111% for roots. The BSAFs in earthworms (Table S1). All PFASs were not detected in the control groups except for PFPPA in shoot. The MDLs of PFASs were in the range of 0.09–0.42 ng g⁻¹ dry weight (dw) for earthworms, 0.46–0.89 ng g⁻¹ dw for root and 0.02–0.89 ng g⁻¹ dw for shoot. Matrix calibration curves were prepared using spiked blank samples, which were extracted in the same way as the samples. Paired-Samples T Test (SPSS for Windows, version 20) was performed to test the differences between PFAS treatments and the controls. There was no obvious toxicity of added PFASs to the earthworms and wheat during the exposure period at all three exposure concentrations. The presence of earthworms in Group III did not affect the biomass of wheat significantly (p > 0.05).

2.9. Data analysis

Bioaccumulation factor is defined as biota-to-soil accumulation factor (BSAF). BSAF values of earthworms were calculated according to the following equation:

\[
\text{BSAF} = \frac{C_e}{C_s} \tag{1}
\]

Where \(C_e\) is the concentration of PFAS in the earthworms (ng g⁻¹ dry organism) and \(C_s\) is the organic carbon-normalized concentration of PFAS in the dry soil (ng g⁻¹ organic C).

Root concentration factor (RCF) was determined as follows:

\[
\text{RCF} = \frac{C_{\text{root}}}{C_e} \tag{2}
\]

where \(C_{\text{root}}\) is the concentration of PFAS in the root of wheat (ng g⁻¹ dry root).

Translocation factor (TF) is defined as the ratio of PFAS concentrations in shoots and in roots (Lin et al., 2006) and can be calculated by:

\[
\text{TF} = \frac{C_{\text{shoot}}}{C_{\text{root}}} \tag{3}
\]

Where \(C_{\text{shoot}}\) is the concentration of PFAS in the shoot of wheat (ng g⁻¹ dry shoot). Bioconcentration factor (BCF) of PFASs in wheat was determined as follows:

\[
\text{BCF} = \frac{C_{\text{wheat}}}{C_s} \tag{4}
\]

Where \(C_{\text{wheat}}\) is the concentration of PFASs in the whole wheat including root and shoot (ng g⁻¹ dry wheat) and \(C_s\) values are listed in Table S2.

3. Results and discussion

3.1. Plant and earthworms mortality and growth

In all test groups, both earthworms and wheat survived the experimental period and appeared in good health. The earthworms were not fed during the exposure period. The average body weight of the earthworms decreased 6–15% at the end of the experiments, but there was no distinct difference in the total body weight of earthworms between the test and control groups (p > 0.05). Similarly, the biomass of wheat was not significantly different between PFAS treatments and the controls. There was no obvious toxicity of added PFASs to the earthworms and wheat during the exposure period at all three exposure concentrations. The presence of earthworms in Group III did not affect the biomass of wheat significantly (p > 0.05).

3.2. Bioaccumulation of PFASs in earthworms and wheat

3.2.1. Earthworm bioaccumulation

Earthworms take up organic pollutants from soil mainly through pore water and ingestion of soil through the gut (Sijm et al., 2000). As can be seen in Table S3, PFPeA displays the lowest BSAF (0.015–0.133) while PFDoA shows the highest BSAF (3.28–5.19). The log BSAFs of PFASs in earthworms increased with the number of perfluorinated carbons at all three spiked levels (\(r^2 = 0.939, \quad p < 0.01\) for PFCAs, taking 200 ng g⁻¹ of group II as an example based on linear regression analysis) and BSAFs of PFASs was higher than those of PFCAs with the same perfluorinated carbon chain length (Fig. 1A). These results found in the present study were similar to our previous work (Zhao et al., 2013) and similar to those found for aquatic organisms such as green mussels (Liu et al., 2011), rainbow trout (Martin et al., 2003), common carp (Inoue et al., 2011), aquatic oligochaete (Lasier et al., 2011), and mammals (Kudo et al., 2001; Lasier et al., 2011). Different from aquatic organisms (Lasier et al., 2011; Martin et al., 2003), PFPeA, PFHxA and PFBS were also bioaccumulated in earthworms. This could be due to the active ingestion of soil through the gut and the high protein content of the earthworms.

The BSAFs of earthworms decreased with increasing soil concentrations, suggesting that the bioaccumulation of PFASs is concentration dependent. Similar result was observed in our previous study (Zhao et al., 2013), which might be caused by the constant binding sites in earthworm and resistant desorption of PFASs from organic matter in soil.

