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ADVANCED REVIEW

The Use of Bayesian Mixing Models for Root Water Uptake: A Critical Review

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ABSTRACT

Isotope-based Bayesian mixing models (BMM) are widely used in ecohydrology to infer where plants acquire water from the soil, yet clear guidance on their application to root water uptake (RWU) remains limited. This review synthesizes existing BMM applications for RWU estimation and critically examines three fundamental challenges that constrain their robustness and interpretability. First, RWU inference is often severely underdetermined because the number of isotopic tracers is far smaller than the number of potential soil water sources or depth layers, placing fundamental limits on parameter identifiability. Second, RWU estimates are sensitive to model configuration choices, particularly source grouping and prior specification. A key conceptual insight emerging from this review is that so-called "non-informative" Dirichlet priors can become strongly informative as the number of sources increases, leading to divergent uptake patterns inferred from the same dataset. Third, inappropriate specification of error structures can misrepresent how isotopic variability is propagated into the likelihood function, inflating posterior uncertainty or biasing inferred RWU proportions. Looking forward, we argue that further progress in BMM-based RWU inference requires moving beyond discrete, depth-resolved formulations toward physically grounded and vertically continuous inference frameworks with well-justified error structures. Such developments, together with explicit consideration of identifiability and model dimensionality, are essential for the robust use of hydrogen and oxygen stable isotopes in quantifying root water uptake patterns.

This article is categorized under:

Science of Water > Hydrological Processes

Science of Water > Methods

1 | Introduction

Root water uptake is a critical process at the intersection of hydrological, ecological, agronomical, and climate sciences (Brantley et al. 2017; Dawson et al. 2002). It is a primary driver of the global water cycle, accounting for around 60% of terrestrial evapotranspiration and connecting precipitation, infiltration, and groundwater recharge (Good et al. 2015; Wei et al. 2017).

Ecologically, quantifying root water uptake is key to understanding plant water-use strategies (Gessler et al. 2022; Zhan et al. 2019), plant community composition and drought tolerance (McCann and Huang 2008), and spatial–temporal dynamics of below-ground water pools (Meunier et al. 2018; Renée Brooks et al. 2010). Furthermore, as plant-water relations are inextricably linked to carbon and nutrient cycles, root water uptake is vital for predicting broader biogeochemical processes (Dubbert et al. 2022). Agronomically, insights into crop root water uptake patterns are essential for improving water-use efficiency, selecting drought-tolerant varieties, and optimizing irrigation strategies (Min et al. 2019; Zhang et al. 2022). A mechanistic understanding of root water uptake is therefore fundamental to addressing grand challenges in water and food security under climate change (Rothfuss and Javaux 2017; van Dusschoten et al. 2020).

Despite its central role, our understanding of root water uptake remains the weak link in our knowledge of the soil–plant-atmosphere continuum. This is primarily due to the challenges of directly observing and measuring processes belowground, in contrast to the more advanced study of aboveground physiology. This knowledge gap impairs our ability to develop an integrated mechanistic understanding of plant-water relations (Jackson et al. 2000) and introduces substantial predictive uncertainty into land surface and earth system models (Sulis et al. 2019).

Among the various techniques for quantifying root water uptake, the use of stable isotopes of oxygen and hydrogen has become a popular method across diverse environments (Dubbert et al. 2022; Popp et al. 2025). Two major isotopic methods are typically employed: direct inference and mixing models. The direct inference, initially proposed by Brunel et al. (1995) and later reviewed by Dawson et al. (2002), identifies the mean root water uptake depth by examining the intersections between soil and plant water isotopic compositions. While this method is intuitive and straightforward, direct inference approaches are highly simplified and can be inaccurate. Inferring a single uptake depth from the intersection between soil and xylem water isotopic compositions implicitly assumes a monotonic or linear relationship between soil water isotopic compositions and depth, an assumption that is often violated under natural conditions. Moreover, root water uptake is not confined to a single depth but is distributed across vertical soil profiles and governed by water potential differences between soil, roots, and the leaves. As a result, direct inference methods cannot capture the distributed and process-driven nature of root water uptake, limiting their ability to resolve plant responses to environmental change or interspecific water competition.

This limitation hinders researchers from studying how plants respond to climate change or how water competition occurs among species. Consequently, more quantitative approaches are needed to robustly assess plant water-use dynamics under changing environmental conditions. In this context, isotope-based mixing models have emerged as a powerful framework to quantify root water uptake.

Mixing models are widely recognized as a significant advancement over direct inference for identifying root water uptake patterns (Dawson et al. 2002). These models not only enable researchers to examine where plants access water across the soil profile but also standardize and enhance mathematical evaluations. However, traditional simple linear mixing models (SLM), which estimate source contributions using isotopic mass balance, are limited to uniquely solvable systems (source number = tracer number + 1). By contrast, root water uptake represents a fundamentally underdetermined problem, with more sources than tracers +1. To address this, Bayesian mixing models (BMM) have been developed over the past two decades to reduce uncertainty in root water uptake proportion estimates by grouping isotopically similar sources and incorporating additional information (e.g., prior knowledge) (Guerrero and Rogers 2020). Despite these advancements, Bayesian mixing models, such as MixSIAR (Stock and Semmens 2016), can still potentially produce substantial errors and uncertainties of estimated root water uptake proportions, arising from source discretization, limited identifiability, sensitivity to prior and error-structure assumptions, and the temporal representativeness of isotopic snapshots (Fu et al. 2024; Neil et al. 2024). Yet a comprehensive review systematically evaluates their correct application and associated sources of uncertainty remain lacking.

Existing reviews examining errors and uncertainties in Bayesian Mixing Models (BMM) for root water uptake estimates typically focus on either the models themselves (Dubbert et al. 2022; Popp et al. 2025) or input data quality (von Freyberg et al. 2020) but rarely integrate both perspectives. This fragmented approach has led to incomplete and sometimes contradictory recommendations. For instance, Phillips et al. (2014) established key recommendations for BMM based on animal dietary studies, including utilizing priors, careful source grouping, and reporting uncertainties etc. However, these general guidelines are not fully applicable to studies of root water uptake, as reducing the number of sources conflicts with the need in ecohydrology to maintain high vertical spatial resolution (i.e., resolving root water uptake across multiple soil depths) for capturing detailed root water uptake patterns. This discrepancy is further illustrated by Rothfuss and Javaux (2017), who, based on simulations using a single isotope (^{18}O), concluded that Bayesian mixing models (BMM) perform best when the number of sources is maximized. This conclusion, however, contradicts both Phillips et al. (2014) and the general principle that increasing sources typically amplifies uncertainty in underdetermined systems (Stock et al. 2018). Moreover, the use of a single isotope, rather than the more robust dual-isotope (δ^2H and $\delta^{18}O$) approach (Evaristo et al. 2017), further limits the generality of the conclusion from Rothfuss and Javaux (2017). Together, these contrasting perspectives show that there is currently no consensus on the optimal configuration and use of BMM for estimating root water uptake.

Beyond model configuration, the critical role of input data quality has been a major source of debate. Studies highlight that variability in the stable isotopes of oxygen and hydrogen in xylem and soil waters, from both natural heterogeneity and extraction methods, introduces significant uncertainty to approximated root water uptake proportions (Penna et al. 2018; von Freyberg et al. 2020). von Freyberg et al. (2020) emphasized that inadequate soil water characterization can lead to misinterpretations of plant water sources and advocate solutions like improved experimental design and labeling techniques. However, an exclusive focus on data quality risks overlooking uncertainty arising from the modeling framework itself. Gai et al. (2023) showed that up to 37% of uncertainty of estimated root water uptake proportions could be attributed to model structure itself, whereas only about 8% originated from data variability. This finding challenges the common assumption that data uncertainty is the dominant limitation in Bayesian mixing model applications. More importantly, the sensitivity of BMM to model configuration and data inputs has not been systematically examined in root water uptake studies. As a result, different modeling configurations applied to the same isotope dataset can yield divergent estimates of root water uptake. For example, Gai et al. (2023) and Shi et al. (2023) analyzed the same soil–plant water isotope dataset but obtained contrasting depth-resolved root water uptake patterns due to differences in source grouping and model settings. Together with recent calls for standardized water extraction protocols (Millar et al. 2022), there is a pressing need for a dedicated synthesis to improve isotope-based estimation of root water uptake. Such an effort is essential to advance methodological transparency, enhance model robustness, and guide future ecohydrological research.

Here we present a systematic review and evaluation of BMM for root water uptake. There is a pressing need for such review now because although the number of studies applying mixing models to quantify root water uptake has increased rapidly, reviews to date in this field remain fragmented and incomplete with open questions remaining, like: How are Bayesian mixing models (BMM) applied in these studies? How reliable are their estimates? And how should models be configured for accurate and consistent results?

To fill these gaps, our review of BMM for root water uptake focused on three elements:

1. A review of methods for quantifying root water uptake, focusing on the widespread use of BMM.
2. A critical examination of the most popular tool, MixSIAR, including its applications in ecohydrology and the methodological challenges associated with its use for root water uptake estimation.
3. Development of a few recommendations for applying MixSIAR on root water uptake quantification and to guide the development of the next-generation BMM.

Our review aims to yield more generalizable recommendations, ultimately supporting more accurate root water uptake assessments and improved water resource management under the changing climate. For transparency, all figures presented in this manuscript are original unless explicitly stated otherwise in the corresponding figure captions.

2 | How to Quantify Root Water Uptake?

Water moves along the soil–plant-atmosphere continuum following gradients in water potential, from regions of higher (less negative) water potential in the soil toward increasingly lower (more negative) water potential in the plant and atmosphere, even though this transport occurs upward from roots to shoots (Jackson et al. 2000). Root water uptake fluxes, defined as the volume of water absorbed through roots per unit soil surface area per unit time ($m\,s^{-1}$), can be partitioned into water fluxes absorbed from different soil depth increments (or sources). For convenience, root water uptake from a given soil depth interval is expressed as a proportion of total uptake. There has been a growing interest in quantifying root water uptake proportions, often referred to as plant water sourcing, plant water partitioning, or plant water apportioning. Here, we consistently use the term “root water uptake” for clarity. Root water uptake studies elegantly answer questions “Where do plants (forest, crop and fruit trees) obtain water?”, which is becoming increasingly important not only for plant ecologists, ecohydrologists, and agronomists (Dawson et al. 2002; Evaristo et al. 2015; Miguez-Macho and Fan 2021; Wang, Wang, et al. 2023; Wang, Gao, et al. 2023), but also for water resource managers and policy makers in agricultural and forested systems in the era of intensified drought due to climate change and global warming (Scandellari et al. 2024).

Roots are sophisticated organs whose morphology and architecture determine their primary functions, including anchorage, transport, and resource acquisition. Key traits such as root diameter, branching, suberization, the presence of root hairs, and rooting depth have been extensively reviewed for their critical role in plant drought tolerance, avoidance strategies, and mortality risk (Freschet et al. 2021; McCormack et al. 2015; Stocker et al. 2023). However, beyond these structural attributes, a second major driver of root water uptake is the ability of root systems to adapt their structure and physiology in response to environmental conditions (Jackson et al. 2000). Roots are highly sensitive to gradients between their internal water potential and that of the surrounding soil, which is itself influenced by soil texture, water content, and osmotic potential (von Freyberg et al. 2020). To optimize water acquisition, roots actively “mine” water by regulating the soil water potential through root exudates or by lowering their own cellular potential via increased solute concentration. They can also “forage” water by growing toward moist zones or abandoning roots in dry soil patches. These subtle uptake dynamics are highly variable across species, time, and space (Müllers et al. 2023). Consequently, root distribution alone cannot be directly equated with the depth distribution of water extraction (Bello et al. 2019).

A major impediment to understanding the link between root distribution and water sourcing lies in the difficulty of characterizing these dynamic root adaptations using traditional sampling methods. While substantial progress has been made in understanding the molecular and physiological controls of root water uptake, translating these insights into spatially resolved root water uptake patterns remains challenging. Nevertheless, the past three decades have witnessed major advances in measurement technologies, including root imaging techniques, soil water content and matric potential sensors, and stable isotope analysis of hydrogen and oxygen, alongside rapid growth in computational capacity. These developments have enabled increasingly sophisticated investigations of root water uptake and have driven a rapid expansion of related studies (Figure 1).

These isotope-based root water uptake studies can be broadly categorized into two groups: process-based isotope models that

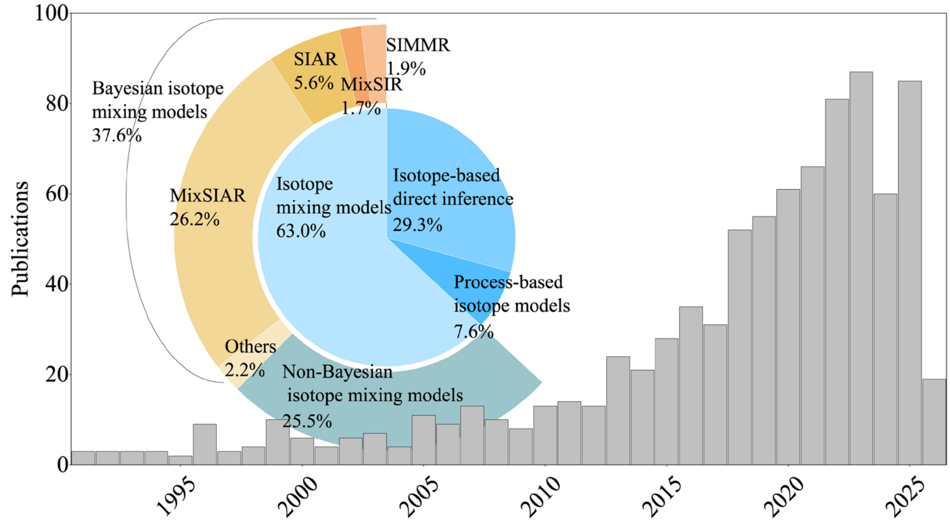

FIGURE 1 | Results of a systematic literature survey conducted in this review using the Web of Science query: ALL = ((“root water uptake” OR “water uptake” OR (“water source” AND (root OR plant)) OR “plant water partitioning”) AND isotope). The pie chart shows the classification and relative proportions of methodological approaches identified in the retrieved publications. Literature coverage is current as of February 28, 2026.

explicitly simulate isotope transport and non-process-based isotope approaches. The latter can be further classified into direct inference and isotope mixing models (Figure 1). The process-based isotope models category, which accounts for approximately 7.6% of the reviewed studies, refers to approaches that simulate root water uptake by coupling process-based hydrological models with hydrogen and oxygen stable isotopes, such as MOIST (Fu et al. 2025), HYDRUS (Zhou et al. 2021), and LWFBrook90 (Bernhard et al. 2025). In these models, root water uptake is primarily governed by physical and physiological processes (Cook and O'Grady 2006; Couvreur et al. 2012; Feddes et al. 1978), while hydrogen and oxygen stable isotopes are incorporated as auxiliary tracers to constrain simulated water fluxes and uptake patterns. In this context, isotopic information serves to reduce equifinality and improve model identifiability, rather than acting as the primary driver of root water uptake estimation.

