INTRODUCTION

Stable isotope analysis (SIA) has become a widely used tool in a variety of ecological and environmental applications. Elements for which stable isotope ratios have been analysed and applied in ecological studies include carbon, nitrogen, hydrogen, oxygen and sulphur. In this context, typically the ratio of heavy to light isotope of a selected element is determined by isotope-ratio mass spectrometry and is then related to that of the relevant international standard material, with the data expressed using the $\delta$ (per mil) notation. The utility of SIA in studies of food web structure in ecosystems (e.g. Jensen et al., 2012) has been greatly enhanced by the use of mixing
models to help identify and quantify food web linkages. Simple stable isotope mixing models can yield very precise outputs for limited circumstances, such as a single consumer using two or three alternative diet sources (Phillips, 2001). However, in practice, most ecological applications of SIA involve much more complex circumstances where a degree of uncertainty is inevitable, and to help deal with these cases. Bayesian mixing models such as, MixSIR, SIAR, simmr and MixSIAR (Moore & Semmens, 2008; Parnell et al., 2010; Stock et al., 2018) have been developed and widely applied. Nevertheless, current models still have several important limitations.

First, available models essentially deal with a single trophic step. Where multiple trophic steps are inherent (e.g. when a secondary consumer ultimately relies on different primary producers), a simple trophic level ‘correction factor’ is then applied. But it is desirable that mixing models can handle multiple trophic steps explicitly, including allowing for ‘latent’ trophic levels. (By latent trophic levels, we mean food web linkages that are known to exist but for which no empirical data are available; for example in aquatic ecosystems when protozoans are included in a consumer network between bacteria and Cladocera even though no isotope data are available for protozoans.) Second, the amount by which the isotope ratio of a particular element changes between diet material and consumer tissue, that is, the trophic discrimination factor (TDF) is a key parameter in mixing models. Available models typically take a constant value for the TDF of a particular isotope based on a broad consensus of ‘average’ values presented in the literature (DeNiro & Epstein, 1977; Deniro & Epstein, 1981). However, this approach ignores the fact that the literature actually displays a wide range of reported trophic discrimination for each isotope (McCutchan Jr et al., 2003; Post, 2002). It also ignores the likelihood that the TDF for any diet may be expected to differ between different food web components. For example, a predator feeding on animal tissue with a biochemical composition similar to its own might be expected to show different isotopic discrimination than a herbivore feeding on plant tissue with a very different biochemical composition from its own. Hence, a mixing model should ideally be able to accommodate specific TDFs for each trophic step. Moreover, existing mixing models typically treat the variance of a consumer’s stable isotope value as an outcome of independent random source measurements as shown in Stock and Semmens (2016), even though this is likely to be an unrealistic assumption. Therefore, it would be important to evaluate this approach by testing how correlations between source measurements (parameters $\Omega$ in Equation 2) affect the end results. Third, with the increased availability of rapid and inexpensive analytical facilities, many studies now routinely analyse the ratio between heavy and light hydrogen stable isotopes ($\delta^2$H) and mixing models should be able to take advantage of this additional information. However, the application of available mixing models in aquatic studies is particularly affected by uncertainties regarding the proportion of H in aquatic consumer tissue that is derived from environmental water rather than from diet (hereafter $\omega$) (but see Solomon et al., 2011). Finally, many ecological studies necessarily involve small sample sizes with little or no replication, so it is desirable that a stable isotope mixing model should be capable of coping with small sample sizes.

During a project investigating variations in food web structure in lakes, we collected a large quantity of data comprising carbon, nitrogen and hydrogen stable isotope ratio values from multiple food web components from around 50 Finnish lakes with different characteristics. These data appeared too complex to be analysed satisfactorily using any existing stable isotope mixing model given the issues outlined above. We therefore developed a modified model which aims to overcome these limitations and which can be run with data that include stable isotope ratios of multiple elements, multiple trophic steps and small sample sizes.

Parnell et al. (2013) reviewed the use of Bayesian stable isotope mixing models for estimating diet proportions and how proportions are affected by different covariate variables. Stock et al. (2018) introduced a flexible framework for Bayesian stable isotope mixing models with one trophic step called MixSIAR. Our work is essentially an expansion of the MixSIAR model for two measured trophic levels where latent trophic links between measured consumers are allowed. In acknowledgment of this background, our modified model is hereafter referred to as two trophic level- or 2TL-MixSIAR. Multiple food web models have been simultaneously modelled earlier by Kadoya et al. (2012), who used a simple isotope model to find limitations of estimating the whole food web when the structure of the food web might not be known beforehand. In contrast, our approach assumes that the structure of the food web is known beforehand but the model considers multiple sources of uncertainty.

Multiple trophic levels have previously been included in Bayesian stable isotope mixing models by Solomon et al. (2011) and we have followed the same principles. The main potential benefit of building a model with multiple trophic levels is efficient use of data. However, when applying such models to data from aquatic food webs, the parameter $\omega$ influences consumer isotope values in multiple levels. Brett et al. (2018) argued that assumptions about $\omega$ have a critical impact on mixing model results in aquatic studies. A model using data from two consumer levels at the same time incorporates more information, which could lead to more realistic estimates of such uncertain parameters and assumptions’ reduced impact on model outputs. In addition, one may have prior information about higher level diet proportions, such as stomach content data for fish, that can also help in estimating diet proportion of a consumer at a lower level. Finally, to get estimates of 2nd level consumption of original sources, we need to model somehow the network between 2nd level consumers and their sources.

In our proposed 2TL-MixSIAR model, informative prior distributions and some simplified assumptions are used for several parameters. Therefore, with simulation experiments, the sensitivity of the posterior distributions to these restrictions can be tested. For data manipulations and analyses, we have used the statistical computing software R (R Core Team, 2019) and for fitting Bayesian models we have used the probabilistic programming language Stan (Carpenter et al., 2017; Stan Development Team, 2019). Here, we first present the stable isotope mixing model with all mathematical notations. We continue with the simulation study to test the robustness of the model with different scenarios and compare these results to those
obtained by present state-of-the-art modelling tool, namely MixSIAR. Finally, we evaluate the benefits and limitations of the new model.

2 | MODEL DESCRIPTION

2.1 | The setup of measurements with some notations

In our setup, the measurements related to sources and consumers are measured from a habitat (a lake for example). We have general symbols for tracer values: $X$ stands for tracer value of a source, $Y$ for tracer value of a 1st trophic level consumer and $Z$ for tracer value of a 2nd trophic level consumer. We have three tracers $j = 1, 2, 3$, which correspond to, for example, stable isotope ratios for carbon (C), hydrogen (H) and nitrogen (N). Moreover, we assume that we have three sources $k = 1, 2, 3$, which are, for example, pelagic phytoplankton, benthic algae and terrestrial plant detritus, respectively (see illustration in Figure 1). Besides tracer values for sources and consumers, the symbol $E$ stands for feature value of a habitat. Below, we introduce more specific notations when considering a source model (Section 2.2, Table 1), a 1st trophic level consumer model (Section 2.3, Tables 2 and 3) and a 2nd trophic level consumer model (Section 2.4, Table 4).

2.2 | Source model

Here, $X_{jk}$ stands for a random tracer $j$ value (i.e. $\delta^{13}C$, when $j = 1$) for a source $k$ from a habitat $h$ in a sample $i$. Habitats are numbered by the index $h = 1, ..., H$, where $H$ is the number of different habitats.

