nature portfolio

Peer Review File

Unravelling the magnitude and drivers of PFAS trophic magnification: a meta-analysis

Corresponding Author: Mr Lorenzo Ricolfi

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This study investigated trophic magnification factors (TMFs) for per- and polyfluoroalkyl substances through a meta-analysis of terrestrial and aquatic food webs. A systematic literature search was conducted, and relevant data was extracted from 64 screened studies for 72 PFAS. From these data, TMFs were calculated as the antilog of log-concentration versus trophic level regression slopes, and statistical modelling and analysis were performed to generate an average TMF for all PFAS as well as individual compounds. The model was also used to evaluate potential predictors of influence on TMFs, including methodological, biological, environmental, and chemical factors. The results of the multilevel meta-analytical model yielded a statistically significant average TMF of 2 for all PFAS investigated, and TMF variability was primarily associated with differences between PFAS compounds, as well as methodological differences such as sample type (whole organism or tissue specific) and lipid or protein normalization of PFAS concentrations.
This study has many strengths including a good sample size of studies from across the globe, clearly laid out hypotheses/study rationale, and key results were accompanied by relevant statistics and quantitative descriptions. Additionally, this meta-analysis highlights that F-53B bioaccumulates more readily than the PFAS it was designed to replace. While the study investigated many suitable and interesting factors that influence bioaccumulation, the authors modelled carbon chain length against TMF instead of perfluoroalkyl chain length. This is an oversight and should be included as a factor since most recent literature correlates perfluoroalkyl chain length with bioaccumulation rather than carbon chain length. Additionally, the main conclusion that PFAS concentrations double with each trophic level is somewhat misleading and could lead to the overestimation of risk for some PFAS and an underestimation of risk for others. PFAS as a class encompasses a wide range of compounds with notable differences in chemical structure and is defined differently by governments. As demonstrated by their model, TMF values vary significantly between different PFAS compounds (Figure 2), and assigning an average TMF value for all PFAS compounds may result in underestimation or overestimation of PFAS concentrations in organisms, contingent on which compounds the organisms are exposed to. Providing a range of TMFs for PFAS compounds as opposed to an average would provide more insight into the variance associated with PFAS type, as well as promote the use of appropriate TMF values in decision making processes. In addition, due to there being numerous definitions of PFAS, it would be helpful for the authors to provide a specific definition for their class of PFAS. Thus, we suggest that the authors consider avoiding treating PFAS as a class in this manuscript as it has a wide range of issues which have been discussed through many reviews and publications such as DOI: 10.1002/etc.5182. However, overall our comments are relatively minor and we do feel this manuscript represents a substantial effort on the part of the authors and presents an interesting and helpful analysis of the available data.

Specific comments:
Abstract:
48-50: This statement is somewhat misleading. Recommend providing a range of TMF values calculated for individual compounds, highlighting differences between PFAS compounds as a key variability driver.
Introduction:
95-97: Please provide more information regarding “PFAS as a class”. There is substantial disagreement between the definition of PFAS as a class and we suggest that at the very least these authors include their definition.
Table 1:
This table is great, and it is extremely helpful to show the different factors/hypotheses in this manner. Typically, a strong relationship is seen between perfluoroalkyl chain length and bioaccumulation, not carbon chain length as currently analyzed in this study. Please conduct the analysis also looking at perfluoroalkyl chain length rather than just the number of carbons

(e.g. PFOA = 7, PFOS = 8, not PFOA and PFOS = 8)

Results:
127-129: Recommend incorporating this language into lines 48-50 to provide more clarity surrounding the interpretation of an average TMF of 2.
138-148: Recommend moving discussions surrounding PFAS type to “Biological, environmental, and chemical factors”, where carbon chain length and PFAS class is discussed.
149-152: Recommend moving discussions surrounding differences in geography to “Biological, environmental, and chemical factors”, where other geographic factors are discussed (ex: latitude, line 213).
152-154: Recommend moving discussions surrounding ecosystem types to “Biological, environmental, and chemical factors”, where other environmental factors are discussed (Ex: lines 204-211).
204-238: Recommend organizing this text so that discussions of biological, environmental, and chemical factors are grouped together.
Discussion:
298-301: If 30% of TMF variability is attributed to compound-specific differences, is it is not sensible for a single TMF value to be applied for all compounds categorized as PFAS? For example, shorter chained PFAS like PFPeA/PFHpA/PFBS/PFHxA have a TMF < 1 and their relative risk would be overestimated, while other PFAS of concern like PFOS/PFDA/PFNA have a TMF >2 and their relative risk would be underestimated.
433-436: Specify the TMF is average.

