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Review

Application of Stable Isotopes in Water Transport Processes of the Soil–Plant–Atmosphere Continuum in *Eucalyptus* Plantations: A Review

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Abstract

The water cycle in forest ecosystems is a core component of ecosystem services, and it is crucial for revealing terrestrial hydrological processes and regulation mechanisms. Investigating water migration across interfaces of the soil–plant–atmosphere continuum (SPAC) has long been a hotspot in ecohydrological research. *Eucalyptus* stands are among the world's major commercial timber species, and their hydrological effects have attracted growing public attention. Naturally occurring hydrogen and oxygen stable isotopes can effectively differentiate water sources, serving as powerful tracers widely applied in hydrological research. Nevertheless, comprehensive systematic reviews on SPAC water dynamics of *Eucalyptus* plantations using stable isotope quantification are still lacking. This study systematically reviews the application of hydrogen and oxygen stable isotopes in analyzing water transport and transformation processes at multiple interfaces of *Eucalyptus* plantation SPAC, covering atmospheric precipitation sources, soil and plant water migration, plant transpiration and groundwater exchange. Future research should strengthen in-situ continuous monitoring of isotopic signatures in diverse water bodies, facilitate the popularization of isotope-based models, and advance quantitative partitioning of evapotranspiration in *Eucalyptus* ecosystems. This review provides theoretical and technical support for exploring hydrological processes and mechanisms in forest ecosystems, and offers scientific references for improving forest water use efficiency and optimizing regional water resource allocation.

Keywords: oxygen and hydrogen stable isotopes; *Eucalyptus* plantation; water transport; soil–plant–atmosphere continuum (SPAC); ecohydrology

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1. Introduction

Stable isotopes (e.g., δ^2H and δ^{18}O) serve as natural tracers to reveal water migration pathways and transformation mechanisms within the Soil–Plant–Atmosphere Continuum (SPAC) [1–3]. Distinct isotopic fractionation occurs when water transforms across the different interfaces of soil, plants, and the atmosphere: evaporation and vapor diffusion selectively mobilize light isotopes, leaving residual liquid water enriched in heavy isotopes. These interface-dependent isotopic signatures act as fingerprints to separate contributions from different water sources and quantify interfacial water exchange fluxes, overcoming the limitation of conventional monitoring methods that cannot directly observe subsurface and in-plant water migration [4,5]. In forest hydrology and plant water use research, stable isotope techniques can explicitly clarify root water uptake, in-plant water transport

and transpiration loss, as well as differentiated contributions of plant transpiration and soil evaporation to atmospheric vapor [6,7]. These techniques provide fundamental data support for quantifying SPAC water transport processes and deepen insights into inherent hydrological cycling patterns of terrestrial ecosystems. Accordingly, they lay a scientific foundation for optimal allocation of forest water resources, targeted vegetation restoration and conservation, and sustainable management decision-making.

Forest ecosystems play a pivotal role in terrestrial water regulation and material cycling, underpinning regional ecological stability and environmental conservation [8–10]. Clarifying isotopic signatures and transformation relationships of water bodies along the SPAC is critical to deciphering underlying forest hydrological processes and driving mechanisms [11,12]. As the primary water input of forest ecosystems, atmospheric precipitation profoundly regulates forest hydrological cycles and plant water utilization [13]. Precipitation infiltration recharges soil water, followed by water redistribution via plant transpiration, soil evaporation and subsurface infiltration within forests (Figure 1). Evapotranspirational vapor exchanges with ambient air sustain forest ecosystem water balance [14,15]. Therefore, water movement and transformation across forest SPAC interfaces have become key research hotspots in hydrology, ecology and soil science in recent decades.

Eucalyptus refers to a genus of flowering plants belonging to the Myrtaceae family. It exhibits rapid growth, strong environmental adaptability and prominent economic value, ranking among the world's three major fast-growing tree species together with poplar and pine. *Eucalyptus* was introduced into China in 1890 and widely cultivated on a large scale starting in the 1950s [16]. Since the 1980s, its economic and ecological values have been fully recognized. The planting area expanded 12.22 times over 35 years, rising from 466,000 hectares in 1986 to 5,694,000 hectares in 2021.