The number of samples per habitat, $l_h$, may vary between habitats and $i = 1, ..., l_h$. Consider a sample $i$ of a habitat $h$, and $K$ sources. A vector $X_{jh} = [X_{j1h}, ..., X_{jkh}]^T$ consists of values for all sources, and it is assumed to follow a multivariate normal distribution. When considering the case $K = 3$, we have, for example,

$$X_{jh} \sim N_3(\mu_{jh}, \Sigma_j),$$

(1)

where $\mu_{jh}$ stands for habitat-specific mean values and

$$\Sigma_j = \begin{bmatrix} \sigma_{j1}^2 & 0 & 0 \\ 0 & \sigma_{j2}^2 & \Omega_j^x \\ 0 & 0 & \sigma_{j3}^2 \end{bmatrix},$$

(2)

for a covariance matrix of different sources for a tracer $j$. In Equation (2), standard deviations $\sigma_{j}^2$ include a measurement error and a (micro-) spatial variation, and $\Omega_j^x$ is a correlation matrix for different sources. Here, the covariance matrix $\Sigma_j$ is assumed to be the same for each habitat within a specific tracer.

Because of the low number of samples, informative prior distributions are needed for within-habitat standard deviation parameters $\sigma_{j}^2$, especially if there is only one sample per habitat, and thus, estimating variance based on data may not be possible. We use informative inverse gamma distributions for parameters $\sigma_{j}^2$. However, for parameters $\Omega_j^x$, we use uninformative LKJ (Lewandowski et al., 2009) distribution.

For tracer $j$, the vector $\mu_{jh}^x = [\mu_{j1h}, \mu_{j2h}, \mu_{j3h}]^T$ has a mean value for each source, and it is treated as a multivariate random effect as follows.

![Diagram of consumer network](image)

**Figure 1** Consumer network in our example. Dashed boxes present sources (tracer value of source $X_{jh}$, where $j$ refers to tracer and $k = 1, 2, 3$ to source). Solid boxes present consumers with measured stable isotope ratios (tracer value of 1st trophic consumer $Y_{j1}$ and tracer value of 2nd trophic consumer $Z_{j1t}$, where $t = 1, 2$ and $tt = 1$ refer to taxon at 1st and 2nd levels, respectively, and $j$ refers to tracer). Ellipses present consumers with no measured stable isotope ratios with trophic discrimination factors $\lambda_{j1s}$, where $t$ refers to taxon, $j$ to tracer, $k = 3$ to source and $s = 2, 3$ to step. For simplicity, habitat $h$, sample $i$ or consumers $c$, $cc$ in the sub-indices were not included in the notations.
TABLE 1 Notations for tracer value $X$ of a source with likelihood distributions and prior distributions for parameters. Informative priors are marked (I). Hyperparameter values for priors are not defined here.

| Notation | Definition |
|---|---|
| $J$ | Tracer index, $j = 1, \ldots, J$ |
| $k$ | Source index, $k = 1, \ldots, K$ |
| $h$ | Habitat index, $h = 1, \ldots, H$ |
| $i$ | Sample index, $i = 1, \ldots, I_h$ |
| $\lambda_{jkh}$ | Tracer $j$, source $k$, habitat $h$ from sample $i$; $N(\mu_{jkh}^*, \sigma_{\mu_k}^*)$ |
| $\mu_{jkh}^*$ | Habitat-specific tracer mean value; $N(\mu_{\mu_k}^*, \Sigma_{\mu_k}^*)$ |
| $(\sigma_{\mu_k}^*)^2$ (I) | Tracer variance; $I - \text{Gamma}(a_{\mu_k}^*, b_{\mu_k}^*)$ |
| $\Sigma_{\mu_k}^*$ | Habitat-specific tracer mean values for sources; $N(\mu_{\mu_k}^*, \Sigma_{\mu_k}^*)$ |
| $\Sigma_{ij}^*$ | Covariance matrix of tracer values for sources, see $\sigma_{\mu_k}^*$ |
| $\Omega_j$ | Correlation matrix of tracer values for sources; $\text{LKJ}(\eta)$ |
| $\mu_{\mu_k}^*$ (I) | Global averages of tracer mean values; $N(\mu_{\mu_k}^*, \Sigma_{\mu_k}^*)$ |
| $(\sigma_{\mu_k}^*)^2$ (I) | Variance of tracer mean value; $I - \text{Gamma}(a_{\mu_k}^*, b_{\mu_k}^*)$ |
| $\Omega_j^*$ | Correlation matrix of tracer mean values; identity matrix |

\[ \mu_{jkh}^* \sim N_3(\mu_{\mu_k}^*, \Sigma_{\mu_k}^*), \]

where $\mu_{\mu_k}^*$ is a vector of global averages and $\Sigma_{\mu_k}^*$ is a covariance matrix with between-habitat standard deviations $\sigma_{\mu_k}^*$. Moreover, for simplifying a complex model structure, habitat means $\mu_{jkh}^*$ are assumed to be independent of each other, thus

\[ \Sigma_{ij}^* = \begin{pmatrix} (\sigma_{\mu_1}^*)^2 & 0 & 0 \\ 0 & (\sigma_{\mu_2}^*)^2 & 0 \\ 0 & 0 & (\sigma_{\mu_3}^*)^2 \end{pmatrix}. \]

With small sample size, informative prior distributions are also needed for $\mu_{\mu_k}^*$ and $\sigma_{\mu_k}^*$ values. We used normal distributions and inverse gamma distributions, respectively. Notations are summarized in Table 1. Note that in the case of raising $\sigma_{\mu_k}^*$ to the power of two, we add parentheses. In the parentheses, Greek letters in superscript is part of the notation, not raising to the power.

In our setup, we assume that there are two levels of consumers in the consumer model (Sections 2.3 and 2.4), which is the main novelty of this work. Any new notations needed are introduced at the beginning of each section.

2.3 First trophic level consumers

2.3.1 Notations of model parameters

A diet proportion of a consumer taxon $t$ from source $k$ in habitat $h$ is denoted by $p_{tkh}^*$, $\sum_{t=1}^{T} p_{tkh}^* = 1$, and a corresponding vector by $\mathbf{p}_{tkh}^* = [p_{1kh}^*, \ldots, p_{Tkh}^*]$. Here, small $x$ refers to sources as a diet. A TDF between diet and a consumer taxon $t$ for tracer $j$ from source $k$ with discrimination $s$ is denoted by $\lambda_{jkh}$. If $s = 1$, it describes the discrimination in a measured consumer. If $s > 1$, it describes the level of latent discrimination between diet and consumer (see Figure 1). The number of trophic level discriminations between a consumer taxon $t$ and measured diet from source $k$ is denoted by $r_{tkh}$. If it has a value 1, this implies no latent trophic discrimination between diet and consumer, otherwise the values in the model are given as prior information.

Parameters $\mu_{tkh}^*$ and $\sigma_{\mu_k}^*$ are the expected value and standard deviation, respectively, of TDF between diet and a consumer for tracer $j$ and step $s$. Individual's TDF-s ($\lambda_{jkh}$) are not known, but their distributions are assumed to be known a priori; $\lambda_{jkh} \sim \text{Gamma}(a_{\lambda_k}^*, b_{\lambda_k}^*)$, where values for parameters $\mu_{tkh}^*$ and $\sigma_{\mu_k}^*$ need to be based on literature and expert knowledge. In the sensitivity analysis in Section 3, for $s = 1$ parameter values are based on McCutchan Jr et al. (2003). For $s > 1$, parameter values are based on the authors' prior knowledge.

Finally, $\xi_{tkh}^*$ is 1st level consumer’s $t$ residual multiplicative error term of tracer $j$. In theory, for perfect integrators (eats ‘everything and everywhere’), $\xi_{tkh}^* = 0$ pushing total consuming variance towards 0 and for perfect specialist (eats only in one specific place in a habitat) $\xi_{tkh}^* = 1$. In reality, this parameter ‘collects’ also other unexplained variability, and thus it is possible that $\xi_{tkh}^* > 1$. As a prior distribution, we used a gamma distribution. See more details in Stock and Semmens (2016).