General comments:
Can the authors comment on the aquatic data being freshwater and/or salt water? Was there and difference between marine and freshwater.
Combine SI excel files into 1 sheet, provide a notes tab to explain the tabs, I might have missed this but include a column somewhere flagging which studies had to have TMFs calculated by the authors of the meta-analysis vs those which were calculated by the cited study (method S5). Include why the natural log and the log10 were used in calculations (assuming its due to the curve it was extracted from but including this is helpful.

(Remarks on code availability)
I can confirm that the code is available and that I can open it up in R.

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)
I was able to access the code and open it within R.

Reviewer #3

(Remarks to the Author)
This is an excellent manuscript that I think will make an important contribution to the scientific literature in foodweb ecology, ecotoxicology, meta-analysis research and the regulation of PFAS and other chemicals on a global scale. The methods are clearly explained and thoroughly reported in the main manuscript, extensive supplementary material with open course code and data sets on GitHub.

The noteworthy results are that PFAS concentrations double with trophic level, as the title describes, but also that these patterns were consistent irrespective of food web types and analytical approaches. The variation in TMF with different PFAS compounds is an important finding that has direct implications for how these chemicals are managed and regulated. This work will be an important publication in this field as it highlights the importance of achieving consistent analytical methods (i.e. whole body versus tissue-specific concentrations) and sampling strategies (e.g. normalising for protein or lipid content). It also highlights the significant knowledge gaps in regions of the world where there are no studies (e.g. southern Hemisphere). The distinction between the different PFAS chemicals and discussion about how this links to sources and regulations makes this an important paper for publication.

I think the data and analysis supports the conclusions. The authors discuss the data limitations and provide reasonable recommendations that will assist in pathways for future research. I don’t have any suggestions on how the data analysis, interpretations or conclusions can be improved. This is high quality research publication. The meta-analysis approach from creating a data set to statistical modelling and checking for publication bias is sound. These are comprehensive and meet the standards of open, transparent, and reliable science.

I feel confident in saying that there is enough detail provided in the methods for the work to be reproduced. The project plan was pre-registered, all search terms and record screening approaches are reported in the supplementary material as well as the code and data sets publicly available on GitHub.

(Remarks on code availability)
I have read the README file and information about the different directories in GitHub. The resources are clearly presented and are a usable resource for the science community. I was able to run the code using the .Rmd files, which included plenty of documentation and code descriptions. I enjoyed reading through the the rendered .html files and I expect users will find these most useful.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The revised manuscript and the authors response to comments are good and the revised manuscript has been strengthened by the review process. I look forward to citing this paper.

(Remarks on code availability)
Code still works

Reviewer #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
The authors have addressed all my comments.

(Remarks on code availability)

Manuscript Number: NCOMMS-25-29154-T

Reviewers' Comments to the Authors:

Reviewer #1

This study investigated trophic magnification factors (TMFs) for per- and polyfluoroalkyl substances through a meta-analysis of terrestrial and aquatic food webs. A systematic literature search was conducted, and relevant data was extracted from 64 screened studies for 72 PFAS. From these data, TMFs were calculated as the antilog of log-concentration versus trophic level regression slopes, and statistical modelling and analysis were performed to generate an average TMF for all PFAS as well as individual compounds. The model was also used to evaluate potential predictors of influence on TMFs, including methodological, biological, environmental, and chemical factors. The results of the multilevel meta-analytical model yielded a statistically significant average TMF of 2 for all PFAS investigated, and TMF variability was primarily associated with differences between PFAS compounds, as well as methodological differences such as sample type (whole organism or tissue specific) and lipid or protein normalization of PFAS concentrations.

