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Prediction of perfluoroalkyl acids (PFAAs) in homegrown eggs: Insights into abiotic and biotic factors affecting bioavailability and derivation of potential remediation measures

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ABSTRACT

Homegrown eggs from free-ranging laying hens often contain elevated concentrations of perfluoroalkyl acids (PFAAs). However, it is unclear which factors contribute to these relatively large exposure risk scenarios. Moreover, existing bioavailability and modeling concepts of conventional organic pollutants cannot be generalized to PFAAs due to their different physicochemical soil interactions. Therefore, there is an urgent need for empirical models, based on real-world data, to provide insights into how (a)biotic factors affect the bioavailability to eggs. To this end, 17 targeted analytes were analyzed in abiotic (i.e. rainwater, soil; both $N = 101$) matrices and homegrown eggs ($N = 101$), which were sampled in 101 private gardens across Flanders (Belgium) in 2019, 2021 and 2022. Various soil characteristics were measured to evaluate their role in affecting PFAA bioavailability to the eggs. Finally, PFAAs were measured in potential feed sources (i.e. homegrown vegetable and earthworm pools; respectively $N = 49$ and $N = 34$) of the laying hens to evaluate their contribution to the egg burden. Modeling suggested that soil was a major exposure source to laying hens, accounting for 16–55% of the total variation in egg concentrations for dominant PFAAs. Moreover, concentrations in vegetables and earthworms for PFBA and PFOS, respectively, were significantly positively related with corresponding egg concentrations. Predictive models based on soil concentrations, total organic carbon (TOC), pH, clay content and exchangeable cations were successfully developed for major PFAAs, providing possibilities for time- and cost-effective risk assessment of PFAAs in homegrown eggs. Among other soil characteristics, TOC and clay content were related with lower and higher egg concentrations for most PFAAs, respectively. This suggests that bioavailability of PFAAs to the eggs is driven by complex physicochemical interactions of PFAAs with TOC and clay. Finally, remediation measures were formulated that are readily applicable to lower PFAA exposure via homegrown eggs.

1. Introduction

Production of self-cultivated food in private gardens has become increasingly popular over recent years (Illieva et al., 2022). Especially, housing of free-ranging laying hens for the production of homegrown eggs has gained worldwide popularity due to its intrinsic economic, nutritional and ecological benefits for humans (Padhi, 2016). However, the presence of organic contaminants in private gardens can pose a significant risk to human health as these can easily enter the food-chain through their bioaccumulative properties, which is also the case for per- and polyfluoroalkylated substances (PFAAs).

Compared to the majority of organic pollutants, PFAAs are exceptional in terms of physicochemical properties. These organofluorine compounds have fully fluorinated alkyl chains characterized by strong

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hydrophobic C-F bonds and a lipophobic ionizable acid group, making them very relevant for a wide range of industrial and commercial applications (Buck et al., 2011; Glüge et al., 2020). However, these properties also result in a very large persistence to degradation combined with a relatively large environmental mobility, varying with the alkyl chain length and type of acid group (Buck et al., 2011). Additionally, their proteinophilic nature leads to a large affinity with protein-rich tissues, including eggs (Wang et al., 2019), while food has generally been identified as the major human exposure source of PFAAs (Roth et al., 2020).

Biomonitoring studies have consistently linked PFAA intake via homegrown egg consumption with elevated human serum PFAA concentrations (Colles et al., 2020) and potential health risks (Lasters et al., 2022; Wang et al., 2019), the latter even in rural areas under very modest egg consumption scenarios (Lasters et al., 2022). Over the last decade, epidemiological studies have increasingly associated human exposure to specific PFAAs, mostly perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), with various adverse health outcomes (Fenton et al., 2021; Shearer et al., 2021). Although there is still ongoing scientific debate about the degree of (mixture) toxicity for many PFAAs to humans at environmentally relevant concentrations (Ducatman al., 2022), elevated exposure to PFOS and PFOA has consistently been linked with increased cholesterol levels, immune suppression (e.g. decreased vaccination response), thyroid disease and cancer (liver, kidney and testicular cancer) (Fenton et al., 2021; Grandjean et al., 2020; Shearer et al., 2021).

In order to decrease PFAA bioaccumulation in homegrown eggs and reduce potential health risks to humans, it is essential to understand how abiotic and biotic factors may affect the bioavailability of these compounds to laying hens and ultimately humans. However, very little knowledge exists on the bioavailability of PFAAs to food from animal origin and the majority of studies is largely limited to plant crop species and performed under experimental conditions, as recently reviewed by Adu et al. (2023). These studies have identified that the soil forms the main sink of PFAAs and that soil physicochemical properties play a decisive role in the bioavailability to terrestrial organisms. In a field experiment on a crop species (common bean, *Phaseolus vulgaris* Linnaeus), Knight et al. (2021) have shown that the bioavailable soil PFAA fraction to plants is largely influenced by the physicochemical properties of both the soil (organic matter, pH, clay content, soil electrical conductivity and cation exchange capacity (CEC)) and the PFAA properties (chain length and functional group). It is likely that these physicochemical properties also play a crucial role in the bioavailability of PFAAs to free-ranging laying hens.

Free-ranging laying hens are geophageous animals that can be directly exposed to pollutants through ingestion of contaminated soil particles (Kijlstra, 2004), which can make up to 40% of their diet (Jurjanz et al., 2015). Homegrown eggs from free-ranging laying hens have been shown to contain elevated PFAA concentrations compared to commercial eggs (Zafeiraki et al., 2016) and eggs from hens housed primarily in indoor conditions (Gazzotti et al., 2021; Mikolajczyk et al., 2022). Grazing of laying hens in outdoor conditions can result in significantly increased soil levels of organic matter, electrical conductivity and CEC (Soares et al., 2022). These soil characteristics can differently affect the bioavailability of PFAAs in the soil, depending on their type of binding interaction with the soil fractions (e.g., clay and organic matter) (Cai et al., 2022). For these reasons, homegrown eggs are an ideal study matrix for examining the role of PFAA bioavailability in the soil on the accumulation in the eggs.

PFAAs dominantly adsorb onto the organic matter and clay fraction via relatively strong hydrophobic and weak electrostatic interactions, respectively (Cai et al., 2022; Li et al., 2018). Therefore, it can be expected that soil organic matter content and clay content would decrease and increase the bioavailability of PFAAs in the soil to the eggs. Moreover, soil properties can affect the binding interaction type (hydrophobic vs electrostatic) of PFAAs and, hence, also the bioavailability of PFAAs in the soil to the eggs. Higher pH and CEC levels may be associated with increased bioavailability by increasing deprotonation of pH-dependent surface charges on the clay matrix fraction. Consequently, relatively weak electrostatic interactions between PFAAs and the clay matrix may increase through bridging of PFAAs with CEC fractions (exchangeable mineral and metal cations) (Cai et al., 2022; 2023), which may result in a higher fraction of PFAAs sorbed onto the clay. As soon as soil particles are ingested by the laying-hen, the low pH values in their glandular stomach (ranging from 3 to 4) (Waegeneers et al., 2009) should theoretically result in large protonation of the clay surface charges, which can result in increased absorption and thus larger bioavailability to the eggs.

Furthermore, biotic components of the terrestrial ecosystem within the private gardens, which may serve as feed items to the laying hens, such as invertebrates (e.g. earthworms) and crop food leftovers, are hypothesized to result in higher egg PFAA burdens. Likewise, rain water, which can be provided as drinking water to the laying hens, may also be related with higher egg PFAA concentrations. However, to the best of our knowledge, no studies have been performed to date that have evaluated the relationships between any of these (a)biotic factors and the bioaccumulation of PFAS in homegrown eggs.

From various perspectives, it is of the utmost importance to characterize these possible relationships and to predict homegrown egg PFAA concentrations, based on these multiple factors. Firstly, preventive what-if risk scenarios can potentially be modeled that may estimate the human exposure risk when free-ranging laying hens would be introduced. Secondly, identification of soil physicochemical properties that potentially affect the bioavailability of PFAAs to the laying hens may enable the opportunity to manipulate these soil physicochemical properties to ultimately lower human exposure. From a fundamental toxicological point of view, existing concepts of processes that affect the bioavailability for conventional organic pollutants cannot be generalized to PFAAs, due to their complex and very different physicochemical interactions with soil matrices (Sigmund et al., 2022). Therefore, there is also an urgent need for empirical models under real-world field conditions that can provide invaluable fundamental knowledge about the interaction of PFAAs with major environmental media, such as soil.

The main objective of this study was to develop and evaluate predictive empirical models for environmentally relevant PFAA concentrations in homegrown eggs, taking into account the potential influence of corresponding soil concentrations, rain water concentrations and multiple soil physicochemical properties (total organic carbon (TOC), clay content, pH, CEC and soil electrical conductivity). Secondly, an explanatory analysis was conducted to gain mechanistic insights into potential associations between these abiotic variables and the egg PFAA concentrations. Finally, relationships between the feed items of the free-ranging laying hens (i.e. pools of self-cultivated vegetables and earthworms) and the egg concentrations were tested to assess their role in the possible transfer of PFAAs to the eggs.

2. Materials and methods

2.1. Volunteer recruitment

Eligible volunteers that met the major study criterium (i.e. private garden with at least two free-ranging laying hens of $\geq$ six months old) were selected throughout Flanders (Belgium) via existing social networks, such as call ups in community groups of Facebook and existing informal contacts. All the personal data were treated confidentially in accordance with the latest privacy regulations (General Data Protection Regulation, GDPR). The privacy policy department of the University of Antwerp approved the data management plan. Each volunteer provided explicit approval for the processing of their data within the context of the research objectives in this study by means of an informed consent. The personal results were communicated to each volunteer via a short report containing background information on PFAAs, a consumption advice based on their individual results and general strategies that may lower overall PFAA exposure.