2.3.2 Consumer model parameters

Tracer values for a 1st tropic level consumer are modelled as equation (4) in Stock and Semmens (2016). When compared with the notation $X_{jkh}$ here a measured individual $Y_{tkh}$ is a consumer $c$ being taxon $t$, instead of a sample $i$ from source $k$, such that $c = 1, \ldots, C_{th}$ and $t = 1, \ldots, T$.

Then, the whole structure is based on diet tracer model as follows (except $j = 2$ in our case, see Equation 10)

\[ Y_{tkh} = \sum_{k=1}^{K} p_{tkh}^* \left( X_{jkh}^c + \sum_{s=1}^{r_{tkh}} \lambda_{jkh} \right). \]
Moreover, in Equation (5), we assume that within latent trophic levels all energy is from a single source. Otherwise, more $\lambda$ parameters are needed.

In these formulations, $X_{jthc}$ and $\lambda_{jks}$ are stochastic variables and other parameters are considered as fixed values. The expected value for $Y_{jthc}$ is then

$$\mu_{jth} = \sum_{k=1}^{K} \lambda_{jkh}^c \left( \phi_{jkh} + \sum_{i=1}^{n} \Phi_{jkh} \right). \quad (6)$$

If

1. $X_{jk}$ and $X_{jk'}$ are independent of each other,
2. $\lambda_{j}$ and $\lambda_{j'}$ terms are independent of each other,
3. $\lambda_{j}$ are independent of $X_{jk}$, an
4. $\text{Var}(X_{jthc}) = \left( \sigma_{\mu_{j}}^2 \right)$ and $\text{Var}(\lambda_{jks}) = \left( \sigma_{\lambda_{j}}^2 \right)$ are independent of $t$, the variance of (5) is $\sum_{k=1}^{K} \left( \sum_{t=1}^{n} \left( \sigma_{\lambda_{j}}^2 \right) \right)$ All of these assumptions may not be valid in some applications. Therefore, the variance is multiplied by $\Phi_{jkh}$, correcting a possible...
TABLE 4 Notations for tracer value $Z$ of a 2nd level consumer and diet tracer model with trophic discrimination factor (TDF) distribution, likelihood distributions, random effect distributions and prior distributions for parameters. Informative priors are marked (I). Hyperparameter values for priors are not defined here.

| Notation | Definition |
|----------|------------|
| $cc$     | 2nd level consumer, $cc = 1, \ldots, C_{th}$ |
| $tt$     | 2nd level consumer taxon, $tt = 1, \ldots, T^t$ |
| $p_{tt,h}^v$ | Diet proportion of taxon $tt$ from taxon $h$ |
| $p_{tt,h}^s$ | Diet proportions of taxon $tt$ in habitat $h$ |
| $\lambda_{j,tt,tt'}$ | TDF between diet and a consumer taxon $tt$ for tracer $j$ from taxon $t$ with step $s = 1$: $N\left(\mu_{j,tt,tt'}^v, \sigma_{j,tt,tt'}^s\right)$ |
| $\mu_{j,tt,tt'}^v$ | Mean value of $\lambda_{j,tt,tt'}$ estimated by literature or by expert knowledge |
| $\sigma_{j,tt,tt'}^s$ | Standard deviation of $\lambda_{j,tt,tt'}$, estimated by literature or by expert knowledge |
| $\xi_{j,tt}$ | 2nd level consumer’s $tt$ multiplicative error of tracer $j$: $\Gamma(a_j^v, b_j^v)$ |
| $Y_{j,tt,h}$ | Diet tracer value $j$ of a consumer taxon $tt$ in habitat $h$ |
| $Z_{j,tt,h,cc}$ | Tracer value $j$ of a consumer taxon $tt$ in a habitat $h$; $N\left(\mu_{j,tt,tt'}^v, \sigma_{j,tt,tt'}^s\right)$ |
| $\mu_{j,tt,h}$ | Tracer mean value of $Y_{j,tt,h}$ |
| $\sigma_{j,tt,h}$ | Tracer standard deviation of $Y_{j,tt,h}$ |
| $l$ | Index used for ILR transformations, $l = 1, \ldots, T^t - 1$ |
| $\Phi_{tt,j}$ | Global average diet proportion of taxon $tt$ from taxon $t$ |
| $\Phi_{tt,j}^v (l)$ | Global average diet proportions; Dirichlet$(a_{tt,j}^v, \ldots, a_{tt,j}^{vl})$ |
| $B_{tt,0}$ | ILR$(\Phi_{tt,j}^v (l))$ |
| $U_{tt,h}^v$ | Random effect: $N\left(0, \sigma_{tt,j}^v\right)$ |
| $\sigma_{tt,j}^v$ | Standard deviation of random effects; HalfCauchy$(0, \sigma_{tt,j}^v)$ |
| $U_{tt,h}^s$ | Random effects when modelling ILR$(p_{tt,h}^s)$ |
| $\Phi_{tt,k}^T$ | Proportion of taxon $tt$ from source $k$ |
| $\Phi_{tt,k}^{TOT}$ | Diet source proportions of taxon $tt$, the vector of $\Phi_{tt,k}^{TOT}$ |

The sensitivity of the assumption (1) is further tested in Section 3. With an assumption of a normal distribution, we have

$$Y_{tt,c} \sim N \left( \mu_{tt,h}^v, \sigma_{tt,h}^s \right)^2, j \in \{1, 3\}. \quad (8)$$

where $h$ is the habitat of individual $c$.

For hydrogen ($j = 2$), the TDF is assumed to be zero in our model, but because environmental water is a significant additional source of $H$ to organism tissue, the proportion of $H$ derived from environmental water needs to be modelled by the parameter $\omega$ (Solomon et al., 2011). First we need to formulate a bias correction for latent trophic level discriminations. Then, consumer tracer values are modelled exploiting information of hydrogen stable isotope values of water in a habitat $h$, notated as $\delta^2H^w_h$.

Let us first consider a simplified case where a consumer has 100% terrestrial diet ($\mu_{tt,h} = 1$) and $t_{13} = 2$:

$$Y_{20bc} = \omega \cdot \delta^2H^w_h + (1 - \omega) \cdot \delta^2H^w_h + (1 - \omega)X_{23bc}$$

which reduces to $\delta^2H^w_h + (1 - \omega)X_{23bc}$ if there is no latent trophic steps ($t_{13} = 1$).

Now expected values and variances for consumer $\delta^2H$ values ($Y_{20bc}$) are given by

$$\mu_{20bc}^v = \sum_{k=1}^K p_{tt,k}^v \left[ \delta^2H^w_h \cdot (1 - (1 - \omega)^n) + (1 - \omega)^n X_{23bc} \right]. \quad (9)$$

The generalized model has a form

$$Y_{20bc} = \sum_{k=1}^K p_{tt,k}^v \left[ \delta^2H^w_h \cdot (1 - (1 - \omega)^n) + (1 - \omega)^n X_{23bc} \right]. \quad (10)$$

With mathematical induction, it is possible to prove that the factor $\omega \sum_{n=1}^\infty (1 - \omega)^{-1} = 1 - (1 - \omega)^n$ (Solomon et al., 2011). Thus, we end up with a model

$$Y_{20bc} = \sum_{k=1}^K p_{tt,k}^v \left[ \delta^2H^w_h \cdot (1 - (1 - \omega)^n) + (1 - \omega)^n X_{23bc} \right]. \quad (11)$$
and

\[
(s'_{2n})^2 = s'_{2n}^2 + \sum_{k=1}^{K} (\rho'_{nk})^2 \left[ (1-\omega)^2 \left( s_{2n}^2 \right) \right].
\]

(12)

Finally, based on an assumption of normality

\[
Y_{2h} \sim N(\mu'_{2h}, (s'_{2n})^2).
\]

(13)

Notations are summarized in Table 2.