This study has many strengths including a good sample size of studies from across the globe, clearly laid out hypotheses/study rationale, and key results were accompanied by relevant statistics and quantitative descriptions. Additionally, this meta-analysis highlights that F-53B bioaccumulates more readily than the PFAS it was designed to replace. While the study investigated many suitable and interesting factors that influence bioaccumulation, the authors modelled carbon chain length against TMF instead of perfluoroalkyl chain length. This is an oversight and should be included as a factor since most recent literature correlates perfluoroalkyl chain length with bioaccumulation rather than carbon chain length. Additionally, the main conclusion that PFAS concentrations double with each trophic level is somewhat misleading and could lead to the overestimation of risk for some PFAS and an underestimation of risk for others. PFAS as a class encompasses a wide range of compounds with notable differences in chemical structure and is defined differently by governments. As demonstrated by their model, TMF values vary significantly between different PFAS compounds (Figure 2), and assigning an average TMF value for all PFAS compounds may result in underestimation or overestimation of PFAS concentrations in organisms, contingent on which compounds the organisms are exposed to. Providing a range of TMFs for PFAS compounds as opposed to an average would provide more insight into the variance associated with PFAS type, as well as promote the use of appropriate TMF values in decision making processes. In addition, due to there being numerous definitions of PFAS, it would be helpful for the authors to provide a specific definition for their class of PFAS. Thus, we suggest that the authors consider avoiding treating PFAS as a class in this manuscript as it has a wide range of issues which have been discussed through many reviews and publications such as DOI: 10.1002/etc.5182. However, overall our comments are relatively minor and we do feel this manuscript represents a substantial effort on the part of the authors and presents an interesting and helpful analysis of the available data.

Author response:

We thank the reviewers for this insightful and constructive feedback. We have revised the manuscript to address these important concerns. Specifically, we have:

- Modified the manuscript title to improve clarity and accessibility by replacing technical jargon with plain language while avoiding overgeneralisation of the study's average finding.
- Made minor changes to the Abstract to avoid overgeneralisation of the study's average finding.
- Added a sentence that provides an explicit definition of PFAS (as a group) used in our study.
- Grouped results on research methodological factors and biological, environmental and chemical factors to improve readability.
- Clarified in the Discussion that the statement "PFAS concentrations double with each trophic level" refers to the average TMF across all PFAS investigated.
- We conducted an additional analysis using perfluoroalkyl chain length as a predictor of change in response to a reviewer's suggestion. While the results are presented in the Results section for transparency, we chose not to interpret them in the Discussion because this analysis was not pre-registered in our research protocol, in accordance with best practices for hypothesis-driven research.
- We conducted an additional analysis using aquatic ecosystem type as a predictor of change in response to a reviewer's suggestion. While the results are presented in the Results section for transparency, we chose not to interpret them in the Discussion because this analysis was not pre-registered in our research protocol, in accordance with best practices for hypothesis-driven research.

- Added a statement in the Discussion acknowledging the limitations of treating PFAS as a class and cautioning against generalizations across structurally distinct compounds.

A detailed response to the reviewers' comments and point-by-point explanation is provided below. We are grateful for the reviewer's suggestions, which have strengthened the clarity and robustness of our conclusions.

Specific comments:

Abstract

1. 48-50: This statement is somewhat misleading. Recommend providing a range of TMF values calculated for individual compounds, highlighting differences between PFAS compounds as a key variability driver.

   Author response: As suggested, we revised this sentence in the Abstract (lines 50–52) to clarify that the reported doubling of PFAS concentrations per trophic level reflects the average TMF across all compounds and that we observed considerable variation across compounds. We now explicitly state the range of individual PFAS TMFs (e.g., from <1 to >3) and highlight that compound identity is a key driver of variability (lines 322-323). This change improves transparency and aligns with the more nuanced interpretation of our findings. Furthermore, we modified the manuscript title to improve clarity.

   Previous title: "A meta-analysis reveals PFAS concentrations double with each trophic level".

   Current title: "Magnitude and drivers of PFAS trophic magnification".

   Lines 50-52 previously: "PFAS concentrations systematically doubled with each trophic level increase (mean TMF=2.00, 95% CI:1.64-2.45), confirming widespread biomagnification across ecosystems."

   Lines 50-52 now: "On average, PFAS concentrations doubled with each trophic level increase (mean TMF=2.00, 95% CI:1.64-2.45), though magnification varied considerably by compound."