By contrast, non-process-based isotope approaches infer root water uptake primarily from the stable isotopes of oxygen ($\delta^{18}O$) and hydrogen ($\delta^{2}H$) in soil and xylem water, whereas other ecohydrological variables, such as soil water content and sap flux, typically enter the inference indirectly as prior constraints or auxiliary information. Under the common assumption that there is no significant isotopic fractionation of stable isotopes of oxygen and hydrogen along the hydraulic continuum from soils to xylem or during xylem water extraction (Barbeta et al. 2022; Chen et al. 2020), the isotopic signals in xylem water (typically not exposed to evaporative enrichment) can be interpreted as a mixture of that in water sources accessed by plants at a given time. Exceptions may occur under conditions of high evaporative demand combined with low sap flow, particularly toward branch tips (Dawson and Ehleringer 1993). Consequently, by comparing the stable isotopes of oxygen and hydrogen in xylem water to that of different water sources, it is possible to determine the percentage of sources used by plants.

Remarkably, 92.4% of the surveyed studies employed non-process-based isotope approaches (Figure 1). This dominance can be attributed to several factors: (1) non-process-based isotope approaches require fewer data inputs and are comparatively easier to implement than process-based hydrodynamic models, particularly under field conditions; (2) stable isotopes of hydrogen and oxygen are ideal tracers for identifying root water uptake because they are integral to water molecules and follow its movement directly (Bachofen et al. 2024; Cui et al. 2023; Moreno-Gutiérrez et al. 2012; Sohel et al. 2023); (3) mass spectrometers and isotope-ratio infrared spectroscopy instruments for $\delta^{18}O$ and $\delta^{2}H$ analyses are widely available, and the associated analytical protocols are well established; and (4) the behavior of $\delta^{18}O$ and $\delta^{2}H$ in plant–soil systems is relatively well understood, allowing root water uptake to be quantified without explicitly resolving the underlying physiological uptake processes (Fan et al. 2019; Ogle et al. 2004, 2014). Additionally, $\delta^{18}O$ and $\delta^{2}H$ in soil and plant water can be measured potentially at high spatial and temporal resolutions (Beyer and Penna 2021; Penna et al. 2018). Together, these advantages have made non-process-based isotope approaches the most widely used tools for quantifying root water uptake.

As with any approach, non-process-based isotope approaches face several challenges, including strong spatial and temporal variability in plant and soil water isotopic compositions, and difficulties in ensuring that sampled waters are representative of the relevant source pools (Penna et al. 2018; von Freyberg et al. 2020). Importantly, these challenges do not imply that non-process-based methods are unreliable, but rather that uncertainty is intrinsic to isotope-based inference and must be treated explicitly.

Many sources of uncertainty can be reduced through an appropriate experimental design, including optimized sampling strategies, careful water extraction, and introducing labelling experiments (Ceperley et al. 2024). However, a substantial component of uncertainty is unavoidable and must be quantified rather than ignored (von Freyberg et al. 2020). In practice, uncertainty in root water uptake estimates can often be constrained by incorporating complementary variables that are routinely measured in plant ecology and soil hydrology, such as soil water content, soil matric potential, fine root length density, and sap flux (Nehemy et al. 2021; Zhu et al. 2024). These complementary data provide independent, process-relevant constraints that enhance the interpretability and robustness of isotope-based root water uptake inference (Couvreur et al. 2012; Nehemy et al. 2021; von Freyberg et al. 2020; Zhu et al. 2024). In this context, stable isotopes of hydrogen and oxygen remain among the most powerful and widely used tools for quantifying root water uptake patterns.

2.1 | Non-Process-Based Isotope Approaches for Root Water Uptake

2.1.1 | Direct Inference

As one of the non-process-based isotope approaches, the direct inference method accounts for 29.3% of root water uptake studies (Figure 1). The direct inference method depicts the mean root water uptake depth as the depth within the root zone at which the soil water isotopic compositions equal the plant water isotopic compositions (Dawson et al. 2002; Ehleringer and Dawson 1992; Miguez-Macho and Fan 2021). Due to its simplicity, intuitiveness, and efficiency, this method is widely used in root water uptake studies (Brunel et al. 1995; Chen et al. 2020; Wang et al. 2019), particularly before the widespread usage of isotope mixing models. The direct inference method relies on a set of simplifying assumptions originally proposed by Brunel et al. (1995): (1) There is no significant fractionation of hydrogen and oxygen stable isotopes during root water uptake; (2) There are no significant sampling and analysis errors of water from plants and soils; (3) Root water uptake is in a steady state; (4) The isotopic composition of the soil water is laterally homogeneous within the rooting area of the tree; and (5) The delays from sampling associated with transport of isotopes are negligible. Together, these assumptions imply that plant water isotopic composition directly reflects a unique and stationary soil water source. However, by construction, this approach reduces root water uptake to a single representative depth, implicitly assuming that root water uptake is spatially concentrated and temporally invariant. As a result, direct inference methods cannot resolve the distribution of root water uptake across depths, nor can they quantify uncertainty in the inferred root water uptake depth, limiting their utility for addressing questions related to plant responses to environmental variability or water competition among species.

2.1.2 | Isotope Mixing Models

The largest proportion of studies, 63.1%, use isotope mixing models and can be divided into non-Bayesian based (25.5%) and Bayesian based (37.6%) (Figure 1), referred to the simple linear mixing model (SLM) and the Bayesian mixing model (BMM), respectively. Since root water uptake problems are transformed to isotope mixing problems by the hypotheses proposed by Brunel et al. (1995), both SLM and BMM can be used to quantify the contributions of different soil segments to plant water by applying isotope mass balance (Phillips and Gregg 2003):

$$\delta_{\text{plant}} = \sum_{i=1}^{n} P_i \delta_i$$

$$\sum_{i=1}^{n} P_i = 1 \tag{1}$$

where n is the number of soil segments (or endmembers); P_i is the uptake proportion from segment i; δ_i is the isotopic composition of soil water in segment i; δ_{plant} is the isotopic composition of xylem water.

Equation (1) forms the mathematical basis for both SLM and BMM to quantify the relative contributions of soil water to plant water. In practice, vertical soil isotope profiles measured within the rooting zone are often partitioned into a finite number of soil segments based on apparent changes or breakpoints in isotopic composition, with each segment treated as a distinct subsurface water source. This discretization enables the application of Equation (1) to quantify root water uptake but introduces two important limitations: (1) it imposes an artificial division on soil water profiles that typically vary continuously with depth. Treating adjacent depths as independent sources neglects vertical connectivity and can misrepresent the distributed nature of root water uptake; and (2) the location of isotopic transition zones is often temporally unstable, shifting in response to precipitation, evaporation, and redistribution processes. These issues can propagate into mixing model inference by altering source definitions across time and space, thereby introducing additional uncertainties and biases in estimated root water uptake proportions (von Freyberg et al. 2020).

SLM typically works best when soil isotopic profiles exhibit pronounced vertical contrasts (Ehleringer and Dawson 1992; von Freyberg et al. 2020). Such gradients commonly develop under prolonged evaporative conditions that enrich shallow soil water relative to deeper layers, or during controlled isotope labelling experiments. However, the soil water isotopic profile typically displays bimodal or multimodal characteristics in the field. Such cases necessitate discretizing the soil profile into multiple segments to approximate plant water sources more realistically. This typically leads to a situation in which the number of water sources exceeds the number of isotopic tracers plus one, resulting in an underdetermined system (Ogle et al. 2014; Phillips and Gregg 2003; Stock et al. 2018). This can occasionally produce physically impossible scenarios, such as P_i is greater than 1 or negative. Moreover, significant uncertainties of approximated P_i can arise from isotopic signals in soil and plant water. These uncertainties can originate from sampling, extraction, ^{2}H offsets (Chen et al. 2020; Millar et al. 2018; Wen, Peng, et al. 2022; Wen, He, et al. 2022), and water transport lags (von Freyberg et al. 2020). Unfortunately, without further treatments such as Tikhonov regularization in the minimization objective function (Si and Kachanoski 2000), SLM cannot account for these uncertainties.

BMM are developed to address several limitations of SLM, particularly their inability to quantify uncertainty and to incorporate additional information in underdetermined systems. A key feature of BMM is the explicit use of prior information, which is

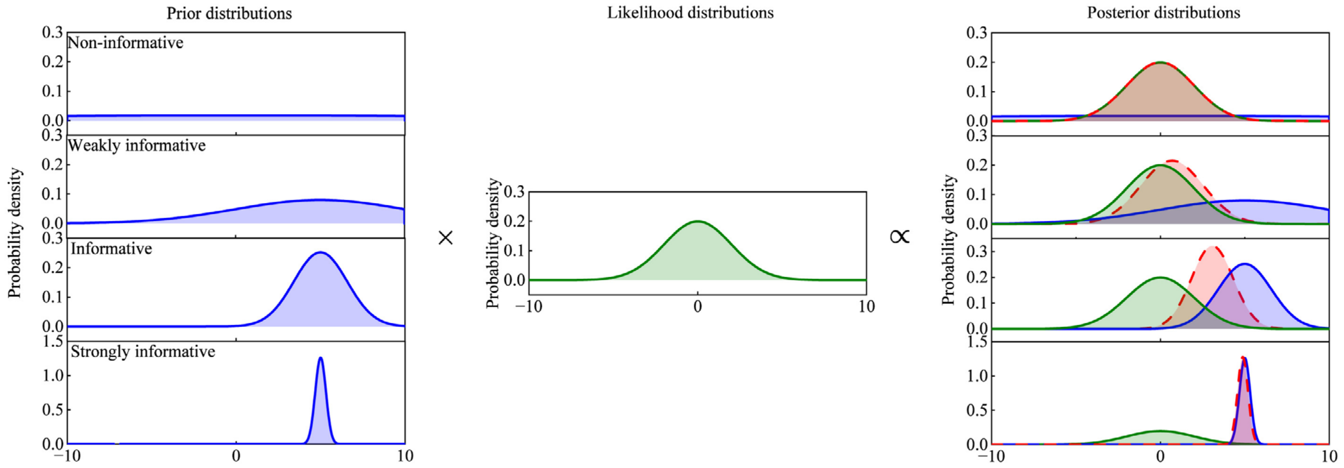

FIGURE 2 | Illustration of the relationship among prior (blue), likelihood (green), and the resulting posterior (red) distributions (replotted from van de Schoot et al. 2021). Note that the likelihood distribution in the bottom-right panel is the same as the likelihood distributions in the middle, it looks flatter because the scale of y axis is larger for accommodating the strongly informative prior distribution.

combined with isotopic observations to update posterior distributions of source contributions (Stock et al. 2018). In ecological and environmental applications, mixing problems are often underdetermined, with the number of potential sources exceeding the number of available tracers plus one. In such cases, specifying prior probability distributions is essential for achieving identifiable solutions (Doucette et al. 2011). BMM allows users to adjust the strength of prior information by modifying the shape parameters of the prior distributions, thereby influencing how strongly the posterior estimates are guided by prior assumptions versus input data. This flexibility enables BMM to formally address underdetermined mixing problems while propagating uncertainty of soil and plant water $\delta^{18}O$ and $\delta^{2}H$ measurements into approximated root water uptake proportions.

However, the solution to the BMM is intractable analytically, and commonly relies on a Markov Chain Monte Carlo (MCMC) sampling algorithm. With the help of dedicated MCMC packages, there are several well-developed BMM packages available, including MixSIAR (Stock and Semmens 2013) (accounting for 26.2%, Figure 1), SIAR (Parnell et al. 2010) (5.6%, Figure 1), and MixSIR (Moore and Semmens 2008) (1.7%, Figure 1). These percentages suggest that MixSIAR is the most popular BMM for quantifying root water uptake proportions.

2.1.3 | MixSIAR

MixSIAR is a Bayesian mixing model widely used for analyzing mixing processes involving bio tracers, such as stable isotopes and fatty acids (Guerrero and Rogers 2020; Stock et al. 2018). It can be employed for studying root water uptake by utilizing isotope mass balance between plants and soil and accounting for errors during the mixing process. Consequently, Equation (1) can be rewritten as (Ogle et al. 2004; Stock et al. 2018):

$$\delta_{\text{plant}} = \sum_{i=1}^{n} P_i \overline{\delta}_{\text{soil},i} + \varepsilon \tag{2}$$

where δ_{plant} is the isotopic composition of plant water; P_i is the unknown uptake proportion from layer i; $\overline{\delta}_{\text{soil},i}$ is the mean isotopic composition of soil water in layer i; Note that values of $\overline{\delta}_{\text{soil},i}$ are fitted hierarchically by soil water isotopic observations (Stock et al. 2018); ε is the error of predicted plant water isotopic compositions.

Root water uptake proportions, P_i, are solved in the Bayesian framework. The core idea of the Bayesian inference involves capturing prior knowledge about a given set of root water uptake proportions through the prior distribution, typically established before data collection (Figure 2); defining the likelihood function based on measured isotopic compositions in plant water (Figure 2); and then combining the prior distribution with the likelihood function using Bayes' theorem to obtain the posterior distributions of root water uptake proportions (Figure 2; van de Schoot et al. 2021):

$$p\left(\boldsymbol{P} \mid \delta_{\text{plant}}\right) \propto p(\boldsymbol{P}) p\left(\delta_{\text{plant}} \mid \boldsymbol{P}\right) \tag{3}$$

where $p\left(\boldsymbol{P} \mid \delta_{\text{plant}}\right)$ is the posterior probability density of unknown uptake proportion vector $\boldsymbol{P}$ given data δ_{plant}. Bayesian inferences are optimal when averaged over this joint probability distribution, with inference for the quantities based on their conditional distribution given the observed δ_{plant}; $p\left(\delta_{\text{plant}} \mid \boldsymbol{P}\right)$ is the likelihood probability density of data δ_{plant} given uptake proportion vector $\boldsymbol{P}$; $p(\boldsymbol{P})$ is the prior probability density of uptake proportion vector $\boldsymbol{P}$.