2.3.3 Model for habitat-specific diet source proportions

Our aim is to estimate habitat-specific unknown diet source proportions, \( p_{th} \), and to determine whether features measured from each habitat \( h \) are needed as covariates. Before modelling, covariates are standardized to obtain convergence of the estimation, and to obtain easier interpretation of results. The standardized \( V \) covariates, called also features, are notated as \( \mathbf{E}_h = \{ e_{h1}, \ldots, e_{hv} \} \). The model for \( p_{th} \) presented in MixSIAR (Stock et al., 2018) is described next.

When considering a habitat with \( \mathbf{E}_h = 0 \), we denote global average diet proportion of a consumer taxon \( t \) for a source \( k \) by \( \Phi_{kt} = \sum_{h=1}^{H} \Phi_{ht} \). With a vector notation we have, for example, \( \Phi_t^K = [\Phi_{t1}, \Phi_{t2}, \ldots, \Phi_{tK}] \) for \( K = 3 \). As a prior distribution for \( \Phi_t^K \), we use a Dirichlet distribution. Isometric log-ratio (ILR) transformation (Egozcue et al., 2003) is used as a link function between modelled proportions and covariates \( \mathbf{E}_h \) as follows:

1. for global average proportion vector \( ILR(\Phi_t^K) = \beta_{t0} \), and
2. for habitat-specific diet proportions

\[
ILR\left( \Phi_t^K \right) = \beta_{t0} + \beta_{t1} E_{h1} + \beta_{t2} E_{h2} + \ldots + \beta_{tv} E_{hv} + U_{th},
\]

(14)

where \( \beta_s \) are \((K-1)\times 1\) vectors, that is, \( \beta_{t0} \) with elements \( \beta_{t0,1} \) and \( \beta_{tv} \) with elements \( \beta_{tv,1} \), \( l = 1, \ldots, K - 1 \), \( v = 1, \ldots, V \). As priors for \( \beta_s \) we use normal distributions. For parameters \( U_{th} \) prior distributions are not needed, because it is transformation \( ILR(\Phi_t^K) \). Moreover, \( U_{th} \) is a \((K-1)\times 1\) vector of habitat-specific random effects \( U_{th} \), \( l = 1, \ldots, K - 1 \), for taxon \( t \). Random effects are thus defined in ILR-space and assumed to be normally distributed \( U_{th} \sim N\left(0, \sum_{v=1}^{V} (\beta_{tv})^2 \right) \) with half Cauchy distribution for \( \beta_{tv} \). Elements on the left of the model (14) are illustrated in the next example.

When \( K = 3 \), each of \( \beta_s \) vectors has 2 (= \( K - 1 \)) elements. ILR is a transformation, where three-dimensional space is transformed to two-dimensional space where two interesting proportions, \( \frac{p_{th1}}{p_{th2}} \) and \( \frac{p_{th3}}{p_{th2}} \), are modelled with the linear model (14) (Pawlowsky-Glahn et al., 2015).

Notations are summarized in Table 3.

2.4 Second trophic level consumers

2.4.1 Notations for model parameters

Tracer values for 2nd level consumers are modelled as for 1st level consumers but now the diet consists of 1st level consumers instead of sources. The diet model shown later in Equation (15) is based on the two-level structure with the following notations (see also Figure 1). Notation \( Z \) refers to a 2nd level consumer and \( Y \) to a 1st level consumer that is considered as food. Notation \( t \) refers to a taxon id of a 2nd level consumer and \( j \) refers to a taxon id of a 1st level consumer. Notation \( cc \) refers both to an individual 2nd level consumer and its food. More precisely, \( Z_{jtt,ht,cc} \) is the measurement of an individual consumer \( cc \) of taxon \( tt \) for tracer \( j \) in a habitat \( h \), and \( Y_{jth,cc} \) belongs its diet being the measured isotope value of taxon \( t \) for tracer \( j \) in a habitat \( h \).

A diet proportion of a consumer taxon \( tt \) from taxon \( t \) in a habitat \( h \) is denoted by \( p_{tt,h} \geq 0 \). \( \sum_{h=1}^{H} p_{tt,h} = 1 \), and a vector of these proportions is denoted by \( \mathbf{p}_{tt,h} = [p_{t1,h}, \ldots, p_{tt,h}] \). Here, small \( y \) refers to a 1st level consumers. A TDF between diet and a consumer taxon \( tt \) for tracer \( j \) from taxon \( t \) with step \( s = 1 \) is denoted by \( \lambda_{1tt,1} \). For simplicity, the latent trophic steps as in the 1st level are not allowed here.

2.4.2 Consumer model

The model for the 2nd trophic level consumers is similar to the 1st level model in Equation (5)

\[
Z_{jtt,ht,cc} = \sum_{t=1}^{T} p_{tt,h} Y_{jtt,cc} + \lambda_{1tt,1}.
\]

(15)

Expected values and variances for 2nd level consumer tracer values are modelled similarly as at 1st level, see Equations (6) and (7), and Sections 2.3.1 and 2.3.2:

\[
\mu_{jtt,h} = \sum_{t=1}^{T} p_{tt,h} \left( \mu_{jt,1} + \lambda_{1tt,1} \right)
\]

(16)

and

\[
(s_{jtt,h})^2 = \sum_{t=1}^{T} \left( p_{tt,h} \sigma_{jt,1}^2 \right) + \sigma_{1tt,1}^2.
\]

(17)

where \( \mu_{jt,1} \) and \( \sigma_{jt,1}^2 \) are the mean and variance of \( Y_{jth,cc} \), and \( \lambda_{1tt,1}^2 \) and \( \sigma_{1tt,1}^2 \) of \( \lambda_{1tt,1} \), respectively. We assume \( \lambda_{1tt,1} \sim N\left(\mu_{1tt,1}, \sigma_{1tt,1}^2 \right) \). Here, \( \gamma_{jt,tt}^y \) is 2nd level consumer’s \( tt \) residual multiplicative error term of tracer \( j \) and has a Gamma distribution as a prior.

Similarly as for the 1st level (Section 2.3.2), for hydrogen \( j = 2 \) the environmental water proportion factor \( \omega \) needs to be included to the model as follows
Now expected values and variances for consumer delta-hydrogen values are defined as

\[ \mu_{2,h}^\gamma = \sum_{t=1}^T \rho_{tt,h}^\gamma \omega \cdot \delta^2 H_t^\gamma + (1 - \omega) \mu_{2h} \]  
\[ \left( \sigma_{2,h}^\gamma \right)^2 = \sum_{t=1}^T \left( \rho_{tt,h}^\gamma \right)^2 (1 - \omega)^2 \left( \sigma_{2h}^\gamma \right)^2. \] 

Then, based on normal assumption

\[ Z_{j,h} \sim N\left( \mu_{j,h}^\gamma, \left( \sigma_{j,h}^\gamma \right)^2 \right). \] 

The whole model and its parameters are described as a directed acyclic graph in Figure 2. All parameters that are estimated are expressed as circles and information that is given by measured data or fixed parameter values are expressed as squares.