3.2.2. Root uptake of PFASs in wheat

Contaminants enter plants via three major pathways that include (Collins et al., 2006): root uptake and subsequent translocation into various plant parts through the transpiration process, vapor uptake from the surrounding atmosphere, and deposition of contaminated dusts on plant cuticles and subsequent contaminant diffusion through plant surfaces (Collins et al., 2006). With the exception of PFPeA, no PFASs were detected in wheat roots and shoots from the control treatment. PFPeA was detected only in control shoots with a mean concentration of 43.4 ng g⁻¹ (dry weight). This amounted to 0.8–1.9% of the total concentrations in the shoots of wheat growing in PFAS spiked soil. PFASs display very low vapor pressure and are not volatile (Hsu et al., 1990). Thus, it is...
assumed that there was no appreciable contribution from foliar uptake to concentrations in the shoots.

The concentrations of PFASs in roots from both Group I and III increased with their concentrations in soil (Table S2, \( r^2 = 0.743 - 0.987, p < 0.01 \)), indicating that wheat roots efficiently accumulate PFASs from soil. To describe the bioaccumulation of PFASs in wheat root, RCF was calculated for each PFAS using Eq (2). Root concentration factors suggest that within both the PFCAs and the PFSAs homologs with fewer perfluorinated carbons accumulate more efficiently in roots (Table S4). Fig. 1B illustrates the relationship between log RCFs of PFASs and their perfluorinated carbon chain length (\( r^2 = 0.990, p < 0.01 \), using group III as an example). The log RCFs of PFASs decreased significantly with the number of carbon chain length from PFPeA to PFDoA, which was contradictory to the accumulation pattern observed in earthworms. Yoo et al. (2011) also noted that the accumulation of PFASs in aquatic worms (Lumbriculus variegatus) increased with chain length (Lasier et al., 2011) but decreased in grass from PFHpA to perfluoro-n-tetradecanoic acid (PFTeDA). These could be accounted for by different accumulation mechanisms of PFASs in plant and animal. The accumulation of PFASs in earthworms is mainly through active ingestion of soil and absorption of contaminants through the gut (Weston et al., 2000). However, the uptake of PFASs in plant from soil is mainly a diffusive process of PFASs in interstitial water and then transport in the water phase of xylem or phloem sap driven by transpiration effect through the following processes: PFASs are desorbed to interstitial water and adsorbed on the root epidermis, and then penetrate through the epidermis and finally be transported to shoots by xylem. Thus, the bioaccumulated concentrations of PFASs in wheat roots are dependent on their concentrations in interstitial soil water, which is defined as \( C_{iw} \) (ng L\(^{-1}\)). According to equilibrium partition theory, the relationship between \( C_{iw} \) and the concentration of PFASs in soil (\( C_e \) organic carbon-normalized, ng g\(^{-1}\)) may be described using following Eq (5).

\[
C_{iw} = C_e / K_{oc}
\]  

where, \( K_{oc} \) is organic carbon-normalized distribution coefficient, which is commonly used to describe the distribution of a contaminant between soil and interstitial water (Collins et al., 2006). According to Eq (5), \( C_{iw} \) is negatively correlated with \( K_{oc} \). Ahrens et al. (2009) reported that PFASs with longer carbon chain length display higher partition potential to sediments/soil, and the short-chained PFCAs (perfluorinated carbons ≤ 7) were found exclusively in pore water while long-chained PFCAs (perfluorinated carbon ≥ 11) were found only in sediment. Zhao et al. (2012) estimated the \( K_{oc} \) of PFASs in sediments and found that log \( K_{oc} \) increases with their carbon chain length. As a result, the bio-accumulated concentrations of PFASs in wheat decrease with the carbon chain length. Fig. 2 illustrates that log RCF of PFASs is negatively correlated with their log \( K_{oc} \) value, suggesting that the uptake of PFASs by wheat is profoundly influenced by their partitioning in soil.