Large-scale *Eucalyptus* plantation cultivation in China delivers dual socioeconomic and ecological benefits: it has effectively improved regional ecological conditions, alleviated the acute supply-demand imbalance of domestic timber, and promoted the development of timber processing and related industries [17]. However, such plantations also embody a typical ecological–economic trade-off. Extensive *Eucalyptus* planting has substantially altered regional vegetation composition, surface coverage, canopy structure and soil physicochemical properties. These changes exert direct and indirect effects on forest ecohydrological processes including precipitation partitioning, soil infiltration, evapotranspiration and plant water consumption. While fast-growing Eucalyptus stands provide essential timber products, their high water demand may shift local water budgets and potentially trigger risks to regional hydrological and ecological security under water-limited conditions [18].

Correspondingly, eco-environmental problems arising from *Eucalyptus* cultivation have attracted increasing academic attention. For instance, heated debates remain over whether large-scale *Eucalyptus* planting aggravates soil drought, lowers groundwater tables and reduces understory plant diversity [19]. To date, no unified consensus has been reached on the hydrological impacts of *Eucalyptus* plantations [20]. Nevertheless, scholars generally agree that extensive *Eucalyptus* cultivation accelerates regional evapotranspiration, further affecting surface runoff generation and soil water distribution [21,22]. In addition, disputes still exist regarding whether *Eucalyptus* planting disrupts regional soil water balance and induces potential ecohydrological security hazards [23,24].

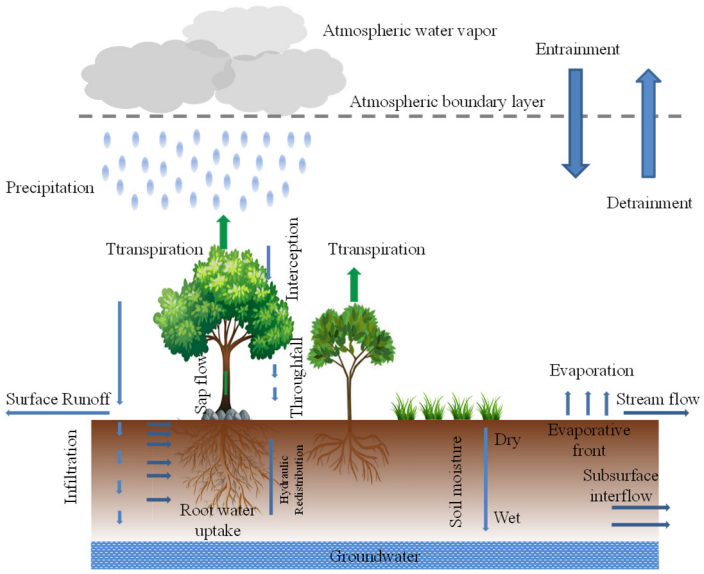


Figure 1. Schematic diagram of water transport processes in the soil–plant–atmosphere continuum (SPAC) [25].

Currently, scholars worldwide, including researchers from Australia, Brazil, India and South Africa, have conducted extensive studies on individual hydrological processes including precipitation, soil infiltration, evaporation and transpiration within *Eucalyptus* plantations [26–28]. Nevertheless, systematic investigations focusing on SPAC-scale water migration in *Eucalyptus* plantations remain inadequate. Accordingly, clarifying how *Eucalyptus* cultivation affects regional hydrological cycles has important scientific implications. However, the underlying mechanisms driving SPAC water transport variations induced by *Eucalyptus* growth have not been fully clarified.

As inherent constituents of natural water molecules, hydrogen and oxygen stable isotopes carry abundant environmental information and serve as effective natural tracers to track water migration across multi-interfaces [29,30]. Crucially, no significant isotopic fractionation occurs during root water uptake and subsequent long-distance water transport in plant xylem, which ensures that the isotopic signatures of xylem water can faithfully retain the original isotopic characteristics of plant water sources. This unique property makes hydrogen and oxygen stable isotopes reliable and powerful tools for quantitatively identifying and partitioning vegetation water sources. This study systematically reviews advances in stable isotope applications for revealing interfacial water dynamics throughout the soil–plant–atmosphere continuum of *Eucalyptus* plantations. It also identifies key research priorities, aiming to deepen our understanding of water migration, transformation and their corresponding driving mechanisms in plantation SPAC. This review can offer valuable references for stable isotope-based research on ecohydrological processes of *Eucalyptus* forests, support sustainable industrial development, and facilitate rational regional water resource regulation.