2.4.3 Model for habitat-specific diet proportions

The aim is to estimate the habitat-specific (h) specific diet proportions \( \rho_{tt,h}^\gamma \) for 2nd level consumer taxon tt. Here, global average diet proportions for 2nd level taxon tt is denoted by a vector \( \Phi_t^\gamma = [\Phi_{tt,t}^\gamma, \ldots, \Phi_{tt,T}^\gamma] \), where \( \Phi_{tt,t}^\gamma \geq 0 \), \( \sum_{t=1}^T \Phi_{tt,t}^\gamma = 1 \), describing proportions of consuming each 1st level consumer taxon t = 1, …, T. Proportions \( \rho_{tt,h}^\gamma \) are modelled with the same idea as in Equation (14) but without covariate information, as follows:

\[ \text{ILR}(\rho_{tt,h}^\gamma) = \beta_{ct,t0} + U_{ct,t}^\gamma. \] 

where \( \beta_{ct,t0} = \text{ILR}(\Phi_t^\gamma) \) is a \((T' - 1) \times 1\) vector of \( \beta_{ct,0}, \) \( l = 1, \ldots, T' - 1, \) and similarly \( U_{ct,t}^\gamma \) is a \((T' - 1) \times 1\) vector of habitat-specific random effects \( U_{ct,t}^\gamma \) for taxon tt. Random effects are defined in ILR-space and assumed to be normally distributed \( U_{ct,t}^\gamma \sim N(0, \left( \sigma_{ct,t}^\gamma \right)^2) \). As priors for \( \Phi_t^\gamma \) is used a Dirichlet distribution and for \( \sigma_{ct,t}^\gamma \) a HalfCauchy distribution as earlier with Equation (14).

Finally, source diet contributions of 2nd level consumers are calculated as a product of diet proportions. For an average lake, we obtain

\[ \Phi_{tt}^\text{TOT} = \sum_{t=1}^{T'} \Phi_{tt,t}^\gamma \cdot \Phi_{tt,t}, \] 

where \( \Phi_{tt}^\text{TOT} \) is the vector of \( \Phi_{tt}^\text{TOT}, \) \( tt = 1, \ldots, T', \) \( k = 1, \ldots, K, \) and \( \Phi_{tt,t}^\gamma \) the vector of \( \Phi_{tt,t}^\gamma, \) \( t = 1, \ldots, T', \) \( k = 1, \ldots, K. \)

Notations are summarized in Table 4.

3 PERFORMANCE ANALYSIS

In this section, we evaluate the proposed Bayesian mixing model using simulation experiments, where the parameters of the simulation model are fixed and compared with the point estimates of posterior distributions obtained from simulated datasets. Furthermore, some parameter values and assumptions of the simulation model are varied to evaluate the sensitivity of posterior distribution to these changes.

As a reference structure for the simulation experiments, we use real data of source values and consumers in Finnish lakes. Our aim is not to make inference as such from this real dataset but instead to use its structure in order to get realistic values when generating values from the proposed model. Next, we introduce key features of the real dataset used in the simulation study.

3.1 Structure of the real data and prior knowledge

We have measurements from 46 Finnish lakes, that is, the number of habitats (H) is 46. Beside isotope values, four available lake features are used as explanatory variables (E) in simulation models:

\[ E_{h}: \text{Catchment area/Lake area ratio} \] 
\[ E_{h2}: \text{Shoreline development} \] 
\[ E_{h3}: \text{Dissolved organic carbon (DOC), mg/L} \] 
\[ E_{h4}: \text{Chlorophyll (mg/m³)/DOC ratio}. \]

We have source measurements (X) from each lake, that is, three stable isotope tracer values: carbon (C), hydrogen (H) and nitrogen (N). Typically, these values were measured once for each lake (41 lakes), but 5 lakes, have 3–6 measurements. In addition, hydrogen stable isotope ratios of water were measured. From 43 lakes, we have also the same tracer measurements from three categories of consumers: Cladocerans and Copepods are 1st level consumers (Y) and small perch (<7.5 cm) are a 2nd level consumer (Z).

The exact number of samples from each lake are shown in Table S1. A sample for sources, Cladoceran and Copepoda, is one bulk measurement of large number of homogenized individuals collected from three locations around the lake. For perch, a sample is one fish. For the pelagic source, \( \delta^{13}C \) isotope ratios were estimated from the \( \delta^{13}C \) ratios of lake water dissolved inorganic carbon, \( \delta^{13}N \) from stable isotope ratios of particulate organic matter (POM), and \( \delta^{15}H \) ratios of pelagic source material was based on those measured for epiphytic algae. Dissolved organic matter and POM from lake inlets were used as proxies for the terrestrial source, and epiphytic algae as the benthic source for all the measured stable isotope ratios.

As described in previous sections and tables (Section 2.2, Table 1, Section 2.3, Tables 2 and 3, and Section 2.4, Table 4), we use the following priors divided into informative, uninformative and
weakly informative priors. Informative prior distributions are used for the following parameters:

1. Global means $\mu_{jk}$ have a normal distribution for each pair $jk$, where $j \in \{1, 2, 3\}$ and $k \in \{1, 2, 3\}$. See Table SI2.
2. Standard deviations between lake $\sigma_{jk}^2$ have an Inverse-gamma distribution for each pair $jk$, where $j \in \{1, 2, 3\}$ and $k \in \{1, 2, 3\}$. See Table SI3.
3. Standard deviations within lake $\sigma_{jk}^2$ have an Inverse-gamma distribution for each pair $jk$, where $j \in \{1, 2, 3\}$ and $k \in \{1, 2, 3\}$. See Table SI4.
4. Environmental water proportion parameter $\omega$ follows Beta$(16, 48)$, where parameters $\alpha = 16$ and $\beta = 48$ are based on prior mean of 0.25 and standard deviation of 0.05.
5. Global average diet proportions $\Phi_{tt} \sim Dirichlet(60, 35)$, $tt = 1$, and describe the information of 95 perch stomach samples, where ratio between Cladocera bulk versus Copepod bulk is 60/35.

Uninformative priors are applied for following parameters:

6. Global average diet proportion $\Phi_{tt} \sim Dirichlet(1, 1, 1)$ for $t = 1, 2, 3$.
7. Correlation matrix $\Omega^2 \sim LKJ(1)$ (Lewandowski et al., 2009).

Weakly informative prior distributions are defined as distributions that push a little bit towards sensible values to evade over-fitting with small data (see McElreath, 2015) but also avoiding taking a strong opinion for the following parameters:

8. Regression coefficients $\beta_{tti} \sim N(0, 10)$ (normal distribution with $\mu = 0$ and $\sigma^2 = 10$), where $\nu \in \{0, 1, 2, 3\}$ and $i \in \{1\}$.
9. Standard deviations $\sigma_{tti}^2 \sim HalfCauchy(3, 0)$ (Cauchy distribution with $\mu = 0$, $c = 3$ and support $[0, \infty)$), where $t \in \{1, 2\}$ and $i \in \{1, 2\}$.
10. A standard deviation $\sigma_{tt}^2 \sim HalfCauchy(3, 0)$, where $tt \in \{1\}$ and $i \in \{1\}$.
11. Multiplicative error $\xi_{tt}^r \sim Gamma(2, 2)$ (gamma distribution with shape $= 2$ and rate $= 2$), where $j \in \{1, 2, 3\}$ and $t \in \{1, 2\}$.
12. Multiplicative error $\xi_{tt}^r \sim Gamma(2, 2)$, where $j \in \{1, 2, 3\}$.

For more specific details of the used probability distributions, see Stan Functions Reference (https://mc-stan.org/docs/functions-reference/index.html).

When constructing the data simulation algorithm, we assume the consumer network illustrated in Figure 1 and follow the model illustrated in Figure 2. All parameters of the model need to be fixed when simulating data.

### 3.2 | A reference structure

First, we tested a performance of the proposed model with the so-called reference structure. Parameters of the reference structure are fixed as follows (shown in the same order as the prior distributions above). The values are manually selected representative values of prior distributions.