3.2.3. Accumulation of PFASs in shoots

The PFAS concentrations in wheat shoots were linearly correlated with the corresponding concentrations in roots (Figure S1, \( r^2 = 0.729 - 0.987, p < 0.05 \)), demonstrating that translocation from roots is the predominant pathway for PFAS accumulation in shoots. The translocation factor describes the efficiency of moving a compound from root to shoot and was calculated by Eq (3) (Lin et al., 2006). Translocation factors of PFCAs increased from PFPeA (0.393–0.580) to PFHxA (0.604–1.91), then decreased with increasing chain length from PFHxA to PFOA (0.078–0.122), and were quite constant from PFOA to PFDoA (0.057–0.176) (Figure S2,}
the PFASs and their lipophilicity (Collins et al., 2006). For contaminants with high water solubility and low log $K_{ow}$, they move easily from the root exterior to root interior where they are drawn to the shoot by the xylem and distributed in the plant depending on their lipophilicity (Collins et al., 2006). For contaminants with high water solubility and low log $K_{ow}$, they don’t partition to soil organic matter to any great extent, and easily move from outer root to inner root, are drawn to the shoot by the xylem and distributed in the plant depending on their lipophilicity. Hydrophobic organic contaminants with log $K_{ow}$ > 4 are strongly sorbed to the root epidermis and have difficulty being translocated from root to shoot (Collins et al., 2006). Fig. 3 depicts the relationship between TFs of the PFASs and their log $K_{ow}$. The TF increased significantly from PFPeA (log $K_{ow} = 2.82$, Table S6) to PFHxA (log $K_{ow} = 3.42$) then decreased exponentially with log $K_{ow}$. Lin et al. (2006) studied tea uptake and translocation of polycyclic aromatic hydrocarbons (PAHs) with log $K_{ow}$ in the range of 3.3–5.4. They also observed an exponential decrease of TF with log $K_{ow}$. The results suggest that translocation of PFASs in plants is mainly dependent on their water solubility and hydrophobicity.

### 3.2.4. Bioconcentration factors of PFASs in wheat

The PFASs are taken up by wheat roots and then translocated to shoots by xylem. The concentrations of PFASs in only roots or shoots may not reflect the bioaccumulation capacity of PFASs in wheat as a whole. In order to compare the difference in bioaccumulation of PFASs between earthworms and wheat, bioconcentration factors (BCFs) of PFASs in wheat were calculated using Eq (4) (Table S7). The log BCFs of PFASs in wheat decreased linearly with the perfluorinated carbon chain length (Fig. 1C, $r^2 = 0.935, p < 0.01$) suggesting that the uptake of PFASs from soil to wheat becomes difficult as the carbon chain increases. The reason for the lower bioconcentration of longer PFASs in wheat could be due to the large molecular size, which may limit their membrane penetration. Molecular weight is a substance property suitable for predicting chemical uptake by plants because both root uptake and translocation involve membrane penetration, a process which is related to molecule size (Calderón-Preciado et al., 2012; Topp et al., 1986). Since PFASs displayed opposite accumulation potentials in earthworms compared to wheat, the accumulation factors of PFASs in earthworms and wheat crossed at around PFHpA, which displayed similar log BSAFs in earthworms and log BCF in wheat (Fig. 1D). When the number of perfluorinated carbon is >7, the BSAFs of PFASs in earthworms were larger than their BCFs in plants, while the BSAFs in earthworms were lower than plant BCFs if the number of perfluorinated carbons was <7. As shown in Table S7, the BCFs of all PFASs decreased with increasing concentrations of PFASs in soil, implying that the bioaccumulation of PFASs in plant is also concentration dependent. Similar decreasing trends in bioaccumulation factors were observed for PAHs in plants (Gao and Ling, 2006; Gao and Zhu, 2004).

### 3.3. Mutual impacts of plant and earthworms on the bioaccumulation of PFASs

When earthworms and wheat are both present in soil, they may affect the accumulation of PFASs within each other by competition or by changing the bioavailability of the contaminants. The number of perfluorinated carbons dictated the different effects that earthworms had on the BCFs of PFASs in wheat (Fig. 4A, Table S7). The BCFs of short-chained PFCAs (≤7) increased while those of long-chained carboxylates (>7) decreased. Wheat BCF values of all three PFASs decreased significantly in the presence of earthworms as well. Kelsey and White (2005) reported that the bioconcentration of $p,p'$-DDE in zucchini was increased three fold when the plant was grown with earthworms. Earthworms burrow and actively ingest soil changing the physical, chemical, and biological properties of soil such as porosity, saturation capacity, and fertility via accelerated organic matter decomposition (Lavelle et al., 1997). Previous research has found that earthworms can promote the bioavailability of organic contaminants by interacting with soil microorganisms and increasing microbial diversities and activities (Hickman and Reid, 2008). In addition, the bioturbation of earthworms may stimulate bacterial communities, which can decompose organic matter releasing the organically-bound PFASs into the soil solution. As a result, earthworms may improve the bioavailability of PFASs to wheat which can explain the increase in the BCFs of short-chained PFCAs. According to Qi and Chen, for...
Group III. The stacked column represents (Group I + Group II) and the comparison of the summed concentrations of PFASs in earthworm and wheat between (Group I + Group II) and Group III (taking exposure concentration of 200 ng g\(^{-1}\) as an example) (C). In Fig. 4C, for each PFAS, the left stack column represents (Group I + Group II) and the right stack column represents Group III.