   Lines 319-328 previously: "We found strong evidence for the amplification of PFAS contamination as it moves up the food chain. Notably, when aggregating results from different chemicals (n = 72) and ecosystems (n = 122), we found that, on average, PFAS concentrations doubled with each increase in trophic level. "

   Lines 319-328 now: "We found strong evidence for the amplification of PFAS contamination as it moves up the food chain. Notably, when aggregating results from different chemicals (n = 72) and ecosystems (n = 122), we found that, on average, PFAS concentrations doubled with each increase in trophic level. However, we observed substantial variation among individual compounds. While F-53B and PFOS showed TMFs exceeding 3, others were below 1 (see Fig. 2B and Fig. S1). ”

Introduction

2. 95-97: Please provide more information regarding “PFAS as a class”. There is substantial disagreement between the definition of PFAS as a class and we suggest that at the very least these authors include their definition.

   Author response: We thank the reviewer for highlighting this important point. We have now included a clear definition of PFAS in the Introduction (lines 97–108). This clarification outlines the scope of compounds considered in our study, including perfluoroalkyl acids (PFAAs), their precursors, and several replacement chemicals.

   Lines 97–108 previously: "By synthesising fragmented evidence into predictive models, this meta-analysis delivers definitive trophic magnification values for PFAS as a class and individual compounds. Doing so establishes a benchmark for harmonising future research and policy, bridging the divide between ecological theory and actionable chemical regulation."

   Lines 97–108 now: "By synthesising fragmented evidence, this meta-analysis delivers trophic magnification values for PFAS, defined here as organic compounds containing at least one perfluorinated methyl or methylene carbon atom, including perfluoroalkyl acids (PFAAs), their precursors with partially fluorinated chains (e.g., fluorotelomer-based compounds), and several replacement substances such as 6:2 chlorinated polyfluorinated ether sulfonate (F-53B) and 4,8-Dioxa-3H-perfluorononanoic acid (ADONA; for a glossary of PFAS acronyms, see Data S3). By providing results for PFAS both as a chemical group and as individual substances, this approach offers comprehensive insights into compound-specific and class-level biomagnification potential, thereby establishing a benchmark for harmonising future research and policy and bridging the divide between ecological theory and actionable chemical regulation.”

3. Table 1: This table is great, and it is extremely helpful to show the different factors/hypotheses in this manner. Typically, a strong relationship is seen between perfluoroalkyl chain length and bioaccumulation, not carbon chain length as currently analyzed in this study. Please conduct the analysis also looking at perfluoroalkyl chain length rather than just the number of carbons (e.g. PFOA = 7, PFOS = 8, not PFOA and PFOS = 8)

Author response: As recommended, we have repeated the analysis using perfluoroalkyl chain length. This reanalysis has been incorporated into the Results (lines 216–220), and the revised model outcomes are shown in Figure S3. Information about the perfluoroalkyl chain length for each PFAS is now reported in the supplementary file "Data_S3" under the column "perfluoroalkyl_ccl". We found that the length of the perfluoroalkyl chain performs comparably to the total carbon chain length in explaining TMF variability. Please note that Table 1 was not modified as it includes predictors listed *a priori* and pre-registered in the research protocol. Instead, the analysis of perfluoroalkyl chain length was added as an additional analysis.

Lines 216–220: "Additionally, the perfluoroalkyl chain length, defined as the number of fully fluorinated carbon atoms ($-CF_2-$ and $-CF_3$ groups) in the linear alkyl chain of a PFAS molecule, excluding any non-fluorinated carbon atoms that are part of the functional group (e.g., carboxylic acid $-COOH$), was not correlated with TMF values ($F(df1 = 1, df2 = 977) = 1.3892$, $p = 0.2388$; Fig. S3)."

Results

4. 127-129: Recommend incorporating this language into lines 48-50 to provide more clarity surrounding the interpretation of an average TMF of 2.

   Author response: We agree and have now revised the Abstract (lines 50-52) to explicitly state that the doubling effect represents an average across all compounds and food webs, and is subject to significant inter-compound variability.

   Lines 50-52 previously: "PFAS concentrations systematically doubled with each trophic level increase (mean TMF=2.00, 95% CI:1.64-2.45), confirming widespread biomagnification across ecosystems."

   Lines 50-52 now: "On average, PFAS concentrations doubled with each trophic level increase (mean TMF=2.00, 95% CI:1.64-2.45), though magnification varied considerably by compound."