- The values of the parameters having informative prior distributions (namely $\mu_{jk}$, $\sigma_{jk}^2$, $\sigma_{tt}^2$, $\alpha$, $\omega$ and $\Phi_{tt}$) have been fixed to a representative value from that specific distribution, see Tables SI2–SI4, and Figures SI1–SI4.
- The parameter values with uninformative priors are fixed as follows:

\[
\Phi_{tt} = [\Phi_{t11}, \Phi_{t12}, \Phi_{t13}] = [0.5, 0.1, 0.4]^T
\]

and

\[
\Phi_{tt} = [\Phi_{t21}, \Phi_{t22}, \Phi_{t23}] = [0.7, 0.2, 0.1]^T.
\]

The sub-index $t = 1$ stands for global average diet proportions for Cladocera and $t = 2$ for Copepoda, when $j = 1, 2, 3$. The correlation $\Omega_{jk} = 1, j = 1, 2, 3$.

- We fix the regression parameters

\[
\beta_1 = [\beta_{11}, \beta_{21}] = [1, -1]^T, \\
\beta_2 = [\beta_{12}, \beta_{22}] = [2, 0.1]^T, \\
\beta_3 = [\beta_{13}, \beta_{23}] = [0.1, -2]^T, \\
\beta_4 = [\beta_{14}, \beta_{24}] = [2, -2]^T,
\]

where the sub-index $\nu = 1, \ldots, 4$ refers to the regression coefficients for one covariate $E_{hi}$. Standard deviations $\sigma_{tt}^2$, $t = 1, 2$, $i = 1, 2$, are fixed as follows

\[
[\sigma_{t11}^2, \sigma_{t21}^2] = [1, 0.5], \quad [\sigma_{t21}^2, \sigma_{t22}^2] = [2, 1],
\]

where the left ones are the residual standard errors for the 1st trophic level taxon, Cladocera, and the right ones for Copepoda. A standard deviation $\sigma_{tt}^2$, $tt = 1, i = 1$, is here fixed to be $\sigma_{t11}^2 = 0.5$. All $\xi_{tt}^r$ and $\xi_{tt}^r$ are set to 1 describing perfect specialist consumers.

After fixing the parameters above being the same as shown in circles outside the larger box in Figure 2, the values of parameters shown in circles inside the box in Figure 2 are obtained after transformation and these values are used in the simulation of the values from the likelihood distributions of $X$, $Y$ and $Z$.

Next, we introduce the structures of scenarios, where a part of the reference structure is changed.

### 3.3 | Test structures

To evaluate how sensitive and non-robust the proposed model is to different assumptions and prior knowledge, we repeated the simulation test altering one parameter/sample size at a time and compared
results with the reference structure. The parameters/assumptions that we altered one at a time were:

1. Scenario NoData: modelling is based only on prior distributions and isotope data are not used.
2. Scenario 2N: sample sizes for each habitat and each taxon are double those of the reference structure.
3. Scenario 8N: sample sizes for each habitat and each taxon are eight times those of the reference structure.
4. Scenario UI-PhiY: prior information of 2nd level consumer diet: instead of an informative one (Dirichlet(60, 35) introduced in Section 3.1), we fit the model with an uninformative prior \( \Phi^y \sim \text{Dirichlet}(1, 1) \). Thus, the simulation model is the same as in the case of the reference structure.
5. Scenario CorX: instead of uncorrelated source measurements, we used in the simulation more realistic correlation matrices as follows

\[
\Omega^x_1 = \begin{bmatrix}
1 & 0.31 & 0.25 \\
0.31 & 1 & 0.25 \\
0.25 & 0.25 & 1
\end{bmatrix}, \quad \Omega^x_2 = \begin{bmatrix}
1 & 0.90 & 0.46 \\
0.90 & 1 & 0.46 \\
0.46 & 0.46 & 1
\end{bmatrix},
\]

\[
\Omega^z_1 = \begin{bmatrix}
1 & 0.65 & 0.63 \\
0.65 & 1 & 0.51 \\
0.63 & 0.51 & 1
\end{bmatrix}.
\]

6. Scenario LatentTL: the latent trophic level, that is Protozoa, is included in a consumer network between Bacteria and Cladocera in Figure 1, that is Cladocera actually consume Protozoa not Bacteria.
7. Scenario Omega15: the value of \( \omega \) is fixed to 0.15 being circa two standard deviations lower than expected value 0.25 based on prior distribution Beta(16, 48).
8. Scenario Omega40: the value of \( \omega \) is fixed to 0.40 being circa three standard deviations higher than expected value 0.25 based on prior distribution Beta(16, 48).
9. Scenario UI-Omega: the value of \( \omega \) is fixed to 0.27 as in the reference scenario. But instead of informative prior, uninformative Beta(1, 1) is used as the prior distribution. Thus, the simulation model is the same as in the case of the reference structure.

In the case of test structures, the reference structure is modified in the scenarios 1–3 and 5–8 changing the simulation process of the reference structure. However, in the case of the scenarios 4 and 9, the simulation process is the same as for the reference structure, but the estimation model is changed using uninformative distributions.

After fixing values for the global parameters (presented as circles outside the larger box in Figure 2), the values of habitat-specific parameters (presented as circles inside the box in Figure 2) are obtained after transformation/simulation and used...
in the simulation of the values from the likelihood distributions of X, Y and Z.

Next, we introduce some well-known performance statistics for more extended sensitivity analysis of the model.

3.4 | Performance statistics

The performance of the proposed model is based on its ability to estimate the important parameters of the four groups shown above. In the following, we use a generic notation \( \theta_m, m = 1, \ldots, M \), for the M parameters in the group. Here, \( \theta_m \) is a real value of the mth parameter and \( \hat{\theta}_m \) is a qth quantile of an estimated posterior distribution; \( l \) is an indicator with value 1 if the following statement is true and 0 otherwise. Using these notations, the performance is measured with the following statistics:

Mean absolute percentage error (MAPE), with posterior median \( \hat{\theta}_m^{0.5} \) was used as a point estimate of the parameter \( \theta_m \). Absolute percentage error (APE) is defined as

\[
\left| \frac{\hat{\theta}_m^{0.5} - \theta_m}{\theta_m} \right|
\]

Average (mean) is taken over APEs of all parameters in the group and simulated datasets. A smaller value is better.

90% coverage probability (CP90), where coverage is defined as

\[
\text{Pr}\left( \theta_m \in (\hat{\theta}_m^{0.05}, \hat{\theta}_m^{0.95}) \right)
\]

Average is taken over CP90s of all parameters in the group and simulated datasets. If CP90 is markedly less than 90%, this is a sign of overconfidence in estimating uncertainty.

Section 3.6 presents aggregated sensitivity analysis results of 10 simulated datasets for each setting, the reference and other settings.

3.5 | Model's convergence in different scenarios

We ran models for each 10 generated datasets within 10 scenarios including one reference structure and nine test structures. The datasets are simulated using R software (R Core Team, 2019). We used RStan (Stan Development Team, 2019) with 4 MCMC chains, each having 5000 iterations and 1500 warm-up iterations. Exceptions were scenarios 2N and 8N where we used 10,000 iterations and 3000 warm-up iterations because of larger sample size.

We used \( \hat{R} \) (see Vehtari et al., 2021) as the criterion for convergence of a model. If a mean \( \hat{R} \) of all parameters in a model was larger than 1.01, a convergence of the model was considered problematic. In scenarios 2N, UI-PhiY, CorX, UI-Omega and in the reference structure, all 10 models converged without problems. In scenarios NoData, 8N, Omega15, Omega40 and LatentTL, we found that 2, 2, 10, 10 and 1 of 10 models had some converging problems, respectively. Especially, when the real value was altered (scenarios Omega15 and Omega40) from representative values of a prior distribution, models had significant problems to converge.