Root water uptake proportions (P_i) range between 0 and 1 and sum to 1 (Equation (1)) when considering soil water in the rooting zone is the only source of plant. Therefore, the joint prior distribution of the source contributions typically follows a Dirichlet distribution (Moore and Semmens 2008; Parnell et al. 2010; Stock and Semmens 2016):

$$\boldsymbol{P} \sim \text{Dirichlet}\left(\alpha_1, \ldots, \alpha_n\right) \tag{4}$$

where $\boldsymbol{P}$ is the vector (length is n) of the root water uptake proportions from each source; α_1 to α_n are shape parameters of the

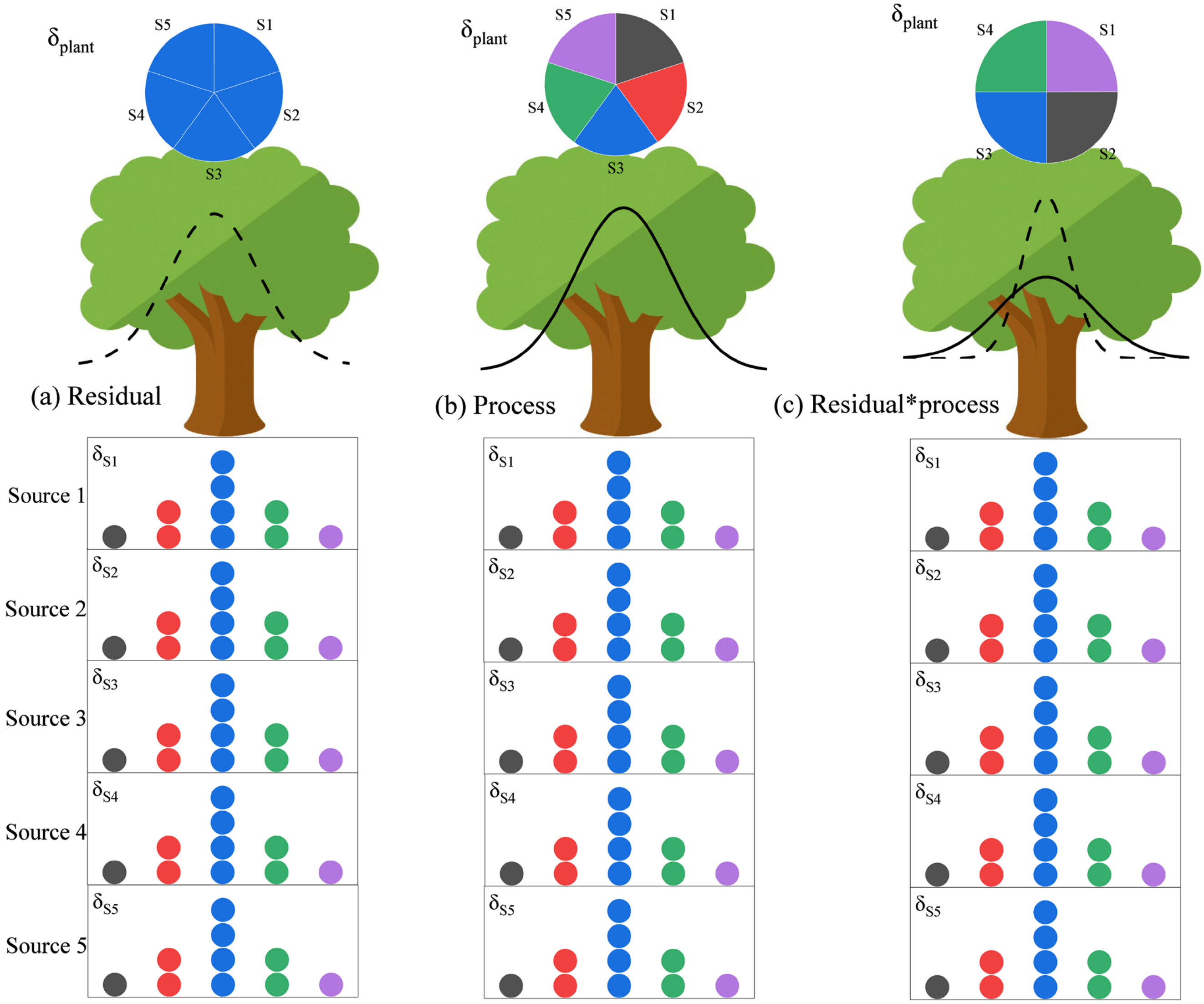

FIGURE 3 | Conceptual illustration of how different error structures in Bayesian mixing models propagate source variability into plant water. Panels (a–c) illustrate the residual, process, and residual*process error structures, respectively. The lower panels intentionally show identical source isotopic distributions across all three cases, emphasizing that differences among error structures arise from how source variability is incorporated into the likelihood formulation, rather than from differences in the source data themselves. Dashed and solid curves schematically indicate whether mixture variance is derived from observational uncertainty or propagated from source variability, respectively. In panel (c), only four source sectors are shown in the plant mixture pie to illustrate that under the residual*process error structure some sources may contribute negligibly or be effectively excluded from uptake, for example when fine roots are absent in each soil layer or when roots preferentially access isotopically extreme soil water. The figure is created by the author team and intended as a conceptual illustration of statistical assumptions underlying different error structures, rather than a depiction of physical root water uptake processes.

Dirichlet distribution. The default setting in MixSIAR of these parameters is $\alpha_1 = \ldots = \alpha_n = 1$, which is called as the "flat" prior or "non-informative" prior. However, other information, such as fine roots and soil water content profiles can be incorporated into the model by adjusting the parameters of the Dirichlet distribution.

For the error term, ε in Equation (2), MixSIAR provides three alternative error structures: residual, process, and residual*process (Figure 3). These error structures differ in how they attribute observed variability in plant water isotopic compositions. Under the residual error structure, water taken up from each source is assumed to have the same mean isotopic compositions as that source (Figure 3a). This corresponds to the assumption that roots sample the full isotopic population of each soil water source. As a result, the expected isotopic composition in plant water is a weighted sum of source means, and variability of isotopic compositions in soil water does not propagate into plant water. Within this framework, source means are treated as fixed values. Theoretically, if root water uptake proportions also remain constant, isotopic compositions in plant water would converge to a single value. In practice, however, variability in measured isotopic compositions in plant water arises from factors unrelated to source variability, such as measurement error, sampling uncertainty, and heterogeneity in water transport and storage within plant tissues. These deviations are represented by the residual error term (Figure 3a), which is typically assumed to be normally distributed with a mean of zero:

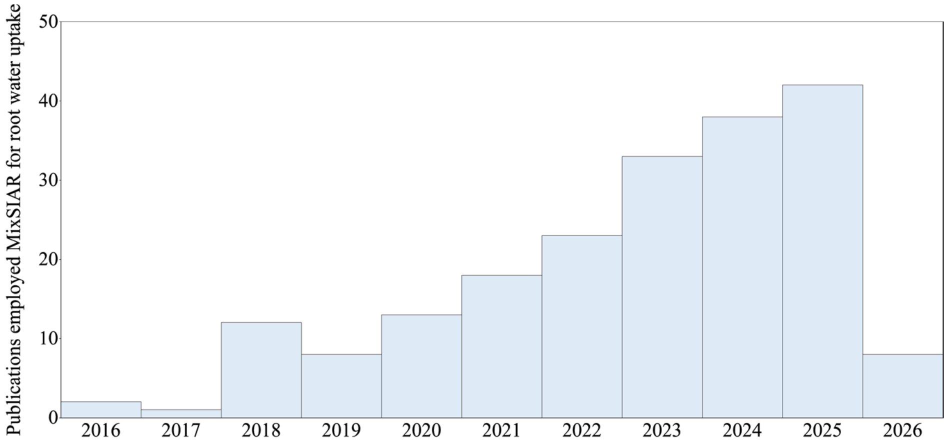

FIGURE 4 | Temporal evolution of studies applying MixSIAR to estimate root water uptake proportions since 2016, based on the literature survey conducted in this review. Data are current as of February 28, 2026.

$$\varepsilon \sim N\left(0, \sigma^2\right) \tag{5}$$

where σ^2 (or Σ for two and more tracers), is the error variance. Thus, the distribution of δ_{plant} under the residual error structure can be written based on Equations (2) and (5) (Stock et al. 2018):

$$\delta_{\text{plant}} \sim N\left(\sum_{i=1}^{n} p_i \bar{\delta}_{\text{soil},i}, \sigma^2\right) \tag{6}$$

In the process error structure, roots are assumed to randomly sample a subset of water molecules from each source, such that the isotopic composition in the sampled water may differ from the source population mean (Figure 3b). When only a finite portion of the source population is sampled, the resulting sample mean can vary among sampling events, leading to variability in the mixture even if root water uptake proportions remain constant. Under this framework, the variability in isotopic compositions in plant water arises directly from the variability in soil water. The expected mean of all possible mixtures is still approximated by the weighted average of source means; however, the variance of the mixture is greater than zero and reflects the propagation of source-level isotopic variability. This variance can be approximated as the sum of source variances weighted by the corresponding root water uptake proportions (Figure 3b; Stock and Semmens 2016):

$$\delta_{\text{plant}} \sim N\left(\sum_{i=1}^{n} p_i \bar{\delta}_{\text{soil},i}, \sum_{i=1}^{n} p_i^2 \sigma_{\text{soil},i}^2\right) \tag{7}$$

where $\sigma_{\text{soil},i}^2$ is the variance of soil water isotopic compositions in layer i.

Equation (7) illustrated that in the process error structure, each soil source's variance is transferred to the plant linearly. However, if the observed σ_{plant}^2 is greater than the maximum or smaller than the minimum of $p_i^2 \sigma_{\text{soil},i}^2$, process error can fail to fit σ_{plant}^2.

To deal with situations when $\sigma_{\text{plant}}^2 > \max\left(p_i^2 \sigma_{\text{soil},i}^2\right)$ with $i = 1 \ldots n$ or $\sigma_{\text{plant}}^2 < \min\left(p_i^2 \sigma_{\text{soil},i}^2\right)$ with $i = 1 \ldots n$, an additional residual error term, ε, is introduced to process error structure, which is known as the residual*process error structure, also known as the multiplication error structure or unified error structure (Stock and Semmens 2016):

$$\delta_{\text{plant}} \sim N\left(\sum_{i=1}^{n} p_i \bar{\delta}_{\text{soil},i}, \varepsilon \sum_{i=1}^{n} p_i^2 \sigma_{\text{soil},i}^2\right) \tag{8}$$

The residual*process error structure adds biological realism to the process error structure and results in wider application scenarios than residual error structure and process error structure. For example, root water uptake may exclude a source due to an insignificant amount of fine roots (Figure 3c), or roots may adsorb extremely depleted or enriched soil water from each source (Figure 3c). In both cases, σ_{plant}^2 cannot be deviated from $\sum_{i=1}^{n} p_i^2 \sigma_{\text{soil},i}^2$. Then, ε can expand ($\varepsilon > 1$) or shrink ($\varepsilon < 1$) the variance estimated from process error ($\sum_{i=1}^{n} p_i^2 \sigma_{\text{soil},i}^2$) to match observed σ_{plant}^2 (Figure 3c).

2.1.4 | How Is MixSIAR Applied?

The versatility of MixSIAR has made it a widely adopted tool in studies across diverse ecosystems for estimating root water uptake proportions and other mixing problems. Since 2016, the number of studies using MixSIAR quantifying root water uptake has increased significantly (Figure 4). MixSIAR allows users to configure their own mixing models by adjusting the source numbers, prior information, and error structures (Stock and Semmens 2013). Additionally, its graphical user interface facilitates ease of use, making it accessible to both novice and advanced users.

Beyond this overall growth, these studies differ in how MixSIAR has been applied and for what purposes. Based on their primary research objectives, the following categories can be identified from our reviewed literatures:

2.1.4.1 | Responses of Plants to Environmental Stressors. MixSIAR has been employed mostly to study root water uptake patterns under various ecosystems and environmental changes to assess how plant species adapt their water use strategies in response to drought or altered precipitation patterns (Granda et al. 2022; He et al. 2024; Ma and Song 2018; Wu et al. 2020, 2022). These studies have generally found that drought and altered precipitation patterns can push plants to shift their water uptake from shallow to deep soil layers, although some species demonstrate limited compensatory uptake from deeper layers during extreme droughts (Antunes et al. 2018; Gessler et al. 2022).

Beyond soil water, non-precipitation water sources such as fog, dew, or atmospheric water can play a critical role in sustaining plant water status for certain functional types, particularly epiphytes and cloud-forest species (Wu et al. 2018). When such sources are not explicitly included as potential contributors in mixing models, their omission may lead to an overestimation of soil water contributions inferred by MixSIAR.

Overall, results derived from MixSIAR highlight substantial interspecific and environmental variability in inferred root water uptake patterns. These findings underscore the context dependence and inherent complexity of root water uptake strategies, while also emphasizing the importance of carefully defining source pools and ecological assumptions when interpreting mixing model outputs.

2.1.4.2 | Agriculture and Forest Water Management. MixSIAR has been increasingly applied to investigate plant water use patterns in both agricultural systems and natural forests, providing insights relevant to water management, ecosystem functioning, and climate adaptation. In managed agroecosystems, these applications have primarily focused on how planting patterns, species combinations, and management practices influence root water uptake strategies and water use efficiency.

In agricultural systems, MixSIAR-based studies consistently highlight the importance of complementary root water uptake patterns for improving productivity and resilience under water-limited conditions. In mixed crop systems, like coffee and shade trees (Muñoz-Villers et al. 2020), as well as rubber and tea (Wu et al. 2016), niche partitioning reduces interspecific competition and enhances overall water exploitation across the soil profile (Schmutz and Schöb 2023), particularly under water-limited conditions (Huang et al. 2023). Similarly, innovative cultivation patterns, such as high-low seed beds for winter wheat, further enhance water use efficiency by directing root water uptake to more productive soil layers (Liu et al. 2021). Irrigation and fertilization experiments further demonstrate that crops such as alfalfa and winter wheat adjust their uptake depths in response to management interventions, with optimized strategies improving both water use efficiency and yield (Wu et al. 2020). Collectively, these findings underscore the potential of intercropping and optimized planting designs for sustainable agricultural water management under increasing climatic variability.

In natural forest ecosystems, MixSIAR applications reveal substantial flexibility and diversity in tree water uptake strategies, which are critical for adaptation to environmental stress. In arid regions, species such as *Caragana korshinskii* and *Reaumuria soongorica* exhibit contrasting strategies, with rapid uptake from shallow soil following precipitation versus progressive reliance on deeper water sources as dry periods intensify (Zhang et al. 2020); in subtropical montane forests, species within *Pinus taiwanensis* communities adjust their uptake depths seasonally in response to soil moisture dynamics, shifting between surface and deeper water sources (Wen, Peng, et al. 2022; Wen, He, et al. 2022). Differences between native and non-native species have also been documented, with non-native species often displaying higher water-use efficiency or deeper water access, potentially conferring competitive advantages under water-limited conditions (Granda et al. 2022).

As competition for water resources intensifies under future climate change, such quantitative insights into root water uptake patterns are increasingly valuable for informing vegetation management, agroecosystem design, and ecosystem restoration strategies (Hiiragi et al. 2024).

2.1.4.3 | Hydrological Processes. Beyond root water uptake, MixSIAR has also been applied to investigate broader hydrological processes, including groundwater recharge pathways and ecosystem-scale water dynamics. In these contexts, Bayesian mixing models are used to partition water sources contributing to soil, groundwater, and surface water pools, thereby providing insights into how precipitation is redistributed within the critical zone.

In subalpine regions of the Qilian Mountains, analyses revealed that shrub plants primarily rely on precipitation and shallow soil water (0–20 cm), whereas groundwater and river water respond more slowly to precipitation inputs, indicating longer storage and transit times (Shi et al. 2021). In the floodplain of the Upper Yellow River, isotopic signatures in soil water revealed the coexistence of piston flow and preferential flow infiltration mechanisms, with river water significantly recharging soil water below 80 cm (Wang, Fu, et al. 2021; Wang, Zhang, et al. 2021). Similarly, on the Loess Plateau, groundwater recharge is found to vary significantly across land use types, with precipitation contributing up to 69.3% of the recharge in plateau regions and surface water contributing 86.9% in hilly-gully regions (Chen et al. 2023).

In these applications, MixSIAR enables the simultaneous consideration of multiple potential water sources, which is particularly valuable when endmembers cannot be reliably distinguished by visual inspection of dual-isotope plots alone. For example, Wang, Fu, et al. (2021) and Wang, Zhang, et al. (2021) showed that groundwater isotopic compositions plotted above the local meteoric water line, suggesting limited evaporative enrichment and a dominant precipitation signal. However, when soil water is explicitly included as a potential source in a Bayesian mixing framework, it contributes approximately 40% to groundwater recharge. This result illustrates that apparent isotopic similarity, or dissimilarity, does not necessarily preclude meaningful hydrological connectivity.