2. Materials and Methods

This review develops its overall research framework based on the complete water transport and transformation pathways within the soil–plant–atmosphere continuum (SPAC) of *Eucalyptus* plantations. Following the SPAC water movement, this framework systematically encompasses the core hydrological processes of *Eucalyptus* plantation ecosystems, including precipitation vapor source identification, soil water infiltration and redistribution, soil surface evaporation, selective root water uptake by *Eucalyptus*, bidirectional soil–plant

hydraulic redistribution, canopy transpiration, and hydrological interactions between the SPAC system and groundwater.

A systematic and standardized literature retrieval and screening strategy was adopted to ensure the comprehensiveness and representativeness of the included studies. Specifically, literature searches were performed across two core databases, namely the China National Knowledge Infrastructure (CNKI) and Web of Science (WoS) Core Collection, with the search period spanning 1958 to 2025. The core search terms included stable isotopes, SPAC, and *Eucalyptus*, while secondary keywords covered precipitation, soil water, plant water uptake, soil evaporation, plant transpiration, and groundwater for multidimensional hierarchical retrieval.

After initial retrieval, more than 300 relevant records were identified. Strict screening criteria were applied according to the core theme of this review, and 100 valid references were ultimately included. Studies unrelated to stable isotope techniques, Eucalyptus plantation ecology, and SPAC water transport processes were excluded, whereas high-quality papers with clear objectives, rigorous experimental designs, and robust conclusions were retained. All eligible references were thoroughly collated, analyzed, and synthesized. Key findings and scientific insights from these studies were incorporated into corresponding sections of the review, offering solid theoretical support, empirical evidence, and a sound research foundation for systematically elaborating each research perspective. Grounded in the complete SPAC water transport framework of *Eucalyptus* plantations, this review systematically summarizes recent advances in stable isotope-based hydrological research, effectively addressing the fragmented research perspectives of previous reviews. Furthermore, this study identifies prominent knowledge gaps in current literature, including insufficient multi-interface coupling analyses, a scarcity of long-term in-situ monitoring, and a general lack of applied research targeting plantation water resource management.

3. Water Transport Processes in the SPAC of Eucalyptus Plantations

3.1. Water Vapor Transport of Atmospheric Precipitation

Atmospheric precipitation constitutes a critical water input to the hydrological cycle of *Eucalyptus* plantations, particularly for the SPAC (Figure 2). The stable isotopic signatures of precipitation can be preserved in soil water, acting as a crucial tracer of environmental hydrological information. Analyzing the spatiotemporal variations in stable isotopes of precipitation helps trace water vapor sources and characterize corresponding environmental conditions. Furthermore, it improves our understanding of interfacial water transport across the soil, plant and atmosphere compartments, as well as the hydraulic connectivity among various water bodies [1]. Numerous studies have demonstrated that the isotopic signatures of regional precipitation are mainly controlled by water vapor sources, vapor transport pathways, and local climatic conditions.

The stable isotopic signatures of atmospheric precipitation exhibit distinct seasonal variations, which are predominantly controlled by multiple meteorological factors, including air temperature, precipitation intensity, relative humidity, evaporation, and condensation processes [31]. These general controls on precipitation isotopes also operate in *Eucalyptus* plantation ecosystems, where pronounced seasonal isotopic patterns have been documented across multiple sites. For instance, precipitation in *E. urophylla* × *E. grandis* plantations in the Leizhou Peninsula and karst areas of Guangxi, China, exhibits notable evaporative isotopic enrichment during the dry season [32,33]. This phenomenon leads to substantial seasonal differences in precipitation δD and δ^{18}O values, with negative values in the rainy season and positive values in the dry season. Analysis of hydrogen and oxygen isotopic signatures in atmospheric precipitation helps elucidate environmental changes during the movement and transformation of precipitation-derived water. The δD

and $\delta^{18}O$ values of rainwater in *Eucalyptus* forests in the eastern mountainous regions of Melbourne, Australia, show considerable variations between 2001–2002 and 2003 [34]. Such variations can be ascribed to differences in atmospheric water vapor transport conditions across different periods. Precipitation isotopes are generally enriched for continental moisture sources and depleted for marine moisture sources. Changes in moisture sources of rainwater further modify its hydrogen and oxygen isotopic signatures. Specifically, $\delta^{18}O$ is highly sensitive to evaporative enrichment, whereas δD is relatively stable against surface evaporation but suffers from larger uncertainties in plant-mediated isotopic fractionation, imposing distinct limitations for tracing hydrological processes.