Since some of the models did not meet the convergence criterion, we replicated them by doubling the number of iterations. The convergence problems in scenario NoData were originally minor (1.010 < \( \hat{R} \) < 1.11), and the higher number of iterations fixed the problems. However, the problematic models in scenarios 8N, Omega15, Omega40 and LatentTL tended to find different solutions between chains and doubling the number of iterations did not offer significant improvement. Especially in scenarios Omega15 and Omega40, the model is clearly miss-specified.

The convergence problems may worsen the performance statistics shown next. Although in practice, it is recommended to base inference only on the chains that have \( \hat{R} < 1.01 \) (Vehtari et al., 2021), in this study all chains were included in performance analysis to evaluate the full effect of a miss-specified model. However, in scenario NoData, we replaced the original solutions because the solutions with more iterations met the convergence criterion.

3.6 | Results of simulation experiments

The main purpose of the Bayesian mixing model is to estimate the following four groups of parameters with used sources and taxa: \( \Phi_{tk}^{l}, t = 1, 2, k = 1, 2, 3, \Phi_{tk}^{l}, tt = 1, 2, \Phi_{tk}^{l}, 1, tt = 1, 2, 3, 4, l = 1, 2. \)

For illustrative purposes, the estimated posterior distributions of these parameters for the reference structure with one simulated dataset are shown in Figures S1-S14. According to those results, the estimation of source diet contributions \( \Phi_{tk}^{l} \) and regression parameters \( \beta_s \) seems to work the best. To add representativeness, this simulation experiment was extended to 10 simulated datasets.

Below, we compare the estimation results of these 10 datasets with the results of nine test structures, when 10 simulated datasets per scenario were analysed. The results are summarized using the performance statistics introduced above.

3.6.1 | Estimation of \( \Phi^y \) parameters

Figure 3 presents performance metrics for a group of parameters \( \Phi_{tk}^{l} \), under different scenarios modelled with the assumptions valid for the reference scenario. We compare the point estimates to the values \( \Phi_{tk}^{l} = [0.5 0.1 0.4]^\top \) and \( \Phi_{tk}^{l} = [0.7 0.2 0.1]^\top \). MAPE increases radically in the NoData scenario indicating that even the small amount of data in the reference scenario greatly increases model predictivity compared with prior information alone. MAPE halves when sample size doubles or be eight times larger. Predictability of \( \Phi^y \) did not change significantly in scenarios where the prior distribution for 2nd level consuming or \( \omega \) is uninformative in the modelling, correlations of source measurements are not zeros in the simulation, or one or more latent trophic level exists in the simulation. However, predictability drops if prior information of \( \omega \) is not correct. In all other scenarios except NoData, Omega15 and Omega40 CP90 stays between 0.85 and 0.95 indicating that 90% Bayesian credible interval is giving sensible estimates for the true parameters.
3.6.2 Estimation of $\Phi^y$ parameters

Figure 4 presents performance metrics for a group of parameters $\Phi^y_{tt, t} = 1, t = 1, 2$, under different scenarios. We compare the point estimates to the values $\Phi^y_1 = [\Phi^y_{1,1}, \Phi^y_{1,2}] = [0.67, 0.33]'$. When informative prior information for 2nd level consumers is not available (scenario UI-PhY), predictive error of $\Phi^y$ parameters increases (MAPE). In all other scenarios, except Omega40, real parameters with every datasets fit to 90% Bayesian credible interval.

3.6.3 Estimation of $\Phi^{TOT}$ parameters

Figure 5 presents performance metrics for parameters, $\Phi^{TOT}_{tt, k}, tt = 1, k = 1, 2, 3$, being total source contributions to 2nd level consumer, under different scenarios. Using Equation (23), we have

$$\Phi^{TOT}_{1} = \sum_{t=1}^{T} \Phi^y_{t,1} \cdot \Phi^x_t = \Phi^y_{1,1} \cdot \Phi^x_1 + \Phi^y_{1,2} \cdot \Phi^x_2. \tag{24}$$

With the true values, we obtain

$$[\Phi^{TOT}_{11} \Phi^{TOT}_{12} \Phi^{TOT}_{13}] = [0.67 \cdot [0.5 0.1 0.4]' + 0.33 \cdot [0.7 0.2 0.1]' = [0.57 0.13 0.30]' \tag{24}.$$

Performance statistics behave as with $\Phi^x$ (Figure 3). Scenarios NoData, Omega15 and Omega40 worsen predictability compared with the reference scenario. Scenarios with larger sample sizes, 2N and 8N, lead to lower MAPE values. Other scenarios lead to only minor changes compared with the reference scenario. With scenarios NoData, Omega15 and Omega40, CP90 values are strikingly lower than 90%.
3.6.4 | Estimation of β parameters

With a group of $\beta_l$ for $v = 1, 2, 3, 4, l = 1, 2$ parameters, we compare the point estimates with the values $\beta_1 = [1 - 1], \beta_2 = [2 - 0.1], \beta_3 = [0.1 - 2], \beta_4 = [2 - 2]$ when the four features of the lakes in the reference data were used as values for $E_l$ of the lake $h$.

MAPE values were mostly larger than with the other parameters because some of the real values were close to the zero and thus small differences were proportionally large. An important remark (Figure 6) is that scenario 8N has notably lower MAPE values than any other scenario. Thus, here there are benefits in having eight times more data, when estimating the regression coefficients. Scenarios Latent-TL, Omega15 and Omega 40 have remarkably larger MAPE values compared with the reference scenario.

Scenarios that had CP90 values outside of [0.85, 0.95] range were NoData, Latent-TL, Omega14 and Omega40. Deviations with Latent-TL were only a minor one having value of 0.84.

3.7 | Comparison to MixSIAR model

To demonstrate how our model compares to original MixSIAR (Stock et al., 2018) model, we replicated modelling with MixSIAR (version 3.1.12) model using the 10 datasets, simulated in scenario UI-PhiY. This scenario was selected as it offers the fairest comparison without informative prior based on additional data source.

MixSIAR is best suited for dietary proportion estimations directly between the consumer and its dietary sources. However, here we had to estimate proportion of initial basal energy sources (Pelagic, Benthic and Terrestrial) in perch diet through two trophic steps. For
the comparison, we did our best to fit our multiple strophic step data structure to MixSIAR framework.

MixSIAR input requires multiple observations to calculate standard deviations for each source within each habitat. However, most of the lakes in our dataset had only one observation for each initial energy source and therefore site-specific standard deviation was unobtainable. For these lakes, we used the mean of standard deviations of lakes with multiple observations of each source (5 lakes). Sample size was set conservatively to 3 which was the minimum of sample sizes in lakes with multiple observations. Single existing observation was used as a mean estimate for these lakes. Hydrogen values of sources were adjusted prior to the input for total contribution of environmental water in tissue $H (\omega_{\text{compound}})$ according to Vander Zanden et al. (2016):

$$\omega_{\text{compound}} = 1 - (1 - \omega)^{\tau},$$

(25)

where $\tau$, the difference in trophic level between the resource and the consumer, was set here as 2. $\omega$ was set to mean of informative prior distribution of 2TL-MixSIAR model, which is 0.25. Real value in this scenario is 0.27 and thus this operation should not cause excessive bias to the results. However, uncertainty of $\omega$ is ignored. Corrected hydrogen value for each source ($k$) within the lake ($h$) was then calculated as:

$$\delta^2 H_{\text{corrected}} := X_{k hi} = \omega_{\text{compound}} \times \delta^2 H_h^k + (1 - \omega_{\text{compound}}) \times X_{khi}. $$

(26)

Means and variances of TDFs of carbon and nitrogen were doubled because of the two trophic steps between the consumer and initial energy sources. We assumed no TDF for hydrogen (means and SDs set to 0).