More generally, these studies highlight that groundwater recharge and subsurface water mixing often involve multiple interacting sources whose relative contributions may not be obvious from isotope plots alone, especially when sampling is sparse or spatially heterogeneous. Bayesian mixing models provide a formal framework for integrating isotope data with prior hydrological knowledge, allowing uncertainty to be quantified and reducing the risk of prematurely excluding plausible sources. As such, MixSIAR and related approaches contribute to a more nuanced understanding of water redistribution processes across landscapes, with implications for water resource management and ecosystem sustainability.

2.1.4.4 | Methodology Evaluation. MixSIAR has also been applied as a diagnostic tool to evaluate how methodological choices influence isotope-based root water uptake estimates. One prominent example concerns the δ^2H offset between xylem and soil water, which was first reported by Barbeta et al. (2019) and mechanistically explained by Chen et al. (2020). This offset has been attributed to isotopic fractionation or compartmental effects during water transport and storage within plants but may also arise from methodological artifacts associated with water extraction techniques (such as cryogenic vacuum distillation, Wen et al. 2021), which can introduce biases in measured δ^2H in xylem water.

If uncorrected, the δ^2H offset violates a core assumption of isotope mixing models: plant water directly reflects mixtures of soil waters. This violation can introduce substantial bias into inferred root water uptake proportions (Wang, Wang, et al. 2023; Wang, Gao, et al. 2023; Wen et al. 2021; Wen, Peng, et al. 2022; Wen, He, et al. 2022). For example, Wen et al. (2021) quantified root water uptake contributions from three depth intervals (0–1 m, 1–2 m, and 2–5 m) using MixSIAR with both corrected and uncorrected xylem δ^2H values. By explicitly comparing model outputs under these two treatments, they showed that neglecting the δ^2H offset led to relative errors of up to 33% in estimated uptake proportions. More recently, a similar offset has also been reported for δ^{18}O and neglecting the δ^{18}O offset led to relative errors of up to 9% in estimated uptake proportions (Yang et al. 2026).

These findings demonstrate that apparent differences in root water uptake patterns may arise not only from ecological or hydrological processes, but also from methodological choices in isotope data processing. More broadly, they highlight the need for careful evaluation of isotopic offsets, correction methods, and underlying assumptions when applying MixSIAR to quantify root water uptake. Without such scrutiny, Bayesian mixing models may yield quantitatively precise but systematically biased estimates of root water uptake proportions.

2.1.4.5 | Model Comparisons. MixSIAR has also been used in comparative studies to evaluate the performance and uncertainty characteristics of different mixing model formulations in root water uptake (Gai et al. 2023; Liu et al. 2023; Stock and Semmens 2016; Wang et al. 2019). These studies generally assess model behavior in terms of goodness-of-fit, sensitivity to environmental conditions, and contributions of model structure to overall uncertainty rather than identifying a universally superior model.

For example, Wang et al. (2019) tested both linear (IsoSource) and Bayesian models (SIAR, MixSIR, and MixSIAR) in a semi-arid ecosystem by comparing the coefficients of determination (R^2) and Nash-Sutcliffe efficiency (NS) values between estimated and observed plant water isotopic compositions. They found that SIAR and MixSIAR provided more stable source apportionment than IsoSource, while MixSIR showed greater sensitivity in capturing seasonal variation. This illustrates that different Bayesian formulations emphasize different aspects of the mixing problem.

Building on this, Liu et al. (2023) evaluated the same Bayesian models under contrasting soil water content conditions for winter wheat. Their results indicated that model performance is context-dependent: MixSIAR performed best under normal and wet conditions, whereas MixSIR yielded more reasonable estimates under dry conditions. These findings highlight that model performance depends not only on model structure but also on hydrological state and data characteristics.

Rather than focusing solely on predictive performance, Gai et al. (2023) explicitly decomposed uncertainty in plant water source partitioning by combining multiple isotopic tracers and Bayesian models for apple trees of different ages. They showed that model structure itself contributed the largest fraction of total uncertainty, exceeding the contribution from isotopic data variability. Based on this analysis, they emphasized that combining dual isotopes (δ^2H and δ^{18}O) with appropriate Bayesian formulations can improve inference robustness but does not eliminate model-induced uncertainty.

Taken together, these comparison studies demonstrate that differences among mixing models can lead to divergent root water uptake estimates even when applied to the same dataset. This underscores that model choice is not neutral: different formulations prioritize different assumptions about source variability, error propagation, and temporal dynamics. Consequently, comparative model evaluation should be viewed as a tool for understanding uncertainty and model behavior, rather than to identify a single "best" mixing model for all root water uptake applications.

Overall, existing applications show that MixSIAR has been widely used to quantify root water uptake across a range of ecosystems and environmental conditions and has provided insights into species-specific water-use strategies and responses to environmental stress (Hill et al. 2021; Schwinning 2020; Sekiya and Yano 2002). These studies demonstrate the practical utility of MixSIAR for root water uptake, while also highlighting that the interpretability of its results depends strongly on model configuration, data quality, and the underlying assumptions.

3 | Problems in Using MIXSIAR Estimate Root Water Uptake Proportions

Note that this review focuses on the root water uptake from soil and its quantifications. Other discrete sources contribute to plant water, such as ground water (Huang et al. 2023; Liu et al. 2018) and fog water (Emery 2016; Emery et al. 2018; Fischer et al. 2016; Zhan et al. 2017) that are not in the scope of this review.

As mentioned earlier, one reason for the widespread adoption of MixSIAR is its graphical user interface (GUI) (Figure 5a),

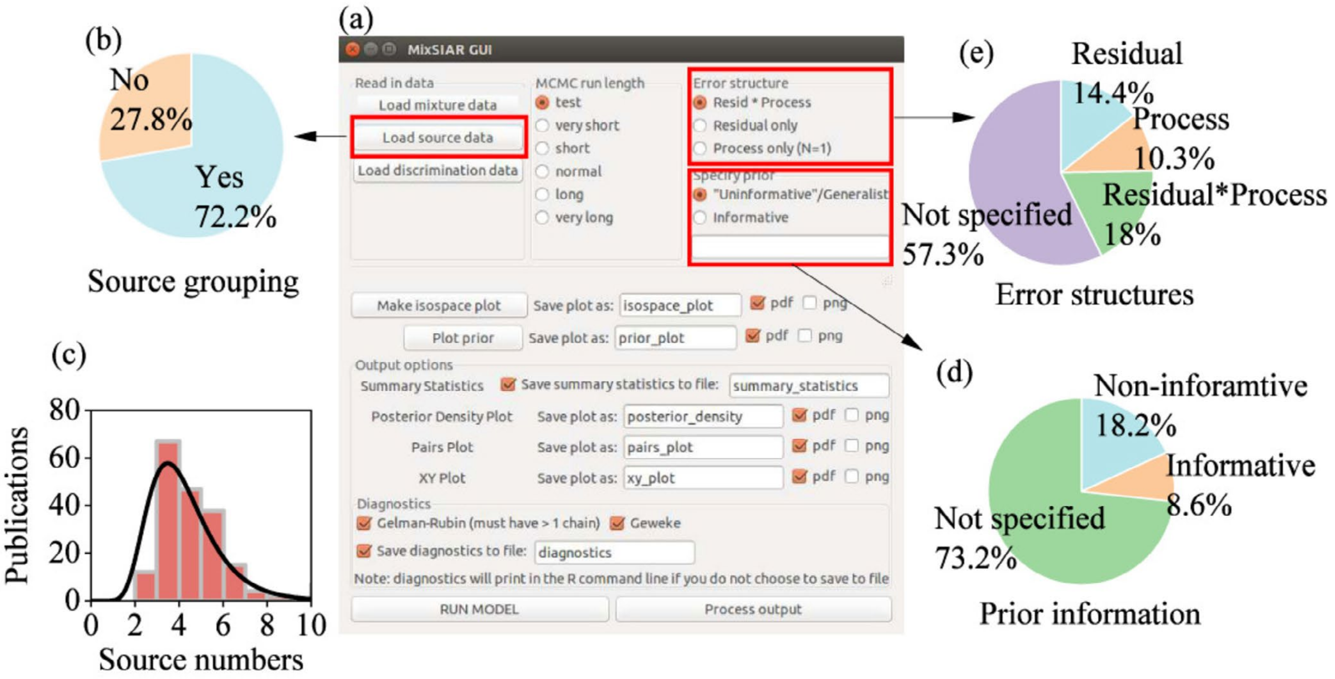

FIGURE 5 | MixSIAR graphical user interface and summary statistics of model configuration choices in root water uptake studies. Panel (a) shows the graphical user interface of MixSIAR and its key input sections (screenshot obtained from the official MixSIAR GitHub repository). Panels (b) and (c) summarize the source grouping strategies and the number of sources used across studies, respectively. Panels (d) and (e) summarize the prior specifications and error structures adopted in these studies, respectively. Data are current as of February 28, 2026.

which provides an accessible framework for configurating Bayesian mixing models. The GUI consists of several components, including "Read in Data", "MCMC Run Length", "Error Structure", "Specify Prior", "Output Options", and "Diagnostic Information". Among these, "Read in Data", "Error Structure", and "Specify Prior" are the most critical for model formulation, as they define the input data, the likelihood structure, and the prior assumptions of the mixing model. Importantly, these settings often require user decisions that are not uniquely constrained by the data alone. Differences in how data are prepared, how error structures are specified, and how prior information is incorporated can therefore lead to divergent results, even when the same dataset is analyzed.

In the "Read in Data" section, mixture data, which represent the observed δ^2H (δ^{18}O) of plant water, are typically used in raw, and discrimination data, which are used to account for potential isotope fractionation, are often assumed to be zero. By contrast, source data are often subject to grouping, which constitutes a major source of error in estimated root water uptake proportions. At present, however, the process of grouping lacks standardized guidelines, leaving decisions about the number of groups and how to group them up to the user.

Similarly, challenges arise in the "Error Structure" and "Specify Prior" sections. MixSIAR offers multiple error structures and flexible prior specifications, but there are no strict standards for these configurations in root water uptake studies. As a result, the setup of error structures and prior information depend entirely on assumptions about data variability, process representation, and model dimensionality. When these perspectives are poorly matched to the research question, substantial differences in estimated root water uptake proportions and associated uncertainties can arise, even when the same dataset is analyzed.

Our review showed that over two-thirds employed source grouping (Figure 5b), with the number of sources ranging from 2 to 10 (mean ± standard deviation = 4.2 ± 1.7; mode = 3; Figure 5c, fitted by log-normal distribution). On the one hand, this variability reflects that the number of sources is constrained by soil profile depth or stratigraphic complexity (e.g., shallow alpine soils versus deep loess profiles); on the other hand, it reflects user choices in discretizing a continuous soil profile.

In addition, among the studies specified prior information settings, approximately 18.2% of studies employed the default non-informative (flat) prior (Figure 5d), which is over 2 times larger than the number of studies applied informative prior (8.6%). In practice, the proportion of studies using the default non-informative prior is likely higher, as studies that did not explicitly report prior settings tend to conservatively use default non-informative prior setting. This choice could be motivated by the intention to minimize subjective assumptions. Our following analysis demonstrates that when the number of sources increases, even non-informative priors can exert strong implicit constraints on posterior estimates due to the high dimensionality of the mixing space (Section 3.3.1). As a result, the distinction between "informative" and "non-informative" priors becomes blurred in practice.

Lastly, although Equations (6–8) indicate that error structure selection should be guided by assumptions about data variability and the underlying mixing process, no clear preference emerges from the literature. Among studies that explicitly specified error structures, residual*process is only marginally preferred over residual and process error structures (Figure 5e). This pattern suggests that error structure selection is often driven by convention or default settings rather than by explicit consideration of their suitability for root water uptake inference.

Taken together, these patterns indicate that applications of MixSIAR to root water uptake are systematically challenged by source grouping choices, prior dimensionality effects, and error structure specification. These challenges are not primarily attributable to individual user decisions. Instead, they reflect unresolved methodological questions and the lack of root water uptake specific guidance for applying MixSIAR.

3.1 | Source Grouping

As discussed above, an undetermined mixing system, where the number of sources exceeds the number of tracers plus one, is common in ecological or hydrological studies (Penna et al. 2018; Phillips et al. 2014; Stock et al. 2018). One way to reduce the degree of underdetermination is source grouping, which reduces the effective number of sources and often leads to narrower and more interpretable posterior distributions (Phillips et al. 2014). A classic example comes from early Holocene human diets, where three marine food sources: pinnipeds (4%–48%), shellfish (0%–36%), and marine fish (0%–68%) are initially treated as separate sources, resulting in very broad and overlapping contribution ranges. However, when these sources are grouped into a general category of "marine foods", the estimated contribution narrowed to 70%–84%, providing more informative and interpretable results (Newsome et al. 2004).

Note that sources in diet studies are generally discrete, whereas root water uptake from soil is theoretically continuous with depth. Approximating a continuous soil water profile using a small number of discrete groups can therefore introduce several limitations, including information loss (Barbeta and Peñuelas 2017; Phillips et al. 2014), increased uncertainties in root water uptake estimates (Phillips et al. 2014; Phillips and Gregg 2003; von Freyberg et al. 2020), reduced vertical resolution of approximated root water uptake profile (Fu et al. 2024), and biased root water uptake proportions (Neil et al. 2024). For example, Wang et al. (2020) and Wang, Fu, et al. (2021) and Wang, Zhang, et al. (2021) applied mixing models to the same study site and dataset but different soil layer grouping strategies (Figure 6A). The resulting absolute mean differences in estimated root water uptake proportions reached 9.4%, 8.5%, and 13.5% for shallow, middle, and deep soil, respectively. These discrepancies indicate that source grouping choices can substantially influence inferred root water uptake patterns, with deeper soil layers often being more sensitive to grouping decisions than shallow layers.

In addition to grouping schemes, approximated root water uptake proportions from MixSIAR are also sensitive to the number of sources specified in the model (Figure 6B).

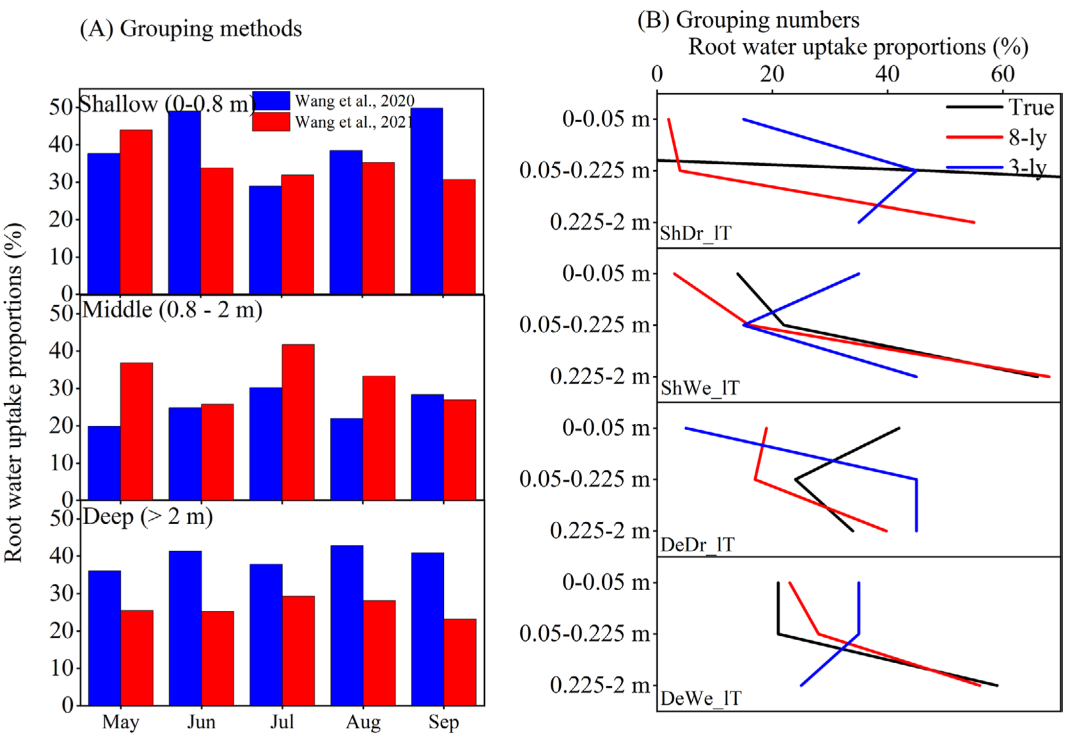

FIGURE 6 | Comparison of estimated root water uptake proportions under different source grouping schemes and grouping numbers. Panel (A) compares root water uptake proportions obtained using different source grouping methods, with data extracted from Wang et al. (2020) (Panel A), Wang, Fu, et al. (2021) and Wang, Zhang, et al. (2021) (Panel A). Panel (B) illustrates the influence of source number on root water uptake estimates, based on data from Rothfuss and Javaux (2017) (Panels B). For consistency, only mean root water uptake proportions reported in the original studies were extracted. When more than three soil layers were used, uptake proportions were re-aggregated into three depth classes according to soil layer thickness to facilitate comparison. In panel (B), Sh, De, Dr, We, and LT denote shallow groundwater level, deep groundwater level, dry soil surface, wet soil surface, and low transpiration rate, respectively.