2.2. Sample collection

Paired environmental and biota samples were collected from 101 private gardens during the summer period of 2019 ($N = 33$), 2021 ($N = 58$) and 2022 ($N = 10$) across Flanders. These samples were collected at various distances within a radius of 25 km from a major fluorochemical plant in Antwerp (Belgium), based on the previously reported spatial distribution of PFAA concentrations in homegrown eggs (Lasters et al., 2022). In this way, a geographically diverse dataset could be obtained with a large contrast in the variables of interest, which was essential for the later data analysis of the predictive model. At all private gardens, a representative composite sample of the top soil layer (three subsamples in polypropylene (PP) tubes from 0 to 5 cm depth) in the chicken enclosure, rain water (50 mL in PP tube), and homegrown eggs (two independent egg samples) were collected. Additionally, free-living earthworms (*Lumbricus terrestris* Linnaeus), i.e. two separate pools of, respectively, three adult (=with clitellum) and three juvenile (=without clitellum) individuals in PP tubes and homegrown vegetables (pool of minimally two crop species in PP containers) were sampled in, respectively, 49 and 34 of the private gardens. For each monitoring campaign, the same standardized sample collection protocol was used for each matrix (detailed in SI: section 1) to minimize sampling bias across the monitoring periods.

Sample matrices were selected so as to explain a maximum amount of variation in egg PFAA concentrations, both at the compound and concentration level. Rain water and soil were selected as both are two major environmental media which can contain a wide variety of PFAA compounds (Liu et al., 2015; Pike et al., 2021). Moreover, rain water is often provided as a drinking water source to free-ranging laying hens (Chung et al., 2020), while the soil is a major feeding source to free-ranging laying hens (Jurjanz et al., 2015). Earthworms and homegrown vegetables can be important feed sources to free-ranging laying hens (Clark et al., 1995). Earthworms can accumulate very large concentrations of long-chain PFAAs (Munoz et al., 2020), while homegrown vegetables are usually enriched with short-chain PFAAs (Liu et al., 2023). Therefore, these potential feed sources were considered to be optimal candidate matrices to comprise most variation in PFAA exposure of the free-ranging laying hens and, hence, accumulation into the eggs. The PFAA concentrations can vary among vegetable species (Liu et al., 2023). Therefore, the vegetable samples were pooled to even out this potential variation. Further details on the collection methodology of these samples are given in the supplementary information (SI section 1).

2.3. Sample processing

The fresh soil samples were mixed thoroughly by hand and divided in separate aliquots for analyses of PFAAs and physicochemical soil characteristics (SI section 2). The homegrown eggs were homogenized with a stainless steel kitchen mixer and pooled into one sample. The earthworms were depurated for ± 24 h in PP containers (height: 8.8 cm, diameter: 12 cm), after which they were rinsed with MQ-water and homogenized with a TissueLyser. The edible parts of the crops were washed with MQ-water, after which they were mixed with a steel kitchen mixer. In between the mixing of each biotic sample, the kitchen mixer and TissueLyser were thoroughly cleaned with acetonitrile (ACN). All the samples were preserved at −20 °C for later analyses.

2.4. Soil physicochemical characteristics

Both fresh and oven-dried soil samples were analyzed for various soil physicochemical characteristics and nutrients, including pH_{KCl}, clay content, TOC, total P/N, inorganic P (PO_4^{3-})/N (NH_4^+ and NO_3^-) fractions, electrical conductivity and exchangeable base cations (mineral cations: Ca^{2+}, Mg^{2+}, K^+, Na^+; metal cations: Fe^{3+}, Mn^{2+} and Al^{3+}). The methodological procedures for the measurement of these soil parameters are detailed in the supplementary information (SI section 2).

2.5. PFAA chemical extraction

For the extraction of the samples, different protocols were used depending on the matrix type. Abiotic matrices, including oven-dried soil (0.30 ± 0.01 g) and unfiltered rain water (10 ± 0.1 mL) samples, were extracted following the protocol described by Groffen et al. (2019). The biotic matrices, which comprised homogenized pooled samples of eggs (0.30 ± 0.01 g), earthworms (0.15 ± 0.01 g) and vegetables (0.30 ± 0.01 g), were extracted following the procedure of Powley et al. (2005). In brief, the biotic samples were extracted based on solvent extraction using ACN, and were cleaned-up with graphitized Envicarb carbon powder and the abiotic samples were extracted using solid-phase extraction with weak-anion exchange (WAX) cartridges. Details of both extraction methodologies are described in the supplementary information (SI section 3).

2.6. Quality control and quality assurance

During the homogenization of the biotic samples, solvent blanks (=10 mL of ACN) were included every 10 samples to check for cross contamination between the samples. For the extraction, one procedural blank (=10 mL ACN spiked with 10 ng of mass-labeled perfluoroalkyl carboxylic acid (PFCA) and perfluoroalkyl sulfonic acid (PFSA) mixture (Internal Standard, ISTD; MPFAC-MXA, Wellington Laboratories, Guelph, Canada) was included per 15 samples to verify any contamination during the extraction. In the case of batch contamination, the procedural blank values were subtracted from the subsequently measured samples. During the PFAA analysis, instrumental blanks (ACN) were regularly injected to rinse the columns and prevent cross contamination across the samples. The samples from the three monitoring campaigns (i.e., 2019, 2021 and 2022) were analyzed separately within each of their corresponding sampling year, but the same extraction protocols were used across these years (SI section 3).

Sadia et al. (2020) reported the presence of taurodeoxycholic acid (TDCA), a bile acid that shares the same diagnostic transition with PFOS (i.e., 499->80) and thus could affect the quantified PFOS concentrations. However, full removal of TDCA was observed with a purification step during the extraction process using graphitized carbon at a ratio of 1:8 (mass graphitized carbon:mass chicken egg sample) (Sadia et al., 2020). In the present study, a ratio of 1:6 was used in the purification step which ensured removal of TDCA from the samples. This was also confirmed with the additional monitoring of the 499->99 transition unique for PFOS, as calculated concentrations based on this transition were not significantly different from those calculated with the 499->80 transition ($P = 0.57$, paired-Wilcox test).

Calibration curves were prepared by adding a constant amount of the ISTD to varying concentrations of an unlabeled PFAA mixture. The serial dilution of this mixture was performed in ACN. A linear regression function with highly significant linear fit (all $R^2 > 0.98$; all $P < 0.001$) described the ratio between concentrations of unlabeled and labeled PFAAs. Individual PFAAs were quantified using their corresponding ISTD with exception of perfluoropentanoic acid (PFPeA), perfluoroheptanoic acid (PFHpA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorobutane sulfonic acid (PFBS), perfluorodecane sulfonic acid (PFDS), hexafluoropropylene oxide-dimer acid (HFPO-DA) and sodium dodecafluoro-3H-4,8-dioxanonanoate (NaDONA). These analytes were all quantified using the ISTD of the compound closest in terms of functional group and size (Table S1), which was validated by Groffen et al. (2021).

2.7. Chemical analysis

In total 11 perfluoroalkyl carboxylic acids (PFCAs) (perfluorobutanoic acid (PFBA), PFPeA, perfluorohexanoic acid (PFHxA), PFHpA, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), PFTrDA and PFTeDA), four perfluoroalkyl sulfonic acids (PFSAs) (PFBS, perfluorohexane sulfonic acid (PFHxS), PFOS and perfluorodecane sulfonic acid (PFDS)) and two emerging fluoroether analytes (NaDONA and HFPO-DA or GenX) were targeted using ultrahigh performance liquid chromatography (ACQUITY, TQD, Waters, Milford, MA, USA) coupled to a tandem quadrupole (TQD) mass spectrometer (UPLC-MS/MS), operating in negative electrospray ionization. The different target analytes were separated using an ACQUITY UPLC BEH C18 VanGuard Precolumn (2.1 × 50 mm; 1.7 µm, Waters, USA). The mobile phase solvents consisted of ACN- and HPLC-grade water, which were both dissolved in 0.1% HPLC-grade formic acid. The solvent gradient started at 65% of water to 0% of water in 3.4 min and back to 65% water at 4.7 min. The flow rate was set to 450 µL/min and the injection volume was 6 µL. PFAA contamination that might originate from the LC-system was retained by insertion of an ACQUITY BEH C18 pre-column (2.1 × 30 mm; 1.7 µm, Waters, USA) between the solvent mixer and the injector. The target PFAA analytes were identified and quantified based on multiple reaction monitoring (MRM) of the diagnostic transitions that are displayed in Table S1. Limits of quantification (LOQs) were calculated for each detected analyte, in matrix, as the concentration corresponding to a peak signal-to-noise ratio of 10. For every matrix, one common LOQ was assigned which corresponded to the maximum LOQ across all the years. In this way, potential bias due to differences in analytical sensitivity across the years was reduced when the datasets of the sampling campaigns were combined for the modeling (see further section 2.9).

2.8. Data processing

The raw dataset consisted of PFAA concentrations from all detected compounds in eggs, soil, rain water, juvenile earthworm pools, adult earthworm pools and vegetable pools along with the soil physicochemical characteristics (TOC, clay content, pH, exchangeable base cations and soil electrical conductivity) from both monitoring campaigns of 2019 and 2021. The dataset from 2022 was only used as validation dataset for the predictive modeling (see further in 2.9.). Prior to the regression analyses, this raw dataset was split into three sub datasets of paired data (Fig. S1), ranging from the most quantitative dataset to the most qualitive dataset (i.e. dataset containing most independent datapoints and least number of variables, and vice versa). In this way, both models with hypothetically the largest predictive power (most quantitative dataset) as well as models with the largest explanatory power (most qualitative dataset) could be selected for the regression analyses.