5. 138-148: Recommend moving discussions surrounding PFAS type to "Biological, environmental, and chemical factors", where carbon chain length and PFAS class is discussed.

   Author response: We thank the reviewer for this organisational suggestion. We have revised the Results section by relocating and grouping discussions of PFAS type (lines 198–220), ecosystem types (lines 221–250), and geographic effects (line 251) into the "Biological, environmental, and chemical factors" section (lines 196–251). We recognise that this restructuring improves the logical flow and readability of the manuscript.

6. 149-152: Recommend moving discussions surrounding differences in geography to “Biological, environmental, and chemical factors”, where other geographic factors are discussed (ex: latitude, line 213).

   Author response: We thank the reviewer for this organisational suggestion. We have revised the Results section by relocating and grouping discussions of PFAS type (lines 198–220), ecosystem types (lines 221–250), and geographic effects (line 251) into the “Biological, environmental, and chemical factors” section (lines 196–251). We recognise that this restructuring improves the logical flow and readability of the manuscript.

7. 152-154: Recommend moving discussions surrounding ecosystem types to “Biological, environmental, and chemical factors”, where other environmental factors are discussed (Ex: lines 204-211).

   Author response: We thank the reviewer for this organisational suggestion. We have revised the Results section by relocating and grouping discussions of PFAS type (lines 198–220), ecosystem types (lines 221–250), and geographic effects (line 251) into the “Biological, environmental, and chemical factors” section (lines 196–251). We recognise that this restructuring improves the logical flow and readability of the manuscript.

8. 204-238: Recommend organizing this text so that discussions of biological, environmental, and chemical factors are grouped together.

   Author response: We thank the reviewer for this organisational suggestion. We have revised the Results section by relocating and grouping discussions of PFAS type (lines 198–220), ecosystem types (lines 221–250), and geographic effects (line 251) into the “Biological, environmental, and chemical factors” section (lines 196–251). We recognise that this restructuring improves the logical flow and readability of the manuscript.

Discussion

9. 433-436: Specify the TMF is average.

   Author response: We thank the reviewer for this clarification. We now explicitly state that the TMF of 2.00 reflects an average across all PFAS compounds.

   Lines 462-465 previously: "A TMF of 2 indicates that PFAS concentrations double at each trophic level, threatening apex predators and humans and potentially destabilising biodiversity and food web resilience."

   Lines 462-465 now: "An average TMF of 2 indicates that, across all compounds and ecosystems analysed, concentrations double at each trophic level, threatening apex predators and humans and potentially destabilising biodiversity and food web resilience."

General comments

10. Can the authors comment on the aquatic data being freshwater and/or salt water? Was there and difference between marine and freshwater.

 Author response: We appreciate this suggestion. We have now included a moderator analysis comparing TMFs between freshwater and marine ecosystems (see Fig. S5). This comparison revealed no significant difference, as described in the Results (lines 223–225). Please note that Table 1 was not modified as it includes predictors listed *a priori* and pre-registered in the research protocol. Instead, the analysis of aquatic food webs was added as an additional analysis. In addition, we added more information about the percentage of food webs falling into the category of marine, freshwater, and estuarine/tidal/brackish water in the Results (lines 121-123)

 Lines 223–225: " Within aquatic ecosystems, no differences emerged between marine and freshwater ecosystems (TMFcontrast = 0.76, CI = (0.51, 1.11), F(df1 = 1, df2 = 635) = 1.9856, p = 0.1593; Fig. S5)."

 Lines 121-123: "Within the aquatic food webs, 49% were freshwater, 34% were marine, and 17% were estuarine, tidal, or brackish water."

11. Combine SI excel files into 1 sheet, provide a notes tab to explain the tabs, I might have missed this but include a column somewhere flagging which studies had to have TMFs calculated by the authors of the meta-analysis vs those which were calculated by the cited study (method S5). Include why the natural log and the log10 were used in calculations (assuming its due to the curve it was extracted from but including this is helpful.

 Author response: We agree and have now combined the SI excel files into one file. A first "README" sheet provides information on the content of the tabs. The column "Note" in Data_S1 indicates which studies required TMFs to be calculated. We have now included a sentence in the Method section (lines 558-560) that clarifies that we converted TMFs reported using logarithm base 10 to natural logarithms prior to analysis to ensure consistency across effect sizes.