Using a virtual dataset with known true uptake proportions (Figure 6B; Rothfuss and Javaux 2017), clear differences in model performance emerge when different numbers of soil layers are used. For an eight-layer discretization, the mean absolute errors between estimated and true root water uptake proportions are 31.9%, 10.1%, and 27.5% for 0–0.05 m, 0.05–0.225 m, and 0.225–2 m, respectively. By contrast, when the same dataset is grouped into only three layers, the corresponding errors increase to 41.1%, 11.0%, and 41.4%, respectively. Notably, different grouping numbers not only altered the magnitude of estimated root water uptake proportions, but in some cases produced qualitatively different, and even reversed, root water uptake patterns (Figure 6B). These results demonstrate that both the number of sources and the adopted grouping strategy can strongly influence inferred root water uptake proportions. The effect is particularly pronounced for deeper soil layers, which typically encompass thicker depth intervals and integrate greater isotopic and hydraulic heterogeneity (He et al. 2023).

Grouping thick soil layers inherently limits the spatial resolution at which root water uptake can be inferred. For example, in the Loess Plateau region, soil sampling can extend to 22 m (He et al. 2023; Xiang et al. 2021), yet soil water from 6 to 22 m is often grouped as a single source when applying mixing models. Such coarse grouping obscures potential depth-specific uptake signals from deep layers and hampers investigations of plant drought responses that critically depend on accessing deep water reserves. This limitation is particularly problematic because key controls on root water uptake, including soil water potential, water fluxes, and fine root distributions, vary continuously with depth (Barbeta and Peñuelas 2017; Braud et al. 2005; Glass et al. 2023; Jury and Horton 2004; López et al. 2001; McElrone et al. 2013; Pregitzer 2002; Rewald et al. 2011; Zhou et al. 2021), By discretizing a continuous soil profile into a small number of sources, source grouping imposes artificial boundaries that break this vertical continuity, leading to a loss of spatial information that is directly relevant to root water uptake processes.

At the same time, severe underdetermination is common in isotope-based root water uptake studies (Doody et al. 2009; Keen et al. 2023; Sanchez-Perez et al. 2008). Source grouping is therefore often adopted as a pragmatic strategy to reduce dimensionality and stabilize mixing model solutions (Newsome et al. 2004; Phillips et al. 2014). However, this review shows that grouping choices involve an inherent trade-off: while grouping alleviates underdetermination, it simultaneously reduces vertical spatial resolution and can substantially influence the accuracy and interpretability of estimated root water uptake proportions, depending on both the grouping scheme and the number of sources used.

3.2 | Prior Information

Non-Bayesian mixing models, such as IsoError, account for source uncertainties through first-order error propagation (Phillips and Gregg 2001; Rothfuss and Javaux 2017). However, these approaches lack mechanisms to incorporate additional physiological information, such as root length density, when the mixing system is underdetermined. By contrast, the Bayesian mixing models allow such information to be incorporated through prior distributions. In Bayesian inference, observed isotope data enter the model via the likelihood, whereas prior distributions encode assumptions or auxiliary information about source contributions. Importantly, while the likelihood is determined by measurements, the specification of priors depends on user choices regarding their form and parameterization and can therefore exert a strong influence on posterior estimates. In general, when priors are diffuse (flat), posterior distributions are dominated by the likelihood and closely reflect the information contained in the isotope data. As priors become more informative (sharp), the posterior distribution increasingly resembles the priors, and the influence of the data diminishes (Figure 2). This prior-likelihood balance is a fundamental feature of Bayesian inference and underpins both its flexibility and its sensitivity to model configuration.

Consequently, inappropriate specification of prior information can bias inference and inflate posterior uncertainty, particularly when the assumed prior structure is inconsistent with actual root water uptake behavior. For example, compensatory root water uptake has been shown to allow relatively small root biomass at depth to extract disproportionately large amounts of water during drought conditions (Thomas et al. 2020; Tzohar et al. 2021). If an exponentially declining root length density profile is imposed as prior information, such compensatory uptake from deep soil layers may be systematically downweighed. In this case, posterior estimates may reflect the assumed root distribution rather than the isotope data, leading to biased uptake proportions, especially in deep soil layers where isotope information is often limited.

Moreover, Bayesian mixing models can be sensitive to prior information, a feature that has been highlighted in several studies. For example, Mahindawansha et al. (2018) found that using a non-informative (flat) prior in MixSIAR resulted in nearly uniformly distributed root water uptake proportions, whereas imposing an exponentially declining fine-root length density as a prior produced posterior uptake profiles with a similar exponential shape. Fu et al. (2024) systematically examined this sensitivity using virtual experiments with known root water uptake profiles. They tested four types of prior information, including uniform uptake density, uniform uptake proportion, fine-root distribution, and soil-leaf water potential gradients. Across all scenarios, the inferred root water uptake profiles closely followed the imposed priors, often regardless of the true uptake pattern. This resulted in biased estimates and wide 95% credible intervals of root water uptake proportions, particularly when isotope information alone is insufficient to constrain the mixing system.

These findings demonstrate that prior information can exert a strong influence on posterior estimates in Bayesian inference (Banner et al. 2020; Gelman et al. 2017). Importantly, this influence is not limited to explicitly informative priors. In high-dimensional mixing problems, even nominally non-informative (flat) priors can become effectively informative by constraining the feasible solution space. Since true root water uptake patterns are generally unobservable in field conditions, such prior dominance often remains implicit and unrecognized.

3.3 | Interactions Between Source Grouping and Prior Information

3.3.1 | Informative Flat Prior

In MixSIAR, the Dirichlet distribution is used as the joint prior distribution for root water uptake proportions, while the marginal prior distribution for each source's uptake proportion follows a Beta distribution (Stock et al. 2018):

$$P_i \sim \text{Beta}\left(\alpha_i, \sum_{i \neq j = 1}^{n} \alpha_j\right) \tag{9}$$

where α_i to α_n are the parameters for the Dirichlet distribution. For example, if there are three sources, the joint prior distribution of all root water uptake proportions is Dirichlet (1, 1, 1) (the flat prior), the prior distributions of root water uptake proportion from each source are Beta (1, 2), Beta (1, 2), and Beta (1, 2), respectively.

Using the Dirichlet distribution as the prior for source contributions is mathematically appropriate, because each root water uptake proportion ranges between 0 and 1, and all proportions sum to one. However, the default flat prior is not necessarily non-informative. As indicated by Equation (9), the marginal prior distribution for each source proportion follows a Beta distribution whose shape depends explicitly on the number of sources. As the number of sources increases, this Beta distribution becomes increasingly peaked, thereby imposing stronger implicit constraints on individual source contributions (Figure 7). In other words, the information content of the prior increases with model dimensionality. In the root water uptake studies using MixSIAR, the number of sources typically ranges from 2 to 10 (Figure 5). Only in the case of 2 sources does the default Dirichlet prior yield truly uniform marginal distributions for each source proportion (Figure 7). When five or more sources are considered, a situation that remains common in practice (Figure 5), the same "flat" prior already exerts influence on posterior estimates. This dimensionality-driven prior informativeness is often overlooked in the literature, where flat Dirichlet priors are routinely interpreted as non-informative. Our results highlight that, in high-dimensional mixing problems, prior informativeness is an emergent property of model structure rather than an explicit user choice, with important implications for interpreting inferred root water uptake proportions.

Additionally, even when the number of sources is fixed, differences in layer thickness can substantially alter the information content of a nominally "flat" prior. This effect is particularly relevant in root water uptake studies, where soil profiles are often discretized unevenly with finer layers near the surface and coarser layers at depth.

To illustrate this, consider a 2-m soil profile divided into four layers of unequal thickness (Figure 8a), where the thickness of the top and bottom layers is 0.03 and 1.5 m, respectively. Under the default flat Dirichlet prior, all layers are assigned the same expected root water uptake proportion, despite representing vastly different soil volumes. When these proportions are normalized by layer thickness (converting uptake proportions into uptake density per unit depth), the top layer exhibits a much higher implied uptake density than the bottom layer (Figure 8b).

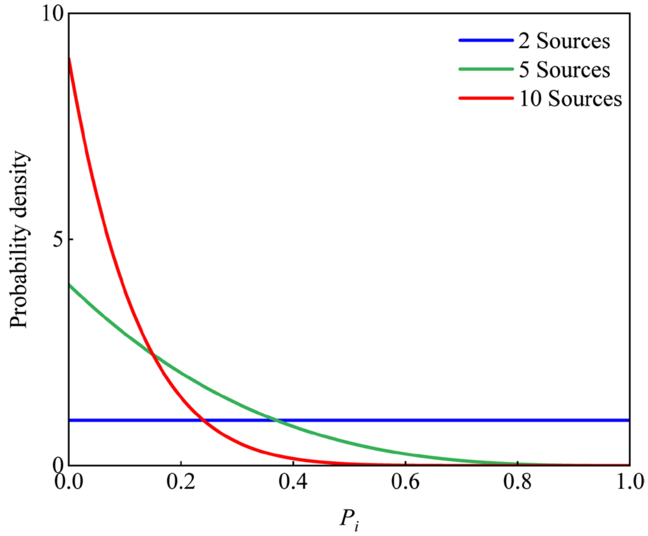

FIGURE 7 | Marginal Beta distributions implied by a nominally non-informative (flat) Dirichlet prior under different numbers of sources. The blue, green, and red curves correspond to Beta (1, 1), Beta (1, 4), and Beta (1, 9), representing the marginal prior distributions of an individual source when the total number of sources is 2, 5, and 10, respectively. When the number of sources equals two, the marginal prior is uniform and can be considered non-informative. As the number of sources increases, however, the marginal distributions become increasingly concentrated, indicating stronger implicit prior information despite the joint Dirichlet prior being specified as flat. This demonstrates that the information content of the marginal prior depends critically on model dimensionality.

This example highlights that a flat prior on uptake proportions is not equivalent to a non-informative prior on uptake density. A truly non-informative prior in a vertically continuous system would imply uniform uptake density across depth, such that water uptake per unit soil thickness is constant regardless of how the profile is discretized (Figure 8c). Consequently, a flat Dirichlet prior is only non-informative when all soil layers have equal thickness. In practice, however, most studies apply fine discretization near the surface and coarse discretization at depth, while simultaneously adopting the default flat prior (Figure 5d). This combination implicitly prefers shallow soil layers in Bayesian inference, even in the absence of supporting data. As a result, prior-induced bias toward shallow uptake can arise purely from discretization choices, rather than reflecting true root water uptake patterns.

3.3.2 | Double Dipping

Double dipping refers to the practice of using the same data both to construct informative prior distributions and to define the likelihood function (Kriegeskorte et al. 2009). This practice introduces circular reasoning, as the data simultaneously inform prior beliefs and posterior updating, thereby violating a core Bayesian principle that prior information and observed evidence should be conceptually independent (van de Schoot et al. 2021).

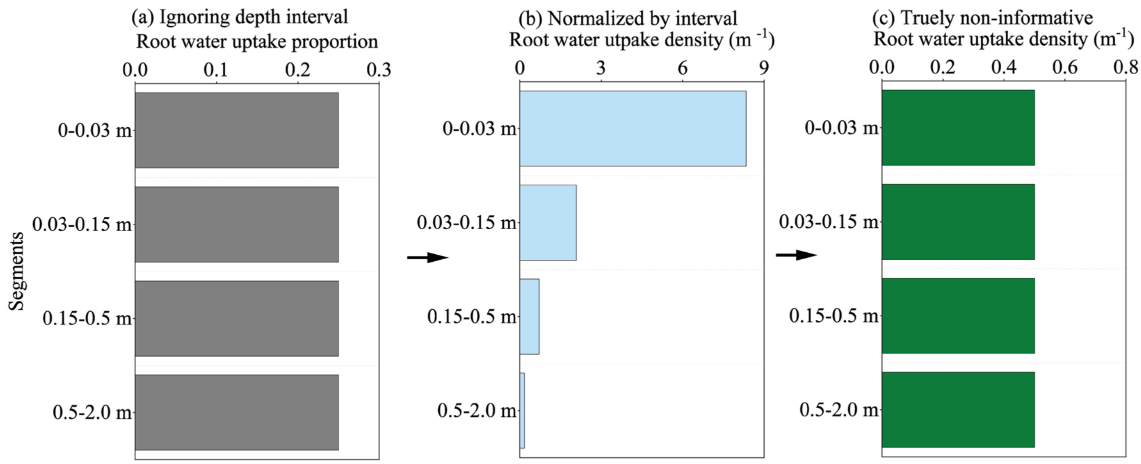

FIGURE 8 | Illustration of how prior specification affects implied root water uptake density under the same source grouping scheme. Panel (a) shows the uniform proportion (nominally non-informative) prior, in which each soil layer is assigned the same initial uptake proportion. Panel (b) shows the corresponding uptake density obtained by normalizing the uptake proportion by layer thickness. Panel (c) shows the uniform density prior, in which each layer is assigned the same initial uptake density, independent of layer thickness.

In isotope-based root water uptake studies, double dipping most commonly arises during source grouping and prior specification. Soil water isotopic data are frequently used to define soil layers or source groups based on similarities in isotopic composition, after which the same grouped isotope data are used again in the likelihood function to estimate root water uptake proportions (e.g., Li et al. 2025; Vega-Grau et al. 2021). Although such practices are often motivated by practical considerations, they effectively reuse the same information at multiple stages of the inference process.

The consequence of double dipping is not merely conceptual but statistical (Spiegelhalter et al. 2002). Reusing data in both priors and likelihood artificially inflates the apparent precision of posterior estimates, leading to overconfident root water uptake proportions and an underestimation of uncertainty (Banner et al. 2020). Moreover, this implicit reinforcement of data-driven structures increases the risk of overfitting, making inferred uptake patterns overly site-specific and less transferable across conditions or studies.