Exchangeable base cations were considered as separate variables for the later statistical analyses as these are known to influence the soil adsorption behavior of PFAAs in a different way, depending on their amount of charges and cation type (cf. mineral vs. metal) (Campos-Pereira et al., 2020), and hence may also affect the bioavailability to the laying hens in a different way. For every sub dataset, PFAA compounds with ≤ 50% detection frequency in any matrix were omitted to minimize left-skewness and prediction inaccuracy, which resulted in a paired dataset for nine PFAAs (PFOS, PFBA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA). For repeatedly sampled locations in 2019 and 2021 ($N = 7$), one independent datapoint was obtained by calculating the average of the variable values from both years to avoid pseudoreplication.

2.9. Data analyses

2.9.1. Predictive modeling

All the statistical analyses were done in R (version 4.2) and the graphical visualization was conducted in GraphPad Prism (version 9.0). The most quantitative dataset (Fig. S1; dataset A) of the monitoring campaigns in 2019 and 2021 was used to evaluate the predictability of PFAA concentrations in homegrown eggs ($N = 89$), as this dataset contained the largest sample size and data contrast relative to the number of predictors. Hereby, the chance of overfitting the predictive model is reduced and the robustness of model predictions is increased. Positively skewed continuous variables were log-transformed as this stabilized the variation in the residual distribution of the datapoints. Model diagnostic plots were run to evaluate model assumptions including linearity, as well as normality and homoscedasticity of the residuals. Variance inflation factors (VIFs) were calculated for all the significant predictors to assess the degree of collinearity among them. If VIF was ≥ 5, the variable with the lowest partial $R2$ was excluded from the model following Akinwande et al. (2015). For all the selected variables, the VIFs ranged between 1.00 and 2.31 indicating no significant multicollinearity problems (Table S4 and Table S5). Regression tree plots (package 'tree') and 3D surface plots (package 'mgcv') were used to identify any potential meaningful interactions among the predictor variables.

For each of the nine PFAAs, multiple regression models were constructed with egg concentrations as the dependent variable and the corresponding soil concentrations, soil physicochemical characteristics and rain water concentrations as independent predictor variables. A stepwise backward selection procedure was used to obtain the best-fit model using the Akaike information criterion (AIC), followed by stepwise elimination of predictors with the highest non-significant P-value ($P \geq 0.1$). This reduction process continued until only significant ($P \leq 0.05$) variables remained in the final model (Steyerberg, 2009). The variable soil concentrations always remained in the model as continuous covariate to have real-world based models. A two-way interaction term between pH and clay content was added to each model for the following reasons: (1) changing pH values can affect the amount of pH-dependent surface charges on the binding sites of clay minerals, which can result in altered PFAA adsorption strength (Nguyen et al., 2020) and hence change the bioavailability of PFAAs to the laying hens; (2) models with inclusion of the interaction term systematically exhibited lower prediction error (lower AIC value) than models with only the main effects of pH and clay content.

Quality metrics were computed for the regression models to assess their overall predictive performance, as outlined by Steyerberg et al. (2010). Goodness-of-fit parameters were constructed which comprised the model fit (adjusted total R^2), the mean absolute error (MAE) and the root mean squared error (RMSE) of the residuals. The uncertainty of the mean slope and individual predictions were captured with, respectively, a 95% confidence interval (CI) and/or prediction interval (PI). The models were calibrated using both an internal validation and an external validation approach to test the degree of similarity between the measured and predicted egg PFAA concentrations. This was done through 10-fold cross-validation with repeatedly random selection of the test sets, after which these predictions were combined with those of the original model and regressed to the measured egg concentrations. Additionally, the performance of the model predictions was externally tested on an entirely new validation dataset of homegrown eggs ($N = 10$) from a monitoring campaign in 2022, which was conducted within the same season but in different private gardens as compared to the ones in 2019 and 2021.

2.9.2. Explanatory analysis

Descriptive statistics were computed for the main soil physicochemical properties of the chicken enclosures (Table 1). In addition, Pearson correlation tests were performed among the soil

Table 1
Descriptive statistics (geometric mean, standard deviation (SD) and min. – max. range) of the soil physicochemical characteristics in the top soil (0–5 cm) composite samples from the chicken enclosures of private gardens ($N = 89$) in Flanders (Belgium). The soil solid components include the total organic carbon content (TOC, in %), total organic nitrogen (TON, in %), total organic phosphorus (TOP, in %) and clay content (in %). The measured physicochemical properties are pH_{KCl}, soil electrical conductivity (in µS/cm), exchangeable cations (mineral base cations: Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and metal cations: Fe^{3+}, Al^{3+}, Mn^{2+} in meq/100 g of dry weighed (dw) soil) and the inorganic N (NH_4^+ and NO_3^-, in mg/kg of dw soil) and P (PO_4^{3-}, in mg/kg of dw soil) fractions. Note that the N and P (in)organic fractions could only be measured on the soil samples of 2021 and 2022.

Soil physicochemical property	Min	Mean	SD	Max
TOC (%)	2.10	2.78	2.68	15.5
TON (mg/kg dw)	820	4790	2368	15,577
TOP (mg/kg dw)	762	1967	615	3585
Clay content (%)	0.933	2.02	0.604	3.84
pH_{KCl}	5.23	6.58	0.461	7.54
Soil electrical conductivity (µS/cm)	41.5	310	32.9	1261
Ca^{2+} (meq/100 g of dw soil)	4.06	15.9	5.82	37.3
Mg^{2+} (meq/100 g of dw soil)	0.498	2.72	1.55	7.89
K^{+} (meq/100 g of dw soil)	0.235	2.49	1.64	7.66
Na^{+} (meq/100 g of dw soil)	0.027	0.429	0.615	3.98
Fe^{3+} (meq/100 g of dw soil)	0.005	0.024	0.018	0.092
Al^{3+} (meq/100 g of dw soil)	0.008	0.052	0.032	0.245
Mn^{2+} (meq/100 g of dw soil)	0.024	0.134	0.102	0.747
NH_4^+ (mg/kg of dw soil)	0.280	62.3	96.8	440
NO_3^- (mg/kg of dw soil)	2.49	216	213	1238
PO_4^{3-} (mg/kg of dw soil)	0.207	20.7	33.1	196

physicochemical characteristics to better understand their relationships (Fig. 3), which was useful as general background information for the interpretation of the further analyses and given that soil properties in the chicken enclosure usually show a distinct pattern (Soares et al., 2022). The quantitative dataset (Fig. S1; dataset A) was used to test significant associations between soil PFAA concentrations, soil physicochemical characteristics (i.e. explanatory variables) and egg PFAA concentrations (i.e. response variable). The dataset was mean-centered and standardized to harmonize the variables and to enable valid comparisons among them. Tree plots were constructed for each of the nine PFAAs to visualize the associations between the tested explanatory variables and the outcome variable. Parameter estimates were reported as standardized Cohen's effect sizes and 95% CIs.

The qualitative dataset (Fig. S1; dataset B) was used to evaluate any significant relationships between the egg PFAA concentrations and the PFAA concentrations in the earthworm pools (juveniles and adults, two separate explanatory variables) and in the vegetable pools. Hereby, the soil PFAA concentrations which explained most variation in the corresponding egg concentrations, based on the partial R^2 of the previous analysis, were controlled for by retaining soil PFAAs as a continuous covariate in these models. A two-way interaction term was tested between the earthworm PFAA concentrations and soil PFAA concentrations as earthworms may synergistically decrease or increase the bioavailability of PFAAs to terrestrial organisms and hence the egg concentrations (Hickman et al., 2008). An interaction term between PFAA concentrations in earthworm pools and vegetable pools was not included in the model to prevent oversaturation of the models, as the number of statistical tests was high relative to the sample size ($N = 34$) of the qualitative dataset.

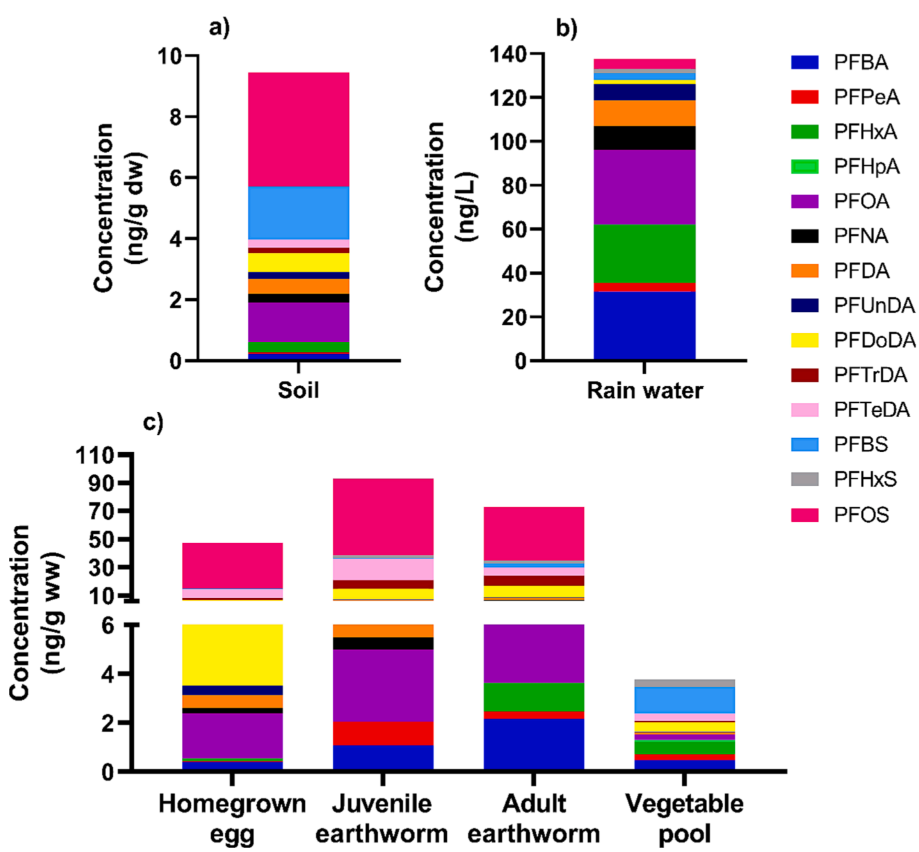

Fig. 1. Overview of the arithmetic mean concentrations of all detected PFAAs in (a) the top soil layer (0–5 cm) of the chicken enclosure soil (in ng/g dry weight (dw)), (b) rain water (in ng/L) and (c) homegrown eggs, juvenile earthworms, adult earthworms and vegetable pools (in ng/g wet weight (ww)) from the private gardens ($N = 89$) in Flanders (Belgium).