Catchment area/Lake area ratio ($E_{hi}$) was selected as the only continuous explanatory variable for both models, because MixSIAR model is limited to one continuous explanatory variable.

MixSIAR model was run with the following settings matching with the simulation model: one random effect factor, the model includes process error and residual error. The number and length of the MCMC chains was defined with run setting 'long'.

Our comparison of models' performance is based on estimation of $\Phi^{\text{TOT}}$ parameters with the same 10 datasets that were simulated for the scenario UI-PhiY. $\Phi^{\text{TOT}}$ is the most interesting parameter that is possible to be estimated also with the original 1-level MixSIAR model. Our modelling exercise showed that MixSIAR MAPE values were roughly twice higher (0.68 vs. 0.32) compared to our 2TL-MixSIAR model (Figure 7a). CP90 values for MixSIAR and 2TL-MixSIAR model were 0.73 and 0.93, respectively (Figure 8b). This indicates that MixSIAR underestimates uncertainty substantially compared to 2TL-MixSIAR model in this particular scenario.

MixSIAR distributions, estimated by MixSIAR, have higher confusion about the real parameter value as seen from an example posterior distribution estimated from a simulated dataset where both models have close to average MAPE values (Figure 8). Furthermore, as said earlier, MixSIAR model still underestimates the uncertainty.

## 4 | DISCUSSION

To our knowledge, this is first attempt to apply a Bayesian isotope mixing model with latent trophic steps and informative priors to a consumer network. With only a small amount of data, and especially for habitats without multiple measurements for estimating standard deviation, more general prior information from different trophic levels helps to obtain a realistic solution to the model. Consequently, we performed a prior sensitivity analysis related to the parameter of 2nd level consumer diet and the value of $\omega$. Besides priors, we also varied sample sizes, modified structures related to the correlation matrices, and added an extra trophic step. Also, a scenario without isotopic data was analysed. With these modifications, we evaluated the sensitivity of the posterior distribution, compared with the reference structure. Of course, the sensitivity analysis had some limitations. Since estimation was time-consuming due to the complex model structure, in simulation experiments, we simulated and analysed 10 datasets per scenario. Thus, random variation may affect the measured performance statistics. Also, only one parameter or assumption was altered at a time and combinations were not studied. There could also be some other possible sources of bias (aka missing scenarios) that were not studied here.

The mixing model we have developed and presented here is able to handle uncertainties related to use of hydrogen stable isotope ratios, which represents a valuable advance over existing models. In many natural systems, overlap or only small differences between stable carbon and nitrogen isotope ratios of dietary sources hinders clear separation of sources based on stable isotope ratios. Therefore, it is common to find that the contribution of multiple diet sources cannot be properly resolved by a mixing model. By adding hydrogen, there is a far greater likelihood of overcoming these problems and thus of obtaining better resolution of the probable contributions of multiple sources, particularly for aquatic ecosystems. However, each additional isotope requires specific prior information regarding its isotopic TDF, and our simulations show that this information can be critical to the accuracy and robustness of model outputs. For example, the results of our simulations confirmed that uncertainty about the $\omega$ value to be used for the hydrogen can greatly affect model outputs (see performance results in Figures 3, 5 and 6 in Section 3.6), as argued by Brett et al. (2018) for simpler models. Hence, our work highlights the risks of using wrong prior information about $\omega$ and suggests that this model can handle the system safely with uninformative priors, when reliable information is not available. As seen in Section 3.7, current state-of-art methods force researchers to take a strong opinion about the value of $\omega$ to use for hydrogen as a tracer.

Available isotope mixing models essentially consider a single trophic step, even if multiple trophic steps are known to be involved. For example, the ultimate reliance of a secondary consumer on different basal resources is modelled directly with a correction for the assumed trophic position of the secondary consumer, even though it is known that one or more primary consumers constitute intermediate trophic linkages. Such an approach basically ignores all the variability in intermediary levels between basal resources.
and the consumer. This ‘black-box’ approach both neglects the opportunity to generate potentially important information about the food web linkages, and by oversimplification can lead to unreliable or even misleading model outputs. In many instances, prior information (SIs, stomach content etc.) from intermediary levels is available and it would be imprudent not to use this information, particularly if information from basal sources is limited. Our application of MixSIAR model (Section 3.7) showed in practise inconvenient steps that have to be taken with current state-of-art methods.

By allowing the inclusion of multiple trophic steps, our model offers enhanced realism and can potentially yield greater information about trophic dynamics. This development extends to the inclusion of what we have termed latent trophic steps, that is linkages that are known to exist but for which no empirical isotope data are available. By including or excluding such latent trophic steps and by simulating the effect of assigning different values to them, the model enables the likely importance of these ‘missing’ linkages to be evaluated. Similarly, our model allows different and specific TDFs to be applied for each isotope and each trophic step, which in principle is a great improvement on the current unrealistic ‘one size fits all’ approach of applying a single, constant TDF for each isotope at every trophic step regardless of the characteristics of the consumer and diet material. Method comparison supported the claim that our 2TL-MixSIAR model offers more accurate estimates about reality and, unlike the original MixSIAR model, does not underestimate uncertainty with 90% Bayesian credible intervals.

Many ecological studies necessarily involve small sample sizes with little or no replication. Therefore, a mixing model should be capable of functioning with small sample sizes. We showed that our model functions satisfactorily even with small sample sizes, although inevitably with a lack of replication the model outputs are weakly constrained. Our simulations showed that even a twofold increase in sample size greatly improved predictive power of the model, which has implications for the design of ecological studies. With limited resources for sample collection and processing, study design has to consider the trade-off between greater replication and the consequent reduction in, for example, spatial and temporal resolution. Decisions about this will need to take account of the study context and the specific questions being addressed, but a model like ours can help with the decision process.

We can conclude that unrealistic assumptions of independent source measurements or one missing latent trophic level did not
markedly reduce the estimation accuracy of diet proportion parameters. However, it is crucial to avoid giving unrealistic prior information about $\omega_i$ for which it is safer to use an uninformative prior distribution. Stomach content data as prior information for 2nd level consumer diet were important for estimating global average diet proportions for the 2nd level consumer ($\Phi^{(2)}$), but not for 1st level consumers ($\Phi^1$). For $\beta$ coefficients of covariate variables, observations are the same except that one missing latent trophic level also reduced predictive accuracy. We also found that even a small increase in the amount of data in the reference scenario led to much more accurate insights compared with only prior information, and doubling sample size improved accuracy much more. Overall, we conclude that our results give confidence that it is possible to get useful insights even when few data are available and the model has some simplified assumptions. There is still work to be done to make such multi-level models more user-friendly and applicable for many type of datasets, but we hope that this work can act as a catalyst for developing such models further and possibly adding such features to existing models.

**AUTHOR CONTRIBUTIONS**

Roger I. Jones conceived the original project and secured the funding. Risto Heikkinen developed the model in discussion with the other authors. Salme Kärkkäinen assisted with statistics and the parametrization of the model. Jos Schilder and Mikko Kiljunen collected the ALLOCARB field data used for the model simulations. Risto Heikkinen and Roger I. Jones prepared a first version of the manuscript, and all authors contributed critically to successive drafts and gave final approval for publication.

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**CONFLICT OF INTEREST**

The authors do not have a conflict of interest to declare.

**PEER REVIEW**

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**DATA AVAILABILITY STATEMENT**

The model codes (R and Stan) and example data are available on https://doi.org/10.5281/zenodo.7079853 (Heikkinen et al., 2022).

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**SUPPORTING INFORMATION**
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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