To preserve the validity of Bayesian inference, prior distributions should be constructed independently of the data used in the likelihood whenever possible (van de Schoot et al. 2021). This may include relying on external information (e.g., root traits, hydraulic constraints, or experimental knowledge) and testing the sensitivity of results to data-informed priors. Addressing double dipping explicitly is therefore essential for ensuring transparent, robust, and interpretable applications of MixSIAR in root water uptake studies.

3.4 | Error Structure Is a Critical but Underexamined Factor

Several studies have compared the performance of different Bayesian mixing models in quantifying root water uptake proportions and have often concluded that MixSIAR outperforms alternative approaches (Gai et al. 2023; Liu et al. 2023; Stock and Semmens 2016; Wang et al. 2019) (Figure 9). These conclusions are typically based on various data sets and error structure configurations. However, such comparisons implicitly assume that differences in performance can be attributed primarily to the mixing model itself, rather than to the assumed error structure.

The innovation of MixSIAR lies in its unified error structure (residual*process), which is typically not utilized in these studies, except Stock and Semmens (2016) (Figure 9). For example, Wang et al. (2019) applied a residual error structure in one dataset and a process error structure in another and concluded that MixSIAR consistently outperformed other models. Similarly, Gai et al. (2023) implemented a process error structure in MixSIAR, effectively making its error formulation identical to that of MixSIR, but did not discuss why MixSIAR and MixSIR yielded markedly different accuracy under the same dataset and nominally equivalent error structure. These examples illustrate that model performance is inseparable from error structure specification, and that comparisons across models using different or inconsistently justified error structures are difficult to interpret.

Consequently, studies summarized in Figure 9 demonstrate that different error structures can yield different performance outcomes depending on the dataset, but they do not provide definitive evidence that MixSIAR is intrinsically superior for root water uptake. Rather, they highlight that error structure selection is a dominant and often underexamined component of Bayesian mixing model performance (von Freyberg et al. 2020).

These findings underscore the need for greater transparency and justification in the selection of error structures when applying and comparing Bayesian mixing models. Without explicitly aligning error structure assumptions with the underlying data-generating processes, model comparison results risk conflating differences in statistical formulation with differences in model performance, thereby limiting their interpretability and generality.

3.5 | Other Problems

Beyond model configuration, the reliability of root water uptake estimates from MixSIAR also depends on the information

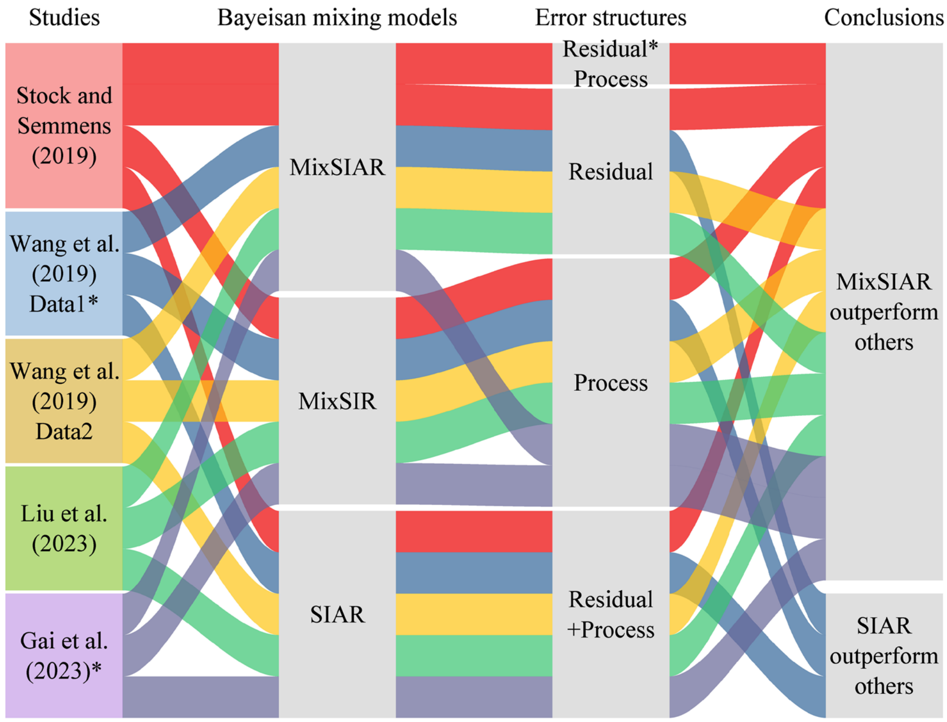

FIGURE 9 | Overview of error structure configurations and reported conclusions from previous model comparison studies in root water uptake. The left column summarizes the error structures used in each study, while the right column summarizes their main conclusions regarding model performance. An asterisk (*) indicates that the non-Bayesian mixing model IsoSource was used for that dataset.

content and uncertainty structure of the input isotope data. Issues related to soil and plant water $\delta^{18}O$ and $\delta^{2}H$ measurements, such as limited isotopic contrast (Hiiragi et al. 2022; Sprenger et al. 2018), spatial and temporal variability (Ceperley et al. 2024; Jiang et al. 2022; Orlowski et al. 2018), primarily affect data identifiability and the validity of likelihood assumptions, rather than representing limitations of Bayesian mixing models themselves. Accordingly, these measurement-related aspects are only briefly acknowledged here, while detailed discussions are available in previous studies (e.g., von Freyberg et al. 2020).

4 | Recommendations and Outlook

We have shown that, despite its widespread use in root water uptake studies, MixSIAR is subject to several recurring methodological challenges. These include (i) model configuration related issues, such as inconsistent source grouping schemes and numbers, strong sensitivity to prior specification and subjective prior selection, and inappropriate or insufficiently justified error structure choices; and (ii) data-related limitations, including weak isotopic contrasts in soil water, measurement uncertainties, unaccounted variability in plant water isotopic signatures, and the influence of unknown or complex root water uptake patterns. Table 1 synthesizes these major pitfalls, clarifies the types of biases they may introduce into root water uptake estimates, and summarizes recommended practices aimed at improving the robustness, interpretability, and reliability of MixSIAR-based root water uptake.

Together, these recommendations highlight the need for model configurations that balance complexity and interpretability, prioritize physically meaningful priors, and employ appropriate error structures tailored to the underlying plant–soil processes.

To provide a quantitative foundation for the recommendations presented in this section, we conducted a series of controlled numerical simulations using MixSIAR. The simulations are based on a virtual dataset derived from Rothfuss and Javaux (2017), in which root water uptake profiles simulated by the physically based Couvreur model are treated as known “true” reference states (benchmark). This framework allows direct evaluation of model performance by comparing inferred uptake proportions against prescribed ground truth under controlled conditions.

Using this virtual test, we systematically examined the effects of key modeling choices commonly encountered in root water uptake studies, including source grouping number, prior specification, and error structure selection. By varying these configurations while holding the underlying root water uptake process constant, the simulations isolate how modeling decisions alone influence bias and uncertainty of estimated root water uptake proportions. Detailed descriptions of the simulation design are provided in Appendix A. Here, we focus on the generalizable insights that inform best practices and motivate the recommendations summarized below.

4.1 | Source Grouping

It is well known that root water uptake is an inherently underdetermined problem (Dubbert et al. 2022). This arises because the root zone can be divided into an infinite number of depth

TABLE 1 | Summary of pitfalls when using MixSIAR for root water uptake and recommendations.

Pitfall	Description	Recommendation
Source grouping numbers	Many sources: Underdetermination (source N exceeds tracer number + 1), inflated uncertainty and overfitting. RMSE may appear to decrease, but DIC increases.	Limit the number of source numbers to 3–4 to balance underdetermination and model stability. Avoid maximizing source numbers purely based on RMSE trends.
Source grouping scheme	Ignoring soil layer thickness causes over-representation of thin layers. Arbitrary grouping can distort depth-resolved root water uptake patterns and amplify biases.	Account for layer thickness when grouping. Consider continuous approaches when fine resolution of root water uptake profile is required.
Prior informativeness	Highly informative priors (e.g., fine root and(or) soil water distribution) may dominate data likelihood.	Use priors with physical justification and minimal informativeness when possible. Adopt uniform-density (UD) priors if independent data are unavailable.
Prior sensitivity	Even flat priors become informative when the number of sources is large; posterior estimates may depend more on priors than data.	Assess prior sensitivity and compare alternative priors. Keep source numbers small (<5) to minimize unintended constraints.
Double dipping	Source grouping and likelihood both derived from isotopic data can cause circular reasoning and overfitting.	Avoid grouping based solely on isotopic similarity; instead, use independent information for source grouping.
Error structure	Using residual*process error structures adds parameters, risks overfitting. Process errors assume active sampling, may be inconsistent with passive root water uptake that generally assumed.	Typically prefer residual error structure. Use residual*process when justified by clear soil–plant variance linkage.

intervals, each potentially contributing to plant water, resulting in far more unknowns than available isotopic constraints. Such an underdetermined system can result in biased (Ghojogh and Crowley 2019) and great uncertainties (Fry 2013) in root water uptake estimates.

A common strategy to mitigate this problem is source grouping, which reduces the dimensionality of the mixing system. Accordingly, we recommend minimizing the number of sources when applying MixSIAR to estimate root water uptake proportions. At first glance, this recommendation appears to contradict our simulation results, which suggest that as the number of source groups increases, the average RMSE decreases (Figure 10a; Rothfuss and Javaux 2017).

However, this apparent improvement reflects a reduction in point-estimate error rather than improved identifiability of the mixing system. Increasing the number of sources inevitably increases the number of unknown parameters relative to the available isotopic tracers, thereby exacerbating underdetermination. As a result, although point estimates may appear closer to the true values on average, the posterior distributions become increasingly sensitive to priors and exhibit larger uncertainty and instability (Fry 2013; Stock et al. 2018). From an inference perspective, this trade-off implies that minimizing source number remains essential for robust and interpretable root water uptake estimation, even when numerical metrics such as RMSE values appear to improve.

Additionally, the necessity of reducing source numbers is supported by the deviance information criterion (DIC) values, which increase as the source number grows in our simulation (Figure 10b). The reason is that the larger the unknown numbers, the more likely MixSIAR is to overfit. Typically, overfitting is undesirable because it makes a model fit not only the underlying signal but also random noise or dataset-specific variability (Ghojogh and Crowley 2019). In a Bayesian context, this instability can further propagate into misleading inference and overconfident conclusions (van de Schoot et al. 2021). Information criteria such as DIC explicitly penalize model complexity and therefore favor models that achieve a balance between goodness of fit and parsimony. Accordingly, when applying MixSIAR to estimate root water uptake proportions, the number of source groups should be minimized to reduce overfitting, improve identifiability, and enhance the robustness of inference.

However, reducing the number of unknowns through source grouping inevitably comes at the cost of vertical spatial resolution in the inferred root water uptake profile (Fu et al. 2024) and can lead to larger approximation errors, as reflected by increased RMSE values (Figure 10a). This highlights a fundamental trade-off between model parsimony and spatial detail in current mixing frameworks.

To alleviate this trade-off, root water uptake can instead be represented using a continuous probability density function (PDF), $f(z; \alpha, \beta)$, along soil depth z, with two unknown shape

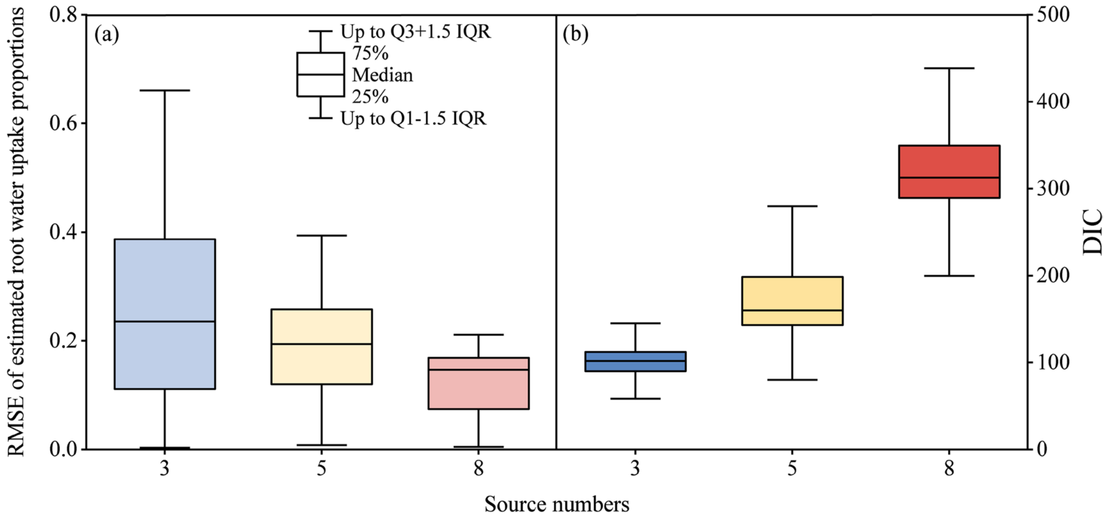

FIGURE 10 | Distributions of root mean squared errors (RMSE; panel a) and deviance information criterion (DIC; panel b) obtained from MixSIAR under different source numbers (three, five, and eight), based on numerical simulations conducted in this study. Boxes indicate the interquartile range (IQR, Q3–Q1), with Q1 and Q3 representing the 25th and 75th percentiles, respectively.

parameters (α, β) (Romero-Saltos et al. 2005), an approach commonly referred to as a continuous isotope mixing model. Unlike MixSIAR, the continuous formulation represents uptake as a smooth function with a limited number of shape parameters, rather than as contributions from predefined soil segments. As a result, it avoids explicit source grouping and preserves vertical continuity in the soil profile.

By reducing the dimensionality of the inference problem while maintaining spatial continuity, continuous mixing approaches minimize information loss and mitigate biases introduced by arbitrary source grouping. Consequently, this approach offers the potential for robust and higher-resolution estimates of root water uptake patterns, particularly when spatial detail is a primary research objective (Fu et al. 2024).

To date, attempts to implement the concept of continuous root water uptake profile remain relatively rare. One example is provided by Romero-Saltos et al. (2005), who represented root water uptake using a normal probability density function along soil depth to estimate the mean uptake depth, an approach later adopted by Yamanaka et al. (2017). This formulation is shown to effectively capture root water uptake depth dynamics.

Another example is RAPID (Ogle et al. 2004), which employs a mixture of two gamma probability density functions along soil depth to describe active root profiles and to compute root water uptake fluxes and proportions across soil layers. Compared to MixSIAR, RAPID offers smaller uncertainties and greater accuracy in estimating root water uptake proportions (Ogle et al. 2004). More recently, Neil et al. (2024) further supported this perspective by incorporating a unimodal probability density function along soil depth into a physically based root water uptake model (Cook and O'Grady 2006), demonstrating improved consistency between estimated root water uptake patterns and observed isotope signals.

Collectively, these studies suggest that continuous approaches hold promise for enhancing accuracy and reducing uncertainty in estimating root water uptake proportions. Extending this concept into a Bayesian framework would allow root water uptake to be modeled as a continuous function of depth, effectively representing an infinite number of sources with a limited set of shape parameters. In doing so, continuous Bayesian mixing models can mitigate the need for arbitrary source grouping and alleviate the trade-off between vertical spatial resolution and source numbers. As a result, such models have the potential to provide more robust, higher-resolution estimates of root water uptake profiles than MixSIAR.