3. Results

3.1. Matrix profile and concentrations

An overview of the profile and mean concentrations of all detected PFAAs in each of the examined abiotic and biotic matrices are shown in Fig. 1. PFOS was the dominant compound in both the soil, homegrown eggs, adult and juvenile earthworms (Fig. 1a-c, mean: 4.61 ng/g dry weight (dw), 32.1 ng/g wet weight (ww), 38.1 ng/ g ww and 54.7 ng/g ww, respectively). On the other hand, PFOA and PFBA were the major compounds in rain water and in the vegetable pools (Fig. 1a-c, mean: 34.0 ng/l and 0.483 ng/g ww, respectively). The polyfluoroalkyl compounds HFPO-DA (GenX) and NaDONA were never detected in any of the samples. In total nine PFAAs (PFOS, PFBA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA) could be detected in > 50% of the samples across all the matrices. These PFAAs were selected for the predictive modeling (see further section 3.2.).

In chicken enclosure soil and rain water, up to 13 and 11 PFAAs could be quantified with a mean total sum concentration of 9.45 ng/g dw and 138 ng/L, respectively (Fig. 1a, Fig. 1b). In chicken enclosure soil, three PFAAs (i.e., PFOS, PFBS and PFOA) contributed for > 62% of the total mean sum concentration. The composition of rainwater was dominated by PFOA (=34.0 ng/L), PFBA (=31.5 ng/L) and PFHxA (26.5 ng/L), which together accounted for > 59% of the total mean sum concentration (Fig. 1b). In the biotic matrices, 12 (vegetable pools), 13 (both homegrown eggs and adult earthworms), 12 (juvenile earthworms) targeted PFAAs could be detected (Fig. 1c). The highest total mean sum concentrations were found in juvenile earthworms (=93.1 ng/g ww), followed by adult earthworms (=72.9 ng/g ww), homegrown eggs (=47.3 ng/g ww) and vegetable pools (=3.78 ng/g ww) (Fig. 1c). In all the animal matrices, PFOS and long-chain PFCAs (PFDoDA, PFTrDA and PFTeDA) were the dominant compounds, whereas short-chain compounds (PFBA, PFPeA and PFHxA) contributed most to the profile of the vegetable pools (Fig. 1c).

3.2. Predictive modeling

The descriptive statistics of the soil physicochemical properties in the chicken enclosure are provided in Table 1. The total organic matter fractions (TOC, total organic nitrogen (TON) and total organic phosphorous (TOP)) and pH were strongly variable among the chicken enclosures, while the clay content exhibited a relatively narrow range (min. – max.: 0.933 – 3.84 %). From the measured exchangeable base cations, Ca^{2+} showed the highest relative soil exchange capacity (15.9 ± 5.82 meq/100 g soil). PFOS, PFBA and the $C_{9\text{-}14}$ carboxylates were all found at quantifiable concentrations in every target matrix (Table 2).

The significant predictors and best-fit predictive equations of the final multiple regression models are summarized for nine PFAAs (PFOS,

Table 2

Overview of the measured PFAA concentrations in the collected top soil (0–5 cm) composite samples of the chicken enclosure (ng/g dry weight (dw)), rain water samples (ng l^{-1}), homegrown eggs (ng/g wet weight (ww)), earthworm pools (adults and adults; ng/g ww) and vegetable pools (ng/g ww) from private gardens (N = 89) in Flanders (Belgium). For each matrix, the arithmetic mean ± standard error (SE) is given along with the minimum (min.) and maximum (max.) range of the PFAA concentrations. LOQ = limit of quantification.

Chicken enclosure soil (ng/g dw)	PFAAs								
	PFOS	PFBA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
LOQ	0.072	0.110	0.079	0.130	0.133	0.139	0.171	0.178	0.230
Min. - max. range	0.080——29.5	<LOQ – 3.60	0.290——6.15	<LOQ – 1.20	<LOQ – 1.45	<LOQ – 0.627	<LOQ – 2.48	<LOQ – 1.82	<LOQ – 1.07
Mean ± SE	3.74 ± 0.610	0.224 ± 0.049	1.31 ± 0.104	0.279 ± 0.027	0.491 ± 0.037	0.217 ± 0.016	0.623 ± 0.058	0.189 ± 0.025	0.256 ± 0.031
Rain water (ng l^{-1})									
LOQ	0.301	1.35	1.63	0.738	1.32	1.28	1.40	1.47	1.51
Min. - max. range	<LOQ – 79.7	<LOQ – 604	<LOQ – 329	<LOQ – 421	<LOQ – 55.8	<LOQ – 86.7	<LOQ –14.1	<LOQ – 28.5	<LOQ – 17.5
Mean ± SE	4.61 ± 1.32	31.5 ± 9.18	34.0 ± 6.61	10.7 ± 1.32	11.9 ± 1.24	7.34 ± 1.46	2.04 ± 0.127	<LOQ ± LOQ	<LOQ ± <LOQ
Homegrown eggs (ng/g ww)									
LOQ	0.073	0.111	0.128	0.098	0.185	0.142	0.141	0.194	0.222
Min. - max. range	0.860——571	<LOQ – 3.72	<LOQ – 8.13	<LOQ – 1.20	<LOQ – 2.34	<LOQ – 3.78	0.187——21.9	<LOQ – 12.3	0.240——147
Mean ± SE	32.1 ± 9.34	0.404 ± 0.063	1.84 ± 0.284	0.223 ± 0.026	0.525 ± 0.049	0.386 ± 0.052	3.07 ± 0.397	1.54 ± 0.194	6.42 ± 1.74
Earthworm pools (ng/g ww)									
Adult									
LOQ	0.518	0.350	0.146	0.167	0.626	0.124	0.782	0.336	0.335
Min. - max. range	2.42——320	<LOQ – 21.6	0.439——5.91	<LOQ – 2.15	<LOQ – 4.26	0.133——1.81	1.64——31.0	1.36——28.2	0.723——30.7
Mean ± SE	38.1 ± 7.78	2.17 ± 0.482	2.57 ± 0.194	0.391 ± 0.051	1.54 ± 0.108	0.649 ± 0.046	8.21 ± 0.927	7.26 ± 0.760	5.66 ± 0.725
Juvenile									
Min. - max. range	1.65——451	<LOQ – 10.1	<LOQ – 72.3	<LOQ – 10.8	<LOQ – 11.2	<LOQ – 3.22	<LOQ – 57.5	0.888——28.5	1.90——79.8
Mean ± SE	54.7 ± 12.3	1.08 ± 0.241	2.95 ± 1.37	0.491 ± 0.205	1.33 ± 0.208	0.646 ± 0.083	7.33 ± 1.19	6.09 ± 0.682	15.2 ± 2.07
Vegetable pools (ng/g ww)									
LOQ	0.028	0.118	0.110	0.044	0.078	0.021	0.232	0.067	0.028
Min. - max. range	<LOQ – 0.259	<LOQ – 5.16	<LOQ – 1.42	<LOQ – 0.063	<LOQ – 0.461	<LOQ – 0.073	<LOQ – 1.28	<LOQ – 0.407	0.090——1.03
Mean ± SE	<LOQ ± <LOQ	0.483 ± 0.162	0.235 ± 0.038	<LOQ ± <LOQ	0.084 ± 0.013	0.028 ± 0.003	0.377 ± 0.037	0.074 ± 0.013	0.282 ± 0.036

PFBA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA) in Table 3. The soil concentration was the best single predictor of the corresponding egg concentrations for PFOS ($P < 0.001$, $R^2_{partial} = 42.2$) and the $C_{4\text{-}9}$ carboxylates ($P < 0.001$, $R^2_{partial} = 16.3–55.3\%$), while it was only marginally significant for the $C_{10\text{-}14}$ carboxylates ($P < 0.1$, $R^2_{partial} \leq 2.8\%$). Moreover, exchangeable Mn^{2+} ($P < 0.01$, $R^2_{partial} = 3.8–27.3\%$), exchangeable Fe^{3+} ($P < 0.01$, $R^2_{partial} = 2.4–11.3\%$), and the two-way interaction term pH:clay content ($P < 0.01$, $R^2_{partial} = 2.4–5.5\%$) and TOC ($P < 0.05$, $R^2_{partial} = 2.7–5.0\%$) were significant predictors of egg concentrations. Rain water concentrations and soil electrical conductivity did not significantly contribute to explaining variation in the egg concentrations for any compound ($P > 0.05$), both in single linear regression as well as in the multiple regression models controlling for the other significant predictors.