**Fig. 4.** The mutual impacts of earthworm and wheat on their bioaccumulation of PFASs from soil (A, B) and the comparison of the summed concentrations of PFASs in earthworm and wheat between (Group I + Group II) and Group III (taking exposure concentration of 200 ng g\(^{-1}\) as an example) (C). In Fig. 4C, for each PFAS, the left stack column represents (Group I + Group II) and the right stack column represents Group III.

readily desorbable contaminants, the primary biouptake route is through pore-water, while enhanced uptake from ingested soil particles becomes important for desorption-resistant contaminants (Qi and Chen, 2010). As discussed above, partition of PFASs to soil increases while desorption becomes difficult as the carbon chain length increases (Zhao et al., 2012), and earthworms display much higher bioaccumulation capacity to long-chained PFCAs than wheat. Thus, earthworms may compete with wheat for bioaccumulation of PFASs with longer carbon chain length, leading to lower BCFs of these long-chained PFASs in wheat in the presence of earthworms. On the other hand, the BSAFs of short-chained PFCAs (≤7) in earthworm were less than unity (log BSAF < 0; BSAF < 1), which indicates that their binding to earthworm is less favored than binding to soil natural organic matter (NOM). As a result, the concentrations of short-chained PFCAs (≤7) in earthworm excrement were higher than the soil NOM. This might cause the diffusion of short-chained PFCAs (≤7) from the worm excrement to soil solution. Hence, the concentrations of short-chained PFCAs (≤7) in the soil solution, which could be uptake by plants, were higher than longer-chained PFCAs. This also promoted the accumulation of short-chained PFCAs in plants.

The uptake of PFASs by earthworms was also affected by the presence of wheat. The BSAFs of all PFASs in earthworms increased dramatically due to the presence of wheat (Table S3 and Fig. 4B, \(p < 0.05\)) and the effects were more remarkable for short-chained PFASs than longer ones. A previous study reported that the biconcentration of \(p,p’\)-DDE by earthworms was increased by more than 2-fold when pumpkin was present (Kelsey et al., 2011). Wheat may also significantly change the physicochemical and biological properties of soil (Pérez-Bejarano et al., 2008). The rhizosphere soil is a unique microenvironment because its properties differ from those of the bulk soil as plant roots exude organic compounds. Root exudation includes the secretion of ions, free oxygen and water, enzymes, and a diverse array of carbon-containing primary and secondary metabolites (Pinton et al., 2007; Zhu et al., 2009). Thus, the adsorption-desorption behaviors of contaminants may be different in the rhizosphere soil with respect to the bulk soil. Studies have demonstrated that root exudates increase the desorption of dichlorodiphenyltrichloroethane (DDT) (Luo et al., 2006) and \(p,p’\)-DDE (White et al., 2003) from soils. The enhanced desorption from rhizosphere soil would increase the bioavailability of PFASs to earthworms. This may explain the effects of wheat on the BSAFs of short-chained PFASs in earthworms. They tend to be weakly sorbed to soil and are much easier to desorb as a result of the root-zone chemistry.

To illustrate the general effects of wheat and earthworms on the bioavailability of PFASs in soil, the summed concentrations of PFASs in earthworms and wheat (ng g\(^{-1}\) dw, \(C_{bio} = C_e + C_{wheat}\)) were compared between Group I + Group II and Group III (Fig. 4C). The \(C_{bio}\) of each PFAS in the treatment with earthworms and wheat grown together (i.e. Group III) was greater than those from treatments where they were grown separately. This suggests that the co-presence of earthworms and wheat may increase the total bioavailability of PFASs in soil.

### 4. Conclusions

PFASs can be bioaccumulated by the roots of wheat from soil and translocated to shoots. The RCF and BCF of PFASs in wheat decreased, while the BSAF in earthworms increased with the perfluorinated carbon chain length. The translocation factors of PFASs in wheat increased from PFPeA to PFHxA, then decreased exponentially with increasing carbon chain length possibly due to the difficulty that larger molecular homologues have in crossing plant membranes. The co-presence of wheat and earthworms mutually
affect their bioaccumulation of PFASs by enhancing the bioavailability of PFASs in soil. These results suggest that PFASs in soil can be bioaccumulated in both earthworms and plants and transferred to species consuming them and pose a risk to human health. The findings are important for predicting the environmental fate and assessing the environmental risk of PFASs in terrestrial ecosystem.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.09.032.

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