4.2 | Prior Information

The prior distribution is a central component of Bayesian mixing models, as it enables users to constrain the unknown root water uptake proportions based on prior knowledge or auxiliary information (Banner et al. 2020; Depaoli et al. 2020). Using controlled numerical simulations (Appendix A), we evaluated the performance of three commonly used prior specifications. Among them, the uniform density (UD, each source has the same initial root water uptake density, where the uptake proportion equals the density multiplied by the thickness of the layer) consistently produced the lowest RMSE values (Figure 11). At the same time, differences in DIC across prior types are minimal, indicating that the improved performance of UD is not driven by overfitting.

The conceptual rationale of the UD prior is straightforward: it removes the implicit influence of unequal layer thickness on the prior information. In the absence of additional constraints, assumption of uniform uptake density implies that thicker soil layers have a higher potential to contribute water because they occupy a larger volume within the root zone (Fu et al. 2024). By contrast, the commonly used uniform proportion (UP) assigns equal initial uptake proportions to all layers, regardless of their thickness. This assumption implicitly exaggerates the contribution of thin soil layers, particularly near the surface, and potentially underestimates the root water uptake proportions from deep soil (Figure 8).

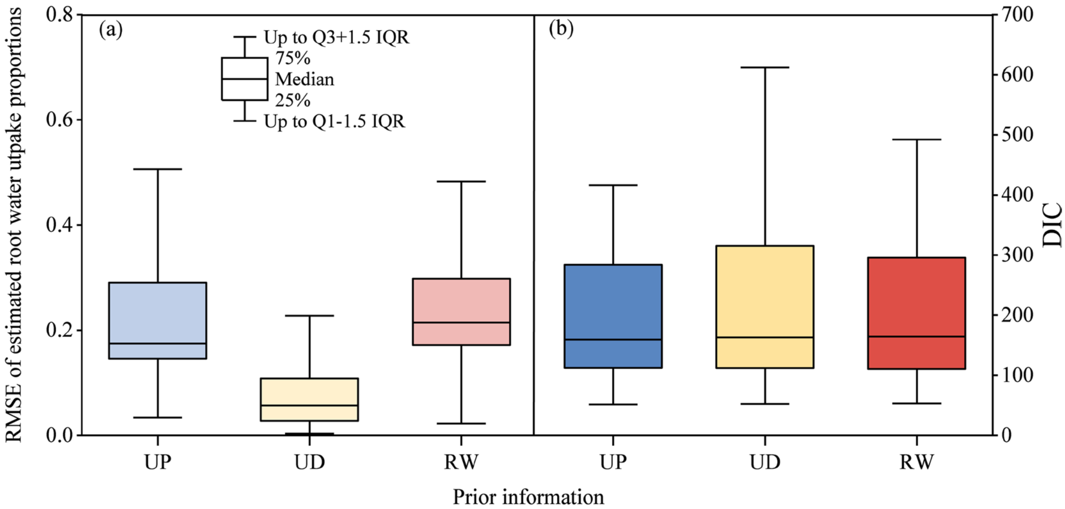

FIGURE 11 | Distributions of root mean squared errors (RMSE; panel a) and deviance information criterion (DIC; panel b) from MixSIAR under different prior specifications, based on numerical simulations conducted in this study. UP, UD, and RW denote uniform proportion, uniform density, and root-soil water-based priors, respectively. Boxes represent the interquartile range (IQR = Q3−Q1), with Q1 and Q3 indicating the 25th and 75th percentiles.

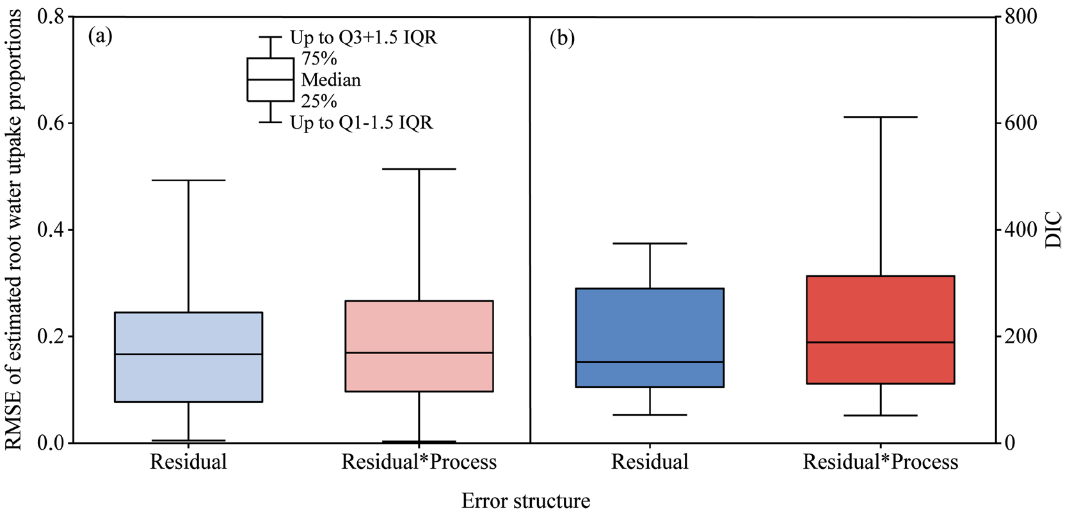

FIGURE 12 | Distributions of root mean squared errors (RMSE; panel a) and deviance information criterion (DIC; panel b) from MixSIAR under different error structure specifications, based on numerical simulations conducted in this study. Boxes represent the interquartile range (IQR = Q3−Q1), with Q1 and Q3 indicating the 25th and 75th percentiles.

Priors based on fine root length density and soil water content have also been widely used (Mahindawansha et al. 2018). However, these priors can be strongly informative and may dominate posterior estimates. Because root water uptake is not necessarily proportional to fine root distribution or soil water content (Zhu et al. 2024), such priors can introduce systematic bias when applied without independent validation.

Therefore, we recommend using a uniform density (UD) prior when estimating root water uptake proportions in the absence of independent auxiliary information (such as root distribution, soil water potential profiles). This choice minimizes unintended constraints imposed by nominally non-informative priors and allows the isotopic mixing data to play a dominant role in posterior inference. When additional information is available and can be independently justified, users may specify informative priors based on that information. Regardless of prior choice, we strongly recommend conducting prior sensitivity analyses to explicitly evaluate how different prior configurations influence posterior root water uptake estimates (Stock et al. 2018).

4.3 | How to Select Error Structures?

Regarding error structures, our simulations indicate that the residual error structure yields RMSE values comparable to those obtained using the residual*process error structure, while consistently resulting in slightly lower DIC values (Figure 12). This pattern suggests that using residual*process tends to introduce additional model complexity without providing commensurate improvements in predictive accuracy, thereby increasing the risk of overfitting. This outcome is expected, as the residual*process formulation includes additional variance parameters to represent source-level variability (Equation (8)), increasing the effective dimensionality of the inference problem. Based on these results, we recommend the residual error structure as a more parsimonious and robust default choice for estimating root water uptake proportions, particularly when the available isotope data are limited and when source-level variability cannot be independently constrained.

It should be noted, however, that compared to the residual error structure, residual*process accounts for source-level variability, and compared to the process-only error structure, residual*process offers greater flexibility and broader applicability (Stock et al. 2018). The feasibility and usefulness of the residual*process error structure have been validated in ecological and dietary mixing studies (Guerrero and Rogers 2020).

In this context, our recommendation in favor of the residual error structure is specific to isotope-based root water uptake studies, where source-level variability is often poorly constrained, sampling is sparse, and the mixing system is strongly underdetermined. Under such conditions, the additional variance components introduced by the residual*process formulation may increase model complexity without sufficient data support, thereby elevating the risk of overfitting. To better guide the selection of error structures in root water uptake applications, it is therefore essential to clearly distinguish the assumptions and implications associated with each error formulation, as summarized in Figure 3 and discussed below.

Mixing models typically distinguish between two fundamental types of error structures: process error and residual error. Process error represents variability arising from incomplete or stochastic sampling of sources, such that variability in the mixture is propagated directly from the variability of source isotopic compositions (Stock et al. 2018). Under a pure process-error formulation, all variance observed in the mixture is attributed to source-level heterogeneity. By contrast, residual error assumes that source means are fixed and that variability in the mixture arises from factors independent of source variability, such as measurement uncertainty, sampling error, or within-organism transport and storage effects. In this case, source variances do not propagate into mixture variance.

Combining these two error structures yields hybrid error structures, such as residual+process (as implemented in SIAR) and residual*process (as implemented in MixSIAR), which increase model flexibility by allowing both source variability and mixture-level noise to contribute to observed tracer variance. While such formulations can provide a more general and biologically realistic representation of uncertainty in some ecological mixing problems, this increased flexibility does not necessarily imply that residual*process is the most appropriate error structure for estimating root water uptake proportions.

If the variability in plant water isotopic compositions can be primarily attributed to variability in soil water isotopic compositions, then process*residual error structure is conceptually appropriate. Such a situation may occur when there is strong evidence that (1) isotopic variance in plant water reflects incomplete or stochastic sampling of heterogeneous soil water pools, or (ii) soil water samples represent only partial sampling of the true source distribution within a given depth interval.

Conversely, if variability in plant isotopic compositions is dominated by factors other than that in soil, such as isotopic heterogeneity during upward water transport (de Deurwaerder et al. 2020; Penna et al. 2018), post-uptake processes within the plant, or uncertainties associated with sampling and extracting (von Freyberg et al. 2020), then a residual error structure is likely more appropriate (Boecklen et al. 2011).

A recent synthesis reported that site-level variances of δ^{18}O and δ^{2}H in plant water are approximately 0.6‰ and 4.2‰ (based on 568 observations), which are substantially smaller than corresponding variances observed in shallow soil water (2.9‰ and 112.4‰, based on 150 observations) and deep soil water (1.0‰ and 50.4‰, based on 8 observations) across a 1 ha area (Goldsmith et al. 2019; von Freyberg et al. 2020). If isotopic variance in plant water is primarily inherited from soil water, one would expect variance of δ^{18}O and δ^{2}H in plant water to fall between the variances of δ^{18}O and δ^{2}H observed in shallow and deep soil water. The fact that variances of δ^{18}O and δ^{2}H in plant water are markedly smaller indicates that variances of δ^{18}O and δ^{2}H in soil water are strongly damped during uptake and transport, supporting the use of a residual error structure in many isotope-based root water uptake applications.

Additionally, root water uptake is generally considered a passive process driven by water potential gradients between soil and leaves (Bowen et al. 2018; McElrone et al. 2013). This mechanism fundamentally differs from the ecological interpretation of process error, which assumes that variability in the mixture arises from active, stochastic sampling of sources. By contrast, variability of δ^{18}O and δ^{2}H in plant water is more plausibly explained by residual error, arising from factors unrelated to source variability, such as differences in transpiration rates (Boecklen et al. 2011), internal water transport and storage, time lags between uptake and sampling, as well as sampling and extraction procedures (von Freyberg et al. 2020).

Moreover, soil water isotope sampling in root water uptake studies typically involves compositing all soil water within a given depth interval, yielding an unbiased estimate of the population mean isotopic composition for each source. Under this sampling strategy, source means are well characterized, and source-level variance should not propagate into mixture variance, which is consistent with the assumptions of a residual error structure (Stock et al. 2018). Consistent with this interpretation, Stock and Semmens (2016) noted that residual error structure is generally preferred for applications beyond diet estimation studies.

Taken together, these considerations provide strong conceptual and practical support for using a residual error structure when estimating root water uptake proportions using isotope-based mixing models.

4.4 | Outlook

4.4.1 | Re-Visiting MixSIAR in Root Water Uptake Studies

MixSIAR has been widely adopted in ecological studies, particularly in animal diet reconstruction, where the assumption of a finite number of discrete and categorical sources (e.g., diet items) is often conceptually and empirically justified (Stock and Semmens 2016). By contrast, in isotope-based root water uptake studies, soil water (the primary source of plant water) is distributed continuously across both depth and time within the rooting zone (Zha et al. 2019). When MixSIAR is applied to quantify depth-based root water uptake, this intrinsically continuous soil water profile is typically discretized into a finite number of soil segments, thereby introducing artificial boundaries between adjacent segments and interrupting the natural continuity of soil water (isotope) profiles (Fu et al. 2024).

The mismatch between a discrete-source mixing framework and a continuous soil water system introduces a fundamental challenge in applying MixSIAR to root water uptake studies. Discretizing the soil profile assumes implicitly that water uptake from different segments can be treated as independent sources. Such an assumption is most defensible when the research objective is to characterize a snapshot of root water uptake under quasi-steady hydraulic conditions; for example, when vertical hydraulic and isotopic gradients are small, or when high-temporal-resolution soil and plant water isotope data are available. Under these conditions, MixSIAR can provide meaningful estimates of instantaneous root water uptake proportions.

However, when soil water isotopic profiles vary rapidly over time, such as following precipitation events or during periods of strong evaporative enrichment, the isotopic distinctness among discretized soil layers may diminish (Fu et al. 2024). In such cases, root water uptake estimates from MixSIAR are only valid at the specific sampling moment, rather than a temporally averaged uptake profile. Consequently, studies that infer monthly or seasonal root water uptake dynamics from sparsely sampled soil and plant water should be interpreted with caution, as they are unlikely to remain stationary over such time scales. Without sufficiently high temporal resolution in isotope measurements, discretized mixing approaches cannot reliably capture time-integrated water uptake behavior.

High-temporal-resolution in situ measurements of $\delta^{18}O$ and $\delta^{2}H$ for soil and plant water offer a partial pathway to mitigate this limitation (Beyer et al. 2020; Dubbert et al. 2022). By narrowing the temporal window over which isotopic profiles are assumed to be stable, such measurements allow MixSIAR to approximate quasi-steady conditions more closely. Recent studies combining high-frequency isotope observations with MixSIAR have demonstrated that temporally dense isotope data can resolve rapid shifts in root water uptake following precipitation and drought events, thereby improving the interpretability of snapshot-based uptake estimates (Kinzinger et al. 2025; Kübert et al. 2023; Sprenger et al. 2025).

Nevertheless, even when temporal resolution is sufficiently high, MixSIAR remains limited in its ability to resolve root water uptake profiles at high vertical spatial resolution. This limitation arises from the underdetermined nature of isotope mixing problems. Increasing the number of discretized soil segments rapidly inflates the number of unknowns relative to the available isotopic information, leading to increased uncertainty of approximated root water uptake proportions (Fu et al. 2024). To overcome this trade-off between vertical spatial resolution and identifiability, recent studies have proposed representing root water uptake as a vertical spatially continuous process using parametric or semi-parametric probability density functions along depth (Fu et al. 2024; Neil et al. 2025). Such continuous formulations can preserve spatial continuity and improve the robustness of inferred root water uptake profiles.

Unfortunately, applications that jointly combine high-temporal-resolution isotope data with continuous root water uptake models remain rare. This scarcity reflects both the limited availability of high-frequency isotope observations and the absence of inference frameworks that can integrate such data with spatially and temporally continuous representations of root water uptake. Addressing this gap will require methodological advances that explicitly link these two perspectives.