All the best-fit predictive equations showed a highly significant linear fit ($P \leq 0.01$), but varied in quality of prediction accuracy and precision of the egg concentrations (Fig. 2). The explained variation in egg concentrations, reflected by the adjusted R^2 values, ranged from 9.12% for PFDoDA to 66.6% for PFOA (Table 3). Importantly, the best predictive models were obtained for PFOS, PFOA and PFNA which together dominantly contributed for > 75% to the total measured egg PFAA burden. The model quality metrics for PFOS, PFOA and PFNA were good to very good, with a MAE of 0.58, 0.28 and 0.07, respectively (Table 3). Moreover, robust and accurate predictions could be made for the models of these compounds, as the slopes of the predicted egg concentrations and measured egg concentrations did not significantly differ (two-sample t-tests, $P > 0.05$), both with the external validation approach (Fig. 2, Table S2) and the internal cross-validation approach (Fig. S3).

The predictive performance for the regression models of the other compounds (PFBA and $\geq C_{10}$ carboxylates) performed less well, with relatively low adjusted R^2 values ranging from 9.12% to 37.1% (Table 3). For PFDA and the $C_{12\text{-}14}$ carboxylates, most prediction error was caused by relatively large overall model-predicted underestimation of the measured egg concentrations and variation in the predictions of measured egg concentrations in the lower part of the concentration range (Fig. 2, range 0—1 log ng/g ww). This was also reflected in the quality metric values (Table 3, low RMSE and MAE) of these compounds and relatively large deviations in the cross-validation slopes for predictions within this lower concentration range (Fig. S2). On the other hand, the prediction uncertainty for PFUnDA and PFBA egg concentrations was mainly due to the large leverage from a few outliers, which was evident from the low adjusted R^2 values, but still good quality metric values (Table 3, e.g. low MAE and RMSE). Moreover, new predictions based on the external validation dataset fell within the 95% prediction interval of the regression curve (Fig. 2).

3.3. Explanatory analysis

The soil concentrations were positively and strongly associated with the egg concentrations for PFOS, PFBA, PFOA and PFNA (all $P < 0.01$ and large mean effect sizes of ≥ 0.35 units). For the $C_{10\text{-}12}$ carboxylates and PFTeDA, only a modestly significant relationship could be observed between the soil concentrations and egg concentrations ($P < 0.05$), while these associations were not significant for the other two carboxylates (all $P > 0.05$, Fig. 2). Lower amounts of soil TOC were significantly related with higher egg concentrations for PFOS ($P < 0.01$) and $C_{9\text{-}14}$ carboxylates ($P < 0.01$), except for PFUnDA (Fig. 3). For TON, only weak negative associations were found with PFUnDA egg concentrations (effect size of −0.13, $P < 0.05$), whereas higher TOP was associated with lower egg concentrations for PFBA and PFOS (resp. effect size of −0.19 to −0.29, $P < 0.05$).

The opposite relationship was observed for the main effects of both clay content and pH, as higher values of both variables were related with

Table 3
Overview of the multiple regression modeling output for the prediction of homegrown egg concentrations (response variable) with respect to nine PFAAs (PFOS, PFBA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA), taking into account the corresponding soil concentrations and significant soil physicochemical characteristics as predictor variables. The mathematic equations of the best-fit multiple regression models are provided along with the model quality metrics (AIC = Akaike information criterion; RMSE = root mean square error of the residuals; MAE = mean absolute error of the model predictions) to estimate the predictive performance of these models.

Response variable	Equation best-fit model	Model quality metrics				
		Model significance level	Adjusted R2	AIC value	RMSE	MAE
Log egg PFOS concentrations	36.6 + 1.07 * log soil PFOS + 23.2 * log Fe^{3+} − 0.934 * log TOC − 37.2 * log clay content – 17.5 * log pH + 5.52 * log Mn^{2+} − 7.92 * log Al^{3+} + 18.7 * log pH:log clay content	$P < 0.0001$ $F_{8,80} = 19.0$	62.1	−28.5	0.77	0.58
Log egg PFBA concentrations	8.41 + 0.633 * log soil PFBA – 9.44 * log clay content – 3.98 * log pH + 1.11 * log Mn^{2+} − 0.174 * log Ca^{2+} + 4.75 * log pH:log clay content	$P < 0.0001$ $F_{6,82} = 7.21$	29.8	−240	0.23	0.18
Log egg PFOA concentrations	−4.30 + 0.799 * log soil PFOA + 25.6 * log Fe^{3+} + 2.10 * log pH – 2.96 * log Al^{3+} – 0.251 * log Mg^{2+}	$P < 0.0001$ $F_{5,83} = 36.1$	66.6	−145	0.41	0.28
Log egg PFNA concentrations	5.25 + 0.672 * log soil PFNA + 2.04 * log Fe^{3+} – 0.104 * log TOC – 5.19 * log clay content – 2.53 * log pH + 0.399 * log Mn^{2+} + 2.56 * log pH:log clay content	$P < 0.0001$ $F_{7,81} = 21.5$	61.9	−389	0.10	0.07
Log egg PFDA concentrations	8.86 + 0.063 * log soil PFDA + 5.08 * log Fe^{3+} – 0.255 * log TOC + 0.882 * log Mn^{2+} – 4.16 * log pH – 8.97 * log clay content + 4.50 * log pH:log clay content	$P < 0.0001$ $F_{7,81} = 3.06$	25.8	−252	0.22	0.17
Log egg PFUnDA concentrations	9.79 – 0.0008 * log soil PFUnDA + 4.93 * log Fe^{3+} + 1.13 * log Mn^{2+} – 10.0 * log clay content – 4.74 * log pH – 0.235 * log Mg^{2+} – 2.16 * log Al^{3+} + 5.15 * log pH:log clay content	$P < 0.0001$ $F_{8,80} = 7.46$	37.1	−273	0.19	0.14
Log egg PFDoDA concentrations	1.86 + 0.319 * log soil PFDoDA – 0.602 * log TOC + 2.28 * log Mn^{2+}	$P < 0.05$ $F_{3,85} = 3.90$	9.12	−87	0.58	0.45
Log egg PFTrDA concentrations	21.1 + 0.315 * log soil PFTrDA – 0.560 * log TOC + 1.83 * log Mn^{2+} −17.4 * log clay content − 10.0 * log pH + 8.95 * log pH:log clay content	$P < 0.001$ $F_{6,82} = 5.22$	22.3	−136	0.43	0.34
Log egg PFTeDA concentrations	38.0 + 0.187 * log soil PFTeDA – 0.728 * log Mg^{2+} + 5.13 * log Mn^{2+} – 37.4 * log clay content – 6.81 * log Al^{3+} − 18.5 * log pH + 19.1 * log pH:log clay content	$P < 0.0001$ $F_{7,81} = 6.54$	30.6	−44.8	0.74	0.61

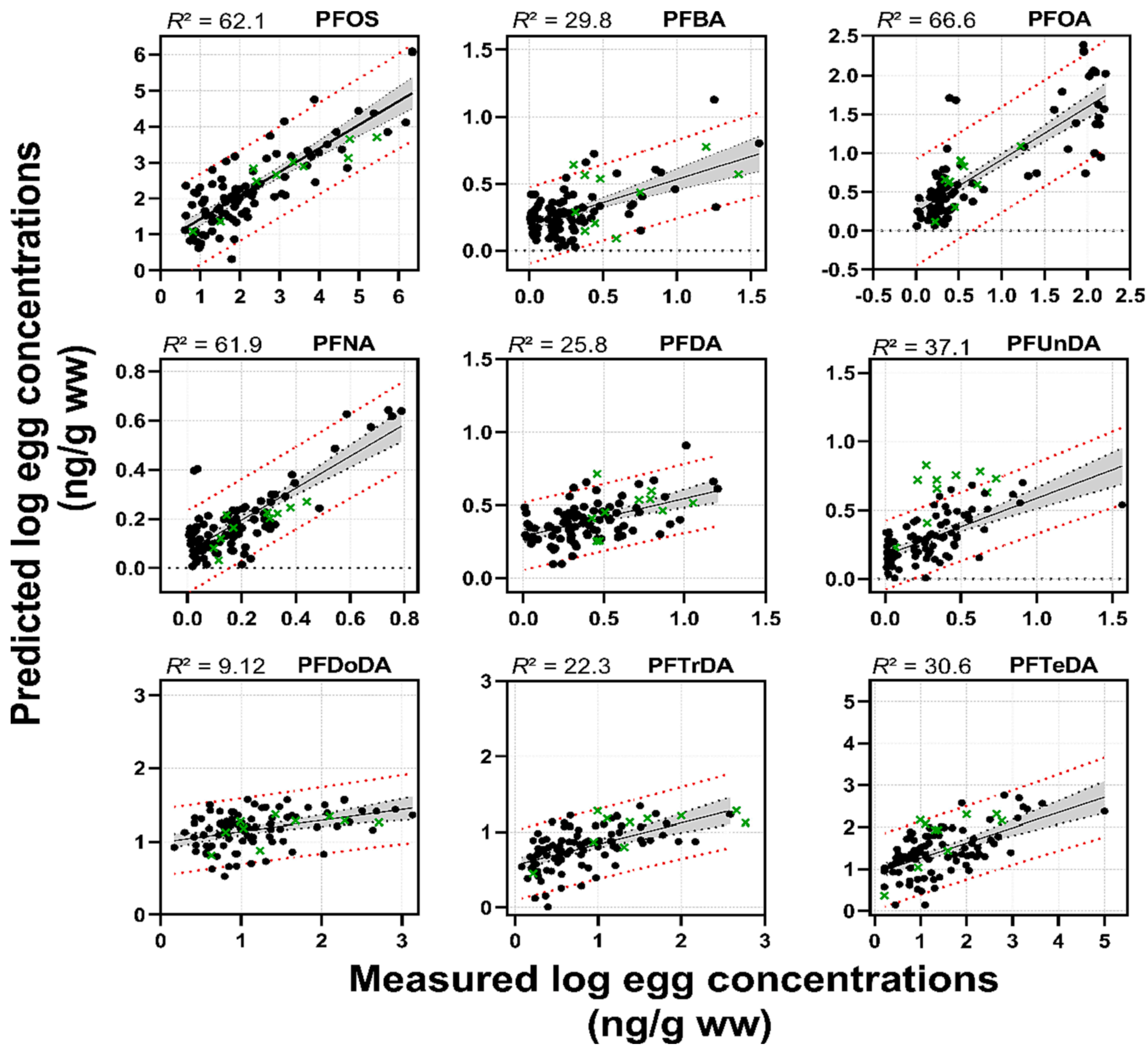

Fig. 2. Multiple regression plots showing the model-predicted concentrations (in log ng/g wet weight (ww) in homegrown eggs of 2021 (training dataset, black dots; $N = 89$) and 2022 (validation dataset, green crosses; $N = 10$) as a function of the measured homegrown egg concentrations (in ng/g ww, log-scale) for nine PFAAs. The adjusted R^2 value of each best-fit model is provided. The black solid line and grey band represent, respectively, the linear regression curve and 95% confidence interval of the average model-predicted log egg concentrations. The red dotted line represent the 95% prediction intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