 Lines 558-560: "When TMFs were reported using logarithm base 10, these were converted to natural logarithms prior to analysis to ensure consistency across effect sizes."

Reviewer #2

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Author response: We thank the co-reviewer for their contribution to this manuscript's assessment as part of the Nature Communications Early Career Researcher initiative. We appreciate the time and expertise invested in co-reviewing our study.

Reviewer #3

This is an excellent manuscript that I think will make an important contribution to the scientific literature in foodweb ecology, ecotoxicology, meta-analysis research and the regulation of PFAS and other chemicals on a global scale. The methods are clearly explained and thoroughly reported in the main manuscript, extensive supplementary material with open course code and data sets on GitHub.

The noteworthy results are that PFAS concentrations double with trophic level, as the title describes, but also that these patterns were consistent irrespective of food web types and analytical approaches. The variation in TMF with different PFAS compounds is an important finding that has direct implications for how these chemicals are managed and regulated. This work will be an important publication in this field as it highlights the importance of achieving consistent analytical methods (i.e. whole body versus tissue-specific concentrations) and sampling strategies (e.g. normalising for protein or lipid content). It also highlights the significant knowledge gaps in regions of the world where there are no studies (e.g. southern Hemisphere). The distinction between the different PFAS chemicals and discussion about how this links to sources and regulations makes this an important paper for publication.

I think the data and analysis supports the conclusions. The authors discuss the data limitations and provide reasonable recommendations that will assist in pathways for future research. I don't have any suggestions on how the data analysis, interpretations or conclusions can be improved. This is high quality research publication. The meta-analysis approach from creating a data set to statistical modelling and checking for publication bias is sound. These are comprehensive and meet the standards of open, transparent, and reliable science.

I feel confident in saying that there is enough detail provided in the methods for the work to be reproduced. The project plan was pre-registered, all search terms and record screening approaches are reported in the supplementary material as well as the code and data sets publicly available on GitHub.

Author response: We thank the reviewer for their positive and encouraging assessment of our manuscript. We are pleased to hear that the methodological transparency, data availability, and analytical approach were appreciated. We are grateful for the recognition of the work's contribution to food web ecology, ecotoxicology, and chemical regulation.

Additional changes

In addition to the changes above, we made the following minor revisions to improve phrasing, spelling, and reporting:

- We have added two additional institutional affiliations for two of the co-authors.
- We changed the phrasing of the following sentence in Table 1 to improve readability and clarity:
    - The sentence before: "There is no clear mechanistic explanation for the greater bioaccumulation potential of acids containing a sulfonyl functional group. Nevertheless, Jones et al.[26] showed that sulfonic acids bind strongly to proteins and thus could show higher bioaccumulative potential."
    - The sentence now: "There is no clear mechanistic explanation for the greater bioaccumulation potential of acids containing a sulfonyl functional group. Nevertheless, Jones et al.[26] demonstrated that sulfonic acids bind strongly to proteins and thus may exhibit higher bioaccumulative potential."
- We corrected a typographical error (line 321):
    - The sentence before: "Notably, when aggregating results from different chemicals (n = 75) and ecosystems (n = 119), we found that, on average, PFAS concentrations doubled with each increase in trophic level."
    - The sentence now: "Notably, when aggregating results from different chemicals (n = 72) and ecosystems (n = 119), we found that, on average, PFAS concentrations doubled with each increase in trophic level."
- We changed some American spellings to British spellings (e.g., 'analyzed' to 'analysed') for consistency (Lines 169, 176, 178, 179, and Tab. 1).
- We corrected the number of food webs included in our main model, which was reported as 122 and is now accurately reported as 119 (lines 48, 92, 119, 130).
- We provided more information on the predictive capacity of our models:

- Lines 286-289 before: “Notably, the full model accounted for 84% of the variation in the dataset ($R^2 = 0.837$)”.
- Lines 286-289 now: “Notably, our model explained 85% of the variation in TMFs when accounting for both fixed and random effects (conditional $R^2 = 0.85$), indicating strong predictive capacity. The fixed effects alone explained around 18% of the variance (marginal $R^2 = 0.18$).”

- The numbering of supplementary figures from Figure S4 onward was shifted by two to accommodate the additional figures resulting from the new additional analyses suggested by the reviewers.