4.4.2 | Integrating Biophysical Processes Into Isotope-Based Bayesian Mixing Models

Although stable isotopes of hydrogen and oxygen are powerful tracers for identifying plant water sources (Popp et al. 2025), isotope-based inference alone is often accompanied by substantial uncertainty, arising from equifinality, limited tracer information, and weak identifiability under field conditions (Allen et al. 2022; von Freyberg et al. 2020). Improving the accuracy and reliability of root water uptake estimates derived from BMM requires the incorporation of additional, process-relevant information (Beria et al. 2020; Ogle et al. 2014).

Such complementary information can be obtained from soil–plant water potential measurements, which are largely independent of isotopic observations (de Deurwaerder et al. 2020). Previous work has demonstrated that water transport from soil to roots and subsequently to leaves and the atmosphere is governed by water potential gradients (Mason Earles et al. 2016; McElrone et al. 2013). This implies that root water uptake profiles inferred from water potential gradients should, in principle, provide complementary and physically consistent constraints on isotope-based root water uptake estimates, as both approaches describe the same underlying process from different perspectives.

Significant progress has been made in representing root water uptake using water potential-based process models (Bernhard et al. 2025). For example, Couvreur et al. (2012) developed an analytical formulation of three-dimensional root water uptake based on an Ohm's law analogy, which can be simplified into an effective one-dimensional representation. Subsequent experimental studies have shown that the Couvreur framework often outperforms alternative models (Thomas et al. 2020, 2024).

Consequently, future modeling efforts should aim to integrate process-based root water uptake formulations, such as the Couvreur model, with BMM. Process-based root water uptake models can provide physically grounded prior information, while hydrogen and oxygen stable isotopes can be used to construct likelihood functions. Such combinations within Bayesian frameworks ensure that inferred uptake profiles are jointly constrained by isotopic evidence and biophysical principles, thereby enhancing robustness and improving the physiological interpretability of isotope-based root water uptake estimates.

4.4.3 | Beyond Process-Based Mixing: Challenges and Opportunities for Root Water Uptake

Even when Bayesian mixing models are augmented with biophysical constraints, important limitations remain. A growing body of evidence suggests that plant water does not represent a simple snapshot mixture of soil water sources along depth but rather integrates water across both space and time (Sprenger et al. 2019; Werner et al. 2021). Consequently, xylem water may reflect not only mixing among different depths but also mixing among waters originating from different times at the same depth (Knighton et al. 2020). This elevates the root water uptake problem from a one-dimensional spatial mixing problem to a two-dimensional (spatiotemporal) mixing problem.

Addressing spatiotemporal integration would require either extending Bayesian inference frameworks to explicitly represent $\delta^{18}O$ and $\delta^{2}H$ variability in soil and plant water across space and time, or deconvolving plant water isotope signals using time series of root-zone isotopic compositions combined with estimates of water travel times (Seeger and Weiler 2021; Werner et al. 2021). However, current Bayesian mixing models for root water uptake are not designed to represent two-dimensional mixing.

Recent advances in representing root water uptake profiles using probability density functions along depth offer a promising pathway toward spatiotemporal mixing by extending these models from one-dimensional to two-dimensional formulations. However, such an extension is not a straightforward modification. Introducing spatiotemporal mixing would inevitably increase model complexity by adding parameters, strengthening constraints, expanding posterior dimensionality, and substantially increasing data requirements. Whether these added degrees of freedom can be supported by available isotope observations remains an open question. Future methodological development is therefore needed to assess when and how spatiotemporal mixing can be represented in a statistically identifiable and practically feasible manner.

Besides spatiotemporal mixing, root water uptake is also shaped by factors that go beyond water availability. Root water uptake reflects a compromise among multiple physiological and ecological constraints (Kakouridis et al. 2022), including carbon investment, nutrient acquisition, and hydraulic efficiency. Water availability not only influences root distribution but also controls the mobility and accessibility of nutrients, thereby coupling root water uptake to nutrient dynamics (Addo-Danso et al. 2020; Cusack and Turner 2021; Kong et al. 2017). During pronounced dry periods, ecosystems often experience a strong spatiotemporal separation between water and nutrient availability, particularly in water-limited environments (Cusack and Turner 2021). As a result, root water uptake strategies may emerge from trade-offs among water uptake, nutrient acquisition, and carbon allocation, rather than being uniquely determined by isotopic observations. These multi-objective controls further limit the extent to which root water uptake can be constrained by isotope-based mixing alone, even when augmented with hydraulic process models.

Finally, root water uptake inference is becoming increasingly high-dimensional because capturing key processes requires integrating spatiotemporal dynamics, hydraulic constraints, nutrient availability, and plant physiological traits. Accounting for these interacting controls inevitably increases both data requirements and model complexity, posing substantial challenges for classical parametric modeling frameworks. As data volume and diversity continue to grow, future root water uptake research may therefore benefit from hybrid approaches that combine process-based mixing models with data-driven methods. Machine learning techniques, for example, offer powerful tools for organizing, integrating, and extracting structure from massive data (Popp et al. 2025). Recent applications of machine learning to isotope hydrology, such as neural-network-based reconstructions of precipitation isotopes at continental scales (Nelson et al. 2021), illustrate the potential for such approaches to complement mechanistic models. Integrating machine learning with isotope-based and process-informed root water uptake models may therefore provide new opportunities to constrain complex uptake dynamics while maintaining consistency with biophysical principles.

Taken together, these considerations suggest that future advances in root water uptake research will depend on inference frameworks that move beyond static and one-dimensional mixing, while remaining explicit about their identifiability limits. Integrating spatiotemporal dynamics and physiological constraints will require not only methodological innovation, but also disciplined evaluation of what isotope observations can and cannot resolve.

5 | Conclusion

Bayesian mixing models have substantially advanced our understanding of root water uptake by enabling quantitative inference of plant water sources and responses to environmental stress. This review synthesizes applications of the most widely used Bayesian mixing model, MixSIAR, in root water uptake studies and identifies several recurring methodological challenges. These include inconsistent source grouping schemes and numbers, strong sensitivity to prior specification, implicit prior dominance under high-dimensional settings, double dipping, and inappropriate error structure selection.

A central methodological insight emerging from this review is that the behavior of priors in Bayesian mixing models is inherently dependent on model dimensionality. Increasing the number of soil layers to improve vertical resolution can unintentionally amplify prior dominance, even when nominally non-informative (flat) priors are used. This effect has important implications for interpreting root water uptake estimates and highlights the need to explicitly consider dimensionality and prior behavior in Bayesian inference.

Based on both literature synthesis and numerical simulations, we provide several practical recommendations. Increasing the number of source groups may reduce approximation error in estimated uptake proportions, but it also increases the risk of overfitting. When prior information is required, accounting for soil layer thickness offers a defensible baseline assumption in the absence of additional constraints. Regarding error structures, a residual-only error formulation is generally preferable for root water uptake studies, as it introduces fewer unknowns and reduces overfitting risk relative to more complex alternatives.

Looking forward, progress in isotope-based root water uptake research is unlikely to come from incremental refinements of existing mixing models alone. Instead, future advances will require inference frameworks that better reflect the physical continuity of soil water, biophysical root water uptake processes, and the information content of available data. Developing physically grounded, spatially continuous mixing or inference models can reduce reliance on arbitrary source grouping and mitigate prior dominance in high-dimensional settings. When combined with appropriate error structures, hydraulic constraints, and auxiliary process information, such approaches offer a promising pathway toward more accurate, robust, and physiologically interpretable estimates of root water uptake. Bayesian methods will therefore remain valuable tools, but only when applied within identifiable regimes and embedded in inference frameworks that explicitly acknowledge dimensionality, spatiotemporal integration, and underlying ecohydrological processes.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The MixSIAR code is publicly available from Stock and Semmens (2016). The virtual data used in our numerical simulations is originally from Rothfuss and Javaux (2017). A complete list of the literature reviewed in this manuscript is accessible from Zenodo repository (http://doi.org/10.5281/zenodo.18975799).

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Appendix A

Numerical Simulations in Section 4

The dataset used for the MixSIAR evaluation originates from Rothfuss and Javaux (2017), where the root water uptake profile estimated by the Couvreur model, a physically based root water uptake model, is treated as the "true" reference. The corresponding soil water content and isotopic distributions, together with the true uptake profile, are shown in Figure A1. For detailed information about the dataset, descriptions of the Couvreur model and its applications, readers are referred to Rothfuss and Javaux (2017) and Couvreur et al. (2012).

MixSIAR Configuration

Based on our review, three variables were identified within the model configuration category: source grouping numbers, prior information, and error structure. Other factors, such as random and fixed effects, were not considered because they are generally ignored in most studies using MixSIAR for root water uptake.

The source grouping number includes three levels: 3, 5, and 8 based on isotope information and the other based on fine root and soil water content distributions using k-means clustering. Plant water isotopic compositions are derived from "true root water uptake" profiles and given soil water isotopic compositions (Figure A1).

While the prior information has three levels: uniform proportion, uniform density, and combined fine root and soil water content distributions. Uniform proportion means that, given the number of sources, each source starts with the same initial uptake proportion. This is the default flat prior in MixSIAR:

$$\boldsymbol{P} \sim \text{Dirichlet}\left(\alpha_1,\ \dots,\alpha_n\right)$$

$$\alpha_1 = \ \dots\ = \alpha_n = 1 \tag{A2}$$

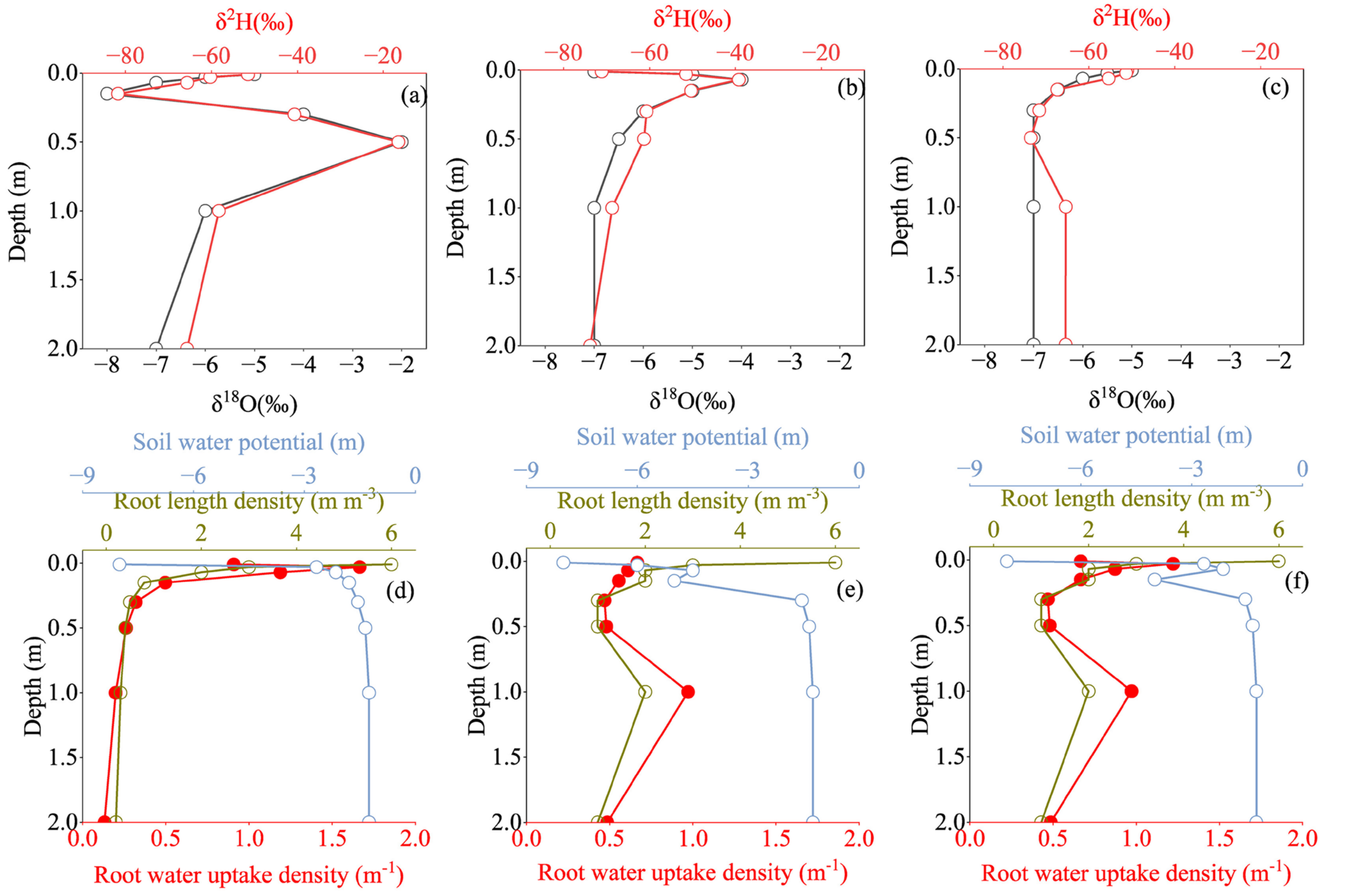

FIGURE A1 | Soil water isotopic profiles at varying levels of isotopic contrast (panels a–c). Fine root length density (dark yellow) and soil water potential (blue) profiles that used in Couvreur model for estimating "True" root water uptake profiles (red) under different levels (panels d, e). Note that panels b and e are originated from Rothfuss and Javaux (2017), other panels are derived with modifications to suit the simulations in this study to cover the most spatial and temporal root water uptake patterns of trees during their growing season (Bachofen et al. 2024).

Uniform density means that, given the number of sources, each source has the same initial root water uptake density, where the uptake proportion equals the density multiplied by the thickness of the layer. In other words, the thicker the layer, the greater it is weighed in the Dirichlet distribution:

$$\boldsymbol{P} \sim \text{Dirichlet}\left(\alpha_1, \ \dots, \alpha_n\right)$$

$$\alpha_i = \frac{\Delta z_i}{z_r} n \tag{A3}$$

where z_r is the rooting depth; Δz_i is the layer thickness of source i; n is the number of sources (unknowns).

Prior information based on combined fine roots and soil water content distributions assumes that the root water uptake profile is proportional to the product of fine root distribution and soil water distribution, with layers containing more fine roots and soil water being weighed more heavily. Shape parameters of the Dirichlet distribution in this prior setting are estimated from the normalized profile of the product of fine root length density (RLD) and soil water content (SWC) distributions (Mahindawansha et al. 2018):

$$\boldsymbol{P} \sim \text{Dirichlet}\left(\alpha_1, \ \dots, \alpha_n\right)$$

$$\alpha_i = \text{RLD}_i \frac{\text{SWC}_i}{\sum_{i=1}^{n} \text{RLD}_i \, \text{SWC}_i} n \tag{A4}$$

Lastly, the error structure includes two levels: residual and process*residual. It is important to note that MixSIAR offers three options, including residual only, process only, and residual*process. However, we excluded "process only" because the process error is already accounted for in the "process*residual" error structure. Additionally, "process only" requires that the mixture (plant water) contains only one data point, which is not the case in majority studies.

MixSIAR is employed to solve the posterior distributions of root water uptake proportions, which are further used to calculate the root mean squared error (RMSE), which is used to quantify the errors of estimated root water uptake proportions from MixSIAR:

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left(P_{E,i} - P_{T,i}\right)^2} \tag{A5}$$

where $P_{E,i}$ and $P_{T,i}$ are estimated and true root water uptake proportion of soil layer i; n is the number of soil layers. Note that another index, deviance information criterion (DIC), is included during MixSIAR evaluation and it can be printed by MixSIAR directly.