higher egg concentrations for most PFAAs (all $P < 0.05$), but in a contrasting way (Fig. 3). Indeed, the effect size of the positive relationship between pH and the egg concentrations was highly significant and very similar, in terms of effect size magnitude, for most compounds (apart from PFDoDA and PFTrDA, all $P < 0.05$ and range of mean effect sizes 0.22 – 0.38). On the other hand, the main effect of clay content was significantly and positively associated with the egg concentrations for most PFAAs ($P < 0.05$), but the effect size magnitude of this relationship tended to increase with increasing alkyl chain-length (Fig. 3). Similarly, the significantly positive two-way interaction term between pH and clay content indicated a combined synergistic relationship of these two variables with the egg concentrations (Fig. S5), except for PFOA and PFDoDA.

For the exchangeable cations, various significant relationships were found between egg PFAA concentrations and di-/tri-valent exchangeable cations, but in a contrasting way (Fig. 3). With respect to the exchangeable metal cations, most PFAAs showed significantly positive relationships between Mn^{2+} and egg concentrations (Fig. 3). Notably, Mn^{2+} was also strongly positively correlated with soil clay content (Pearson $R = 0.68$, $P < 0.01$; Fig. S2) and significantly positive interaction terms between Mn^{2+} and clay content were found (Fig. S5). Moreover, higher Fe^{3+} was strongly related with higher egg PFOA concentrations (mean effect size of 0.55, $P < 0.01$; Fig. 3), while this metal cation was also positively associated with higher PFOS and PFUnDA egg concentrations ($P < 0.05$; Fig. 3). Remarkably, and in contrast to Mn^{2+} and Fe^{3+}, the metal cation Al^{3+} was strongly negatively related with egg concentrations for most PFAAs ($P < 0.05$; Fig. 3). Moreover, Al^{3+} was strongly positively correlated with TOC content (Pearson $R = 0.51$, $P < 0.01$; Fig. S2). Likewise, this negative relationship was also found between mineral cations (Mg^{2+} and Ca^{2+}) and PFAA egg concentrations, but for less compounds and often less strong relationships compared to those found for Al^{3+} (Fig. 3). Lastly, monovalent exchangeable mineral cations K^+ and Na^+ were unrelated with egg PFAA concentrations.

PFOS was the dominant compound in homegrown eggs, earthworms and in the chicken enclosure soil, while PFBA was the major compound detected in vegetables (Table 2). For PFOS and PFOA, significantly positive relationships could be observed between adult worm concentrations and egg concentrations (both $P \leq 0.05$), whereas the relationships for the other compounds were not significant (all $P > 0.05$). For

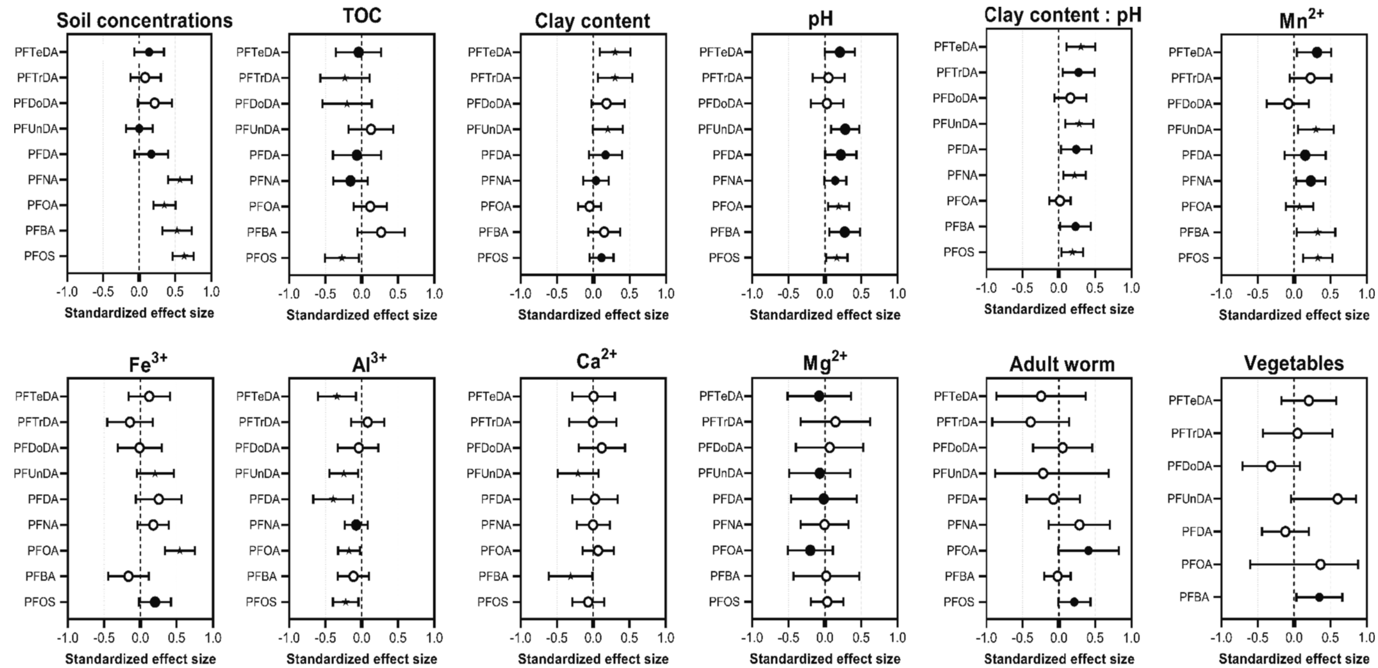

Fig. 3. Standardized effect size estimates and 95% confidence intervals (CIs) of the relationships between the dependent variable (=homegrown egg concentrations for nine PFAAs) and relevant explanatory variables (soil concentrations, total organic carbon (TOC), clay content, pH, two-way interaction term of clay content * pH, exchangeable metal cations (Mn^{2+}, Fe^{3+} and Al^{3+}) and exchangeable mineral cations (Ca^{2+} and Mg^{2+}), based on the outcome of the predictive regression models. Rain water PFAA concentrations, soil electrical conductivity, exchangeable Na^{+} and K^{+} are not shown as none of these explanatory variables was significantly related with any of the egg PFAA concentrations. Symbols represent the significance level of the relationship between the independent and dependent variable (asterisk: $P <$ 0.01; filled circle: $P \leq 0.05$; hollow circle: $P \geq 0.05$ or not significant).

PFBA, the vegetable concentrations were significantly associated with the corresponding egg concentrations ($P < 0.05$) but not for the other compounds (all $P > 0.05$). Interestingly, juvenile worms contained significantly higher PFOS and PFTeDA concentrations than adult worms, while the reverse was true for PFBA and PFOA (two-sample t-tests; all P < 0.05).

4. Discussion

4.1. Matrix profile and concentrations

From the 17 targeted analytes, up to 13 PFAAs could be detected in the soil from the chicken enclosure (Fig. 1). Compared to general soil data at non-suspected sites across Europe, the mean soil concentrations for the ∑PFCAs and ∑PFSAs in the chicken enclosure (∑PFCAs = 3.58 ng/g dw; ∑PFSAs = 7.66 ng/g dw) largely exceeded the mean concentrations of soil for the ∑PFCAs and ∑PFSAs in Europe, respectively 1.00 ng/g dw and 0.808 ng/g dw (Rankin et al., 2016). Moreover, the soil short-chain PFAAs concentrations of the present study were similar to those in residential garden soil from Minnesota (USA), which were sampled both nearby and remotely from a fluorochemical waste disposal site (Scher et al., 2018). However, soil PFOS and PFOA concentrations of the present study were more than twice as high as those reported by Scher et al. (2018). In rainwater, PFOA and PFBA were the major compounds with concentrations ranging between < LOQ- 329 ng/L and < LOQ-604 ng/L, respectively (Table 2). The rainwater concentrations for most detected compounds were in the same order of magnitude as those reported in some urban regions, as recently *meta*-analyzed by Cousins et al. (2022). Notably, trifluoroacetic acid is frequently the most abundant compound detected in rainwater (Pike et al., 2021), which was not included as a targeted analyte in the present study.

The homegrown egg concentrations of the present study were among the highest ever reported in homegrown chicken eggs (Gazzotti et al., 2021; Su et al., 2017; Wang et al., 2019), especially for PFOS, which was the dominant compound in the eggs with concentrations ranging between 0.860 and 571 ng/g ww (Table 2). The current European regulatory limits for PFOS (=1.0 ng/g ww), PFOA (=0.30 ng/g ww) and PFNA (=0.70 ng/g ww) concentrations in commercial eggs (EC, 2022) were exceeded in 94%, 76% and 25% of the egg samples. This clearly confirms previous findings that homegrown egg consumption can be a major PFAA exposure source presenting potential health risks to humans (Lasters et al., 2022).

Likewise, PFOS was the dominant compound in the earthworms, with concentrations ranging between 1.65 and 451 ng/g ww and 2.42–320 ng/g ww in juvenile and adult life-stages (Table 2). This in agreement with other studies on earthworms at aqueous film-forming foam impacted sites, which found large accumulation of PFOS, although at concentrations>100x higher compared to those of the present study (Munoz et al., 2020; Rich et al., 2015). The vegetable pool concentrations were dominated by PFBA (range: <LOQ − 5.16 ng/g ww), which is in agreement with other studies that examined PFAAs in field-grown vegetables (Liu et al., 2023; Scher et al., 2018). However, long-chain PFCAs were frequently detected in the vegetable pool samples at quantifiable concentrations (range: <LOQ − 1.28), while these compounds were rarely reported field-grown vegetables at other sites (Liu et al., 2023; Scher et al., 2018).

4.2. Predictive modeling

To the best of our knowledge, no studies have been conducted that evaluated the predictability of PFAA concentrations in (homegrown) food, which makes it difficult to compare the obtained predictive models of the present study with literature data. Based on the extensive set of applied quality metrics to evaluate the model performance, good predictive models were obtained in terms of robustness (successful internal and external validation, resp. Fig. S4 and Fig. 3), precision and accuracy (relatively high adjusted R^2, low MAE, Table 3) for prominent PFAAs (e.g. PFOS, PFOA and PFNA). These three compounds are often major contributors to the total PFAA content in dietary food (Klenow et al., 2013; Lasters et al., 2022), which is considered to be the most important exposure source of bioaccumulative long-chain PFAAs to the general human population (Roth et al., 2020). Consequently, they are very frequently detected in humans at concentrations associated with potential health risks (Colles et al., 2020; Fenton et al., 2021; Richterova et al., 2023).

For most PFAAs, the soil concentration was the most important predictor of their corresponding egg concentrations, which is in agreement with studies on other persistent organic pollutants, such as dioxins and polychlorinated biphenyls (PCBs), that also exhibit strong soil adsorption properties and hence a relatively large exposure risk to free-ranging laying hens (Waegeneers et al., 2009; Windal et al., 2009). Importantly, for these aforementioned classic organic pollutants, the soil concentration could often be used as a single predictor for egg concentrations (Schoeters and Hoogenboom, 2006; Waegeneers et al., 2009), whereas this is clearly not the case for PFAAs. In the present study, predictions often significantly improved by adding additional physicochemical properties to the models (Table S3), which clearly demonstrates the complex and distinct sorption behavior of PFAAs compared to other groups of organic pollutants. Therefore, the present study highlights the necessity of evaluating multiple parameters to adequately predict PFAA accumulation in terrestrial organisms.

The established predictive models for PFOS, PFOA and PFNA in the present study show promising potential for effective usage in monitoring and human risk assessment of PFAAs. Importantly, the construction of these models was underpinned by a large dataset (N = 89) and thoroughly validated (Fig. 3, Fig. S3) across a large geographical range, resulting in a well-covered contrast for most of the examined variables. Therefore, the models for those compounds that showed good overall performance (e.g. PFOS, PFOA and PFNA) should be sufficiently accurate for large-scale application by regulatory agencies for decision-making processes to rapidly estimate the exposure risk via consumption of homegrown eggs in any given private garden of Flanders. Since soil is present in virtually every private garden, it enables the development of what-if risk scenarios that could clarify which exposure risk would be posed to the owners when free-ranging laying hens would be introduced. Furthermore, soil data of PFAA concentrations have become increasingly available over the last years in several countries (Brusseau et al., 2020), including in Flanders (City of Antwerp, 2021; Department Environment and Health, 2022b; Flanders Environment Agency, 2022) due to intensified (ongoing) monitoring efforts, which could potentially be inserted into the models. In this way, the available soil data can be complementarily used together with the models to evaluate conditional human exposure risk scenarios with respect to homegrown egg consumption. Within this particular context, the models should at least include some basic soil parameters for the aforementioned PFAA (e.g. soil concentration, pH and clay content and their interaction effect), of which the first one can increasingly be adopted from existing soil databanks and the latter two are relatively low in cost and readily measurable (Wäldchen et al., 2012).

As a general remark, it should also be noted that the relative weight of a predictive model in decision-making processes should be in proportion to the amount of verification and validation of the model (Ellis, 2012). Therefore, the potential application of these models on a global scale should be interpreted with caution. For instance, some gardens in tropical climate regions can be characterized by much higher clay content ranges than those measured in Flanders (Akihiko and Wagai, 2017), which may result in an increased uncertainty of the model predictions. Moreover, for the remaining compounds (i.e. PFBA and $C_{10\text{-}14}$ carboxylates), which performed considerably less well than the aforementioned compounds (PFOS, PFOA and PFNA), some of the constructed predictive models in the present study have to be considered as an interim step for which future revision is highly recommended.

Clearly, soil concentrations and the other examined predictors did explain much less variation in egg concentrations for these compounds (min.-max. range total R^2: 9.12 – 37.1%). This poor model performance is probably due to the lower variability of the concentration range for these compounds (e.g., often one order of magnitude difference between lower and higher order concentrations), which would make it intrinsically more difficult to predict the variability in egg concentrations. However, it cannot be ruled out that other potential exposure sources than the soil are could contribute to explaining additional variation in egg PFAA concentrations, such as inhalation of contaminated dust. The enclosures of laying hens are known for the accumulation of fine, airborne particulate dust matter, through preening, molting of feathers and deposition of fecal residues (Maffia et al., 2021; Viegas et al., 2013), to which PFAAs in theory can be adsorbed (Gustafsson et al., 2022). Prior to and during the timing of sample collection for the present study, these compounds were actively emitted (Peters et al., 2022) and circulating (Department Environment and Health, 2022a) in the atmosphere for a substantial part of the study area. For these reasons, it can be hypothesized that laying hens, through soil scratching and dust bathing behavior, can be substantially exposed to contaminated dust particles via inhalation.

Based on preliminary calculations with available literature data (detailed for PFDoDA as an example in section 6 of the SI), the contribution of PFAA intake via dust inhalation would be much lower than via soil consumption. Even for the worse-case scenario of dust intake, the intake (i.e., 0.120 ng/day) would be almost 8x lower than the intake (i.e., 0.945 ng/day) via a modal soil consumption scenario. However, we could not take into account the additional intake of dust via typical free-ranging laying hen behavioral activities, e.g. dust-bathing and feather preening behavior. Therefore, the above calculations for dust inhalation are probably still an underestimation of the total exposure via dust. It would be beneficial that future modeling efforts quantify these potentially important PFAA exposure sources.

Another important exposure source that may likely explain additional variation of the egg concentrations is feed of the laying hens other than earthworms and vegetables, which were considered in the present study. Notably, the explained variation in egg PFBA and PFOS concentrations substantially improved when the predictive models were run with the homegrown vegetables and adult earthworms as additional, significant predictors for PFBA and PFOS, respectively (e.g. total adjusted R^2 for PFBA increased from 29.8 to 43.9%). This result is in agreement with other studies on PFAAs (Lasters et al., 2022) and dioxins (Kijlstra, 2004; Waegeneers et al., 2009) in homegrown eggs. Together, these results indicate that homegrown vegetables and adult earthworms can be an important exposure source of PFBA and PFOS to free-ranging laying hens, respectively.

Lastly, it should be noted that seasonal fluctuations with respect to some of the soil physicochemical characteristics and grazing patterns of the free-ranging laying hens may affect the model outcomes. Nevertheless, soil TOC, pH and exchangeable cation levels usually vary only to a small extent within sites (Soares et al., 2022) and the variation among private gardens was relatively large in the present study due to the large spatial range that was considered. Therefore, it is unlikely that fluctuations in soil physicochemical characteristics would significantly alter the outcome of the modeling. Nevertheless, it is known that seasonal variation in grazing patterns and activity of free-ranging laying hens can be relatively large as longer days and warmer temperatures in the summer period result in more outside foraging and feeding activity (Ferreira et al., 2022; Taylor et al., 2017). Therefore, one might expect that soil and water intake would be higher in summer compared to spring (i.e., the onset of the egg-laying cycle) and may result in higher intake of PFAAs during summer. However, to the best of our knowledge, this has not yet been investigated and thus remains speculative.

4.3. Explanatory analysis

In line with the expectations, soil physicochemical characteristics that should result in lower (e.g., TOC) and higher (e.g., clay content, pH, exchangeable cations) bioavailability were often associated with lower and higher egg concentrations, respectively (Fig. 3). While TOC and clay content are often identified as the dominant soil solid components governing PFAA adsorption and retention in the soil matrix (Li et al., 2018; Millinovic et al., 2015), their association with egg concentrations was consistently negative and positive for all the compounds, respectively. Therefore, given that the soil could also be identified as a major exposure source to the laying-hens, it is likely that opposite associations for TOC and clay content with egg concentrations result from adsorption affinity differences of PFAAs with both matrices (Li et al., 2018; Cai et al., 2022), resulting in bioavailability differences. Consequently, the degree of PFAA absorption from the digestive tract to the systemic circulation may be altered and hence also the accumulation in the eggs.

Strong hydrophobic interactions dominate the PFAA adsorption onto TOC, whereas weaker and more reversible, electrostatic interactions are predominant on the clay fraction (Li et al., 2018). Furthermore, the sorption reversibility on TOC decreases with increasing alkyl chain-length, whereas PFOS typically shows almost negligible reversibility, once adsorbed onto the TOC matrix (Millinovic et al., 2015). Therefore, it is hypothesized that TOC-adsorbed PFAAs could largely not be absorbed from the digestive tract into the eggs, ultimately leading to lower egg concentrations. This is reflected in the present study as the largest effect size between TOC and egg concentrations was observed for more hydrophobic compounds with high sorption coefficients, such as PFOS (Fig. 3). Furthermore, as PFOS and many of the long-chain carboxylates have been phased-out (UNEP, 2019), a substantial proportion of environmental contamination of these compounds originates from historical pollution (Lasters et al., 2022). However, polyfluorinated precursor compounds, which are still being produced, can be biotransformed to PFBA and PFOA (Dhore and Murthy, 2021; Prevedourous et al., 2006). This may additionally explain the absence of relationships between the soil solid components and PFBA as well as PFOA.

On the other hand, the positive relationship between clay content and egg PFAA concentrations could be explained by weak, reversible electrostatic interactions resulting in successful absorption of PFAAs from the digestive tract to the eggs. All the examined PFAAs in the present study have such low pKa values that they are dominantly present in their anionic form under modal environmental conditions (Goss, 2008). Therefore, the charged nature of the electrostatic interaction between PFAAs and clay particles implies that the ad-/desorption is largely prone to pH changes, in contrast to the dominant hydrophobic interactions of PFAAs with TOC. As soon as soil particles are ingested by the laying-hen, the low pH values in their glandular stomach (ranging from 3 to 4) (Waegeneers et al., 2009) should theoretically result in large protonation of the clay surface charges, which can result in increased absorption. This is also in agreement with another study on PCB exposure to piglets, which has reported a much larger retention of pollutants in the digestive tract by TOC, compared to clay, resulting in lower bioavailability to adipose tissue (Delannoy et al., 2015).

The suggested higher bioavailability to the eggs of ingested PFAAs adsorbed to the clay content, is further supported by the observed significantly positive interaction between clay content and pH linked with higher egg concentrations. Higher pH levels result in increased deprotonation of pH-dependent surface charges on the clay mineral and TOC surface, which promotes binding of positively charged di- and trivalent cations onto the clay matrix (Wang et al., 2023). On their turn, these cations can interact with negatively charged PFAAs via cation bridging and ligand exchange mechanisms (Li et al., 2018; You et al., 2010). Indeed, exchangeable Mn^{2+} and Fe^{3+} were positively correlated with egg concentrations for major PFAAs (Fig. 3) and also showed strong positive interactions with clay content and pH, respectively (Fig. S5). Moreover, exchangeable Mn^{2+} correlated strongly and significantly with clay content while no significant correlations were found between these cations and TOC (Fig. S2). This could imply that the PFAA fraction sorbed to the clay content is larger than the fraction sorbed to the TOC, which would also explain the larger statistical effect sizes of the relationships between clay content and most PFAAs (Fig. 3) Notably, the statistical interaction effect between pH and clay content tended to increase with increasing chain-length, which may indicate that electrostatic sorption increases with increasing chain-length (Cai et al. 2022). Then, when the ingested clay particles are absorbed from the digestive tract to the liver, a proportionally larger amount of longer chain PFAAs may be transferred to the eggs.

Unexpectedly, for the exchangeable mineral cations (Ca^{2+} and Mg^{2+}) and Al^{3+}, which nevertheless show similar electrostatic interactions as described earlier for the metal cations (Li et al., 2018), significantly negative associations were found with egg concentrations for several compounds. The soil chemistry of the chicken enclosures in the present study could be characterized by a mean pH of 6.58, combined with large concentrations of exchangeable Ca^{2+} and PO_4^{3-} (resp. mean 15.9 meq/100 g soil and 20.7 mg/kg soil). Under these specific soil conditions, which are typical for soils impacted by grazing of laying-hens (Soares et al., 2022), formation of precipitated $CaPO_4^{3-}$ and $MgPO_4^{3-}$ complexes is promoted (Shen et al., 2011). These complexes can potentially repulse PFAAs through competition for binding sites (Qian et al., 2017), leading indirectly to lower bioavailability to the laying-hens and hence lower egg concentrations. This hypothesis is partly supported by the observed positive and strong correlation between Ca^{2+} and PO_4^{3-} (Fig. S2), although such correlations with PO_4^{3-} were absent for Mg^{2+} and Al^{3+}. However, all of these three cations were significantly positively correlated with TOC (Fig. S2). Therefore, it is also plausible that the negative associations between these exchangeable cations and egg concentrations are, in fact, a reflection of the negative relationship between TOC and egg concentrations. In other words, soils with higher TOC levels also contained higher exchangeable Ca^{2+}, Mg^{2+} and Al^{3+} levels.

In conclusion, although the sorption behavior of PFAAs with soil solid components has been relatively well described (Cai et al., 2022), very little is known to date on how these soil ad- and desorption mechanisms can affect bioavailability of PFAAs in terrestrial organisms, including laying-hens. Nevertheless, it is well known for other pollutants (e.g. metals, PCBs and dioxins) that varying amounts of organic matter, clay content and other soil characteristics can have a profound effect on the bioavailability to geophageous animals (Delannoy et al., 2015; Waegeneers et al., 2009; Zhang et al., 2019). Due to the amphiphilic properties of PFAAs, the present study emphasizes that multiple soil properties may affect the bioavailability of these compounds to terrestrial organisms. Evidently, the interplay of the suggested mechanistic soil interactions in the present study should be further elucidated under controlled lab conditions. With respect to the electrostatic clay content-PFAA interactions, this can be achieved by *in vitro* digestion models with simulated pH conditions of those found in the chicken stomach (pH = 3–4) and in the digestive tract (pH = 6.5) (Waegeneers et al., 2009). Alternatively, but more demanding from a practical point of view, semi-controlled lab experiments could be conducted with exposure of chickens to equally spiked soil concentrations but varying soil physicochemical properties. Within this setting, measurements in faeces and eggs of the exposed chickens could further elucidate to which extent PFAAs are bioavailable to the eggs.

4.4. Potential remediation implications

In comparison to eggs from commercial origin, which often contain non-detectable PFAA concentrations (Zafeiraki et al., 2016), it is clear that homegrown eggs are generally more susceptible to PFAA contamination. The present study shows that the soil can play both directly (i.e. food source) and indirectly (i.e. through soil-PFAA physicochemical interactions and as medium for prey, such as earthworms) a crucial role in this exposure context. Particularly, as the clay mineral fraction is associated with higher egg PFAA accumulation, it could be useful to introduce a sand parcel within the chicken enclosure as a readily applicable and relatively cheap measure. PFAAs show only very weak interactions with quartz (SiO_2), the main component of sand, which are readily desorbed with rainfall (Hellsing et al., 2016). In addition, sandy soils tend to contain lower amounts of soil invertebrates, including earthworms (Bedano et al., 2016), which could be identified as a significant exposure source of some PFAAs to the laying hens. Thus, considering that soil and soil physicochemical characteristics often explained > 50% of the total variation in egg concentrations of abundant PFAAs in eggs, implementation of these measures could result in a substantial decrease in homegrown egg concentrations.

Notably, rain water PFAA concentrations were not significantly related with egg concentrations for any of the compounds in the present study. Although most target PFAAs were frequently detected in rain water, relatively low concentrations (mean ranged from < LOQ – 0.034 ng/ml, Table 2) were measured compared to the soil or feed concentrations. Moreover, laying hens have an average drinking water intake of 185 mL per day (Howard, 1975), while soil and feed intake during foraging can be up to 35 g per day (Kijlstra, 2004), which implies that the intake of PFAAs via water would be negligibly small compared to the feed. Furthermore, it should be noted that some chicken owners did also provide tap water to the laying hens, especially during dry summer periods at the time of sampling. Nevertheless, it should be noted that the relative importance of exposure sources to terrestrial biota is generally site-specific. In the present study, a considerable part of the locations had been prone to industrial emissions and deposition onto the soil, which may have masked the role of water in the PFAA exposure to the free-ranging laying hens. In study areas with another PFAA contamination history, rain water can still be an important exposure source, for instance at study sites with accidental release of PFAAs into the air.

5. Conclusions

In the present study, we successfully developed and validated empirical models that accurately predict homegrown egg concentrations for some environmentally widespread and abundant PFAAs, e.g. PFOS, PFOA and PFNA. Based on these model outcomes, we proposed regulatory implications as part of time and cost-effective risk assessment of PFAAs in homegrown food, which is a dominant human exposure source of PFAAs. The present study highlighted that soil can be a major exposure source of PFAAs to free-ranging laying hens and that accumulation in homegrown eggs from soil intake is highly dependent on the internal bioavailability of the compounds, which is likely influenced by the interaction type (hydrophobic versus electrostatic) of PFAAs with the soil component (organic versus mineral composition) and potentially governed by the soil pH and exchangeable cations. The constructed predictive models of the present study can be further refined in future research efforts with additional data of other potential exposure pathways (e.g., dust ingestion and inhalation) and by evaluating the applicability of the models in regions with other PFAA contamination sources. Important local remediation measures were formulated to substantially lower the PFAA exposure to free-ranging laying hens and hence lower human exposure via homegrown egg consumption.

CRediT authorship contribution statement

Robin Lasters: Conceptualization, Investigation, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Kevin Van Sundert:** Formal analysis, Writing – review & editing. **Thimo Groffen:** Conceptualization, Investigation, Writing – review & editing. **Jodie Buytaert:** Investigation, Writing – review & editing. **Marcel Eens:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Lieven Bervoets:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

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

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary material

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