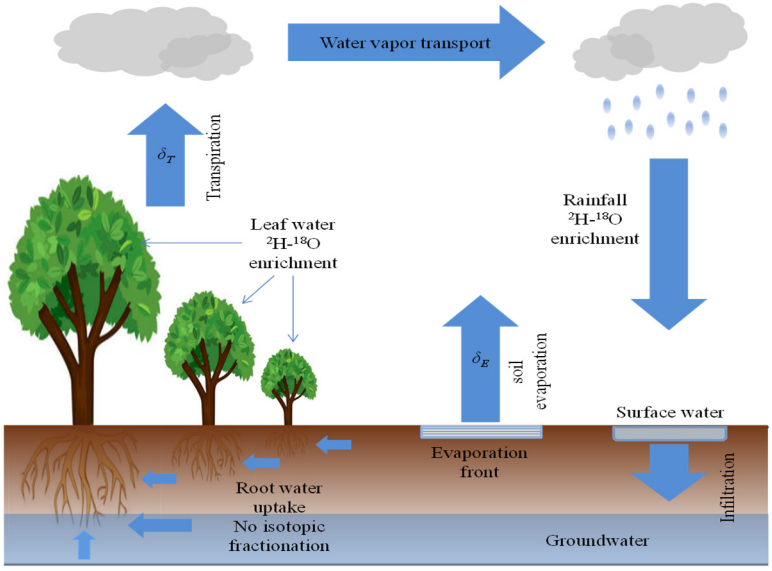


Figure 2. Schematic diagram illustrating the use of isotopic signatures to trace diverse plant water sources.

3.2. Soil Water Transport

3.2.1. Sources of Soil Water

Soil water acts as a critical hydrological linkage among atmospheric precipitation, groundwater, and plant water, and dominates water transformation processes at the soil-atmosphere interface within the SPAC [35]. Precipitation represents the primary source of soil water recharge, and its seasonal fluctuations substantially modulate soil water regimes and transport processes [36]. The hydrogen and oxygen isotopic signatures of soil water are regulated by multiple factors, including precipitation input, vegetation coverage, surface evaporation, and horizontal movement and vertical percolation of soil water [37,38]. Accordingly, analyzing the spatiotemporal distribution of stable isotopic signatures in soil water enables quantitative differentiation of soil water sources and clarifies the mechanisms through which precipitation infiltration modulates soil water transport and transformation [39,40].

These general principles governing soil water isotopes have been widely verified in *Eucalyptus* plantation ecosystems, showing unique vertical recharge characteristics along soil profiles. In *Eucalyptus* plantations, precipitation-derived soil water recharge intensity declines with soil depth. The isotopic signatures of shallow soil water are consistent with those of recent rainwater, indicating that shallow soil layers are predominantly recharged by precipitation. In contrast, deep soil water is weakly affected by precipitation recharge, with relatively stable isotopic signatures that differ substantially from those of shallow soil water [41]. Ding et al. (2016) reported that frequent rainfall in the rainy season effectively replenishes shallow soil water (0–50 cm), with isotopic signatures closely matching those

of concurrent precipitation [32]. Conversely, deep soil layers (50–100 cm) receive limited rainfall recharge, with distinct isotopic signatures relative to shallow soil layers. Accordingly, comparative analysis of isotopic signatures across different water bodies and vertical soil profiles can clarify the intrinsic relationships among hydrological components and reveal how precipitation infiltration regulates soil water transport. However, such isotopic signals are complicated by evaporative fractionation in topsoil, which selectively enriches heavy isotopes and may distort source partitioning if fractionation effects are not explicitly incorporated into hydrological interpretations.

3.2.2. Soil Evaporation

Evapotranspiration is a core component of the hydrological and energy cycles in terrestrial ecosystems and constitutes a major pathway for ecosystem water output. As a key component of evapotranspiration, soil evaporation acts as an indispensable hydrological component in terrestrial ecosystems [42,43]. Soil water evaporation is constrained by atmospheric evaporative demand and soil water supply capacity, and these two factors jointly drive stable isotope enrichment in surface soil water [44]. During soil water evaporation, light isotopes preferentially escape from the soil water phase, leading to enrichment of heavy isotopes in residual soil water [45,46].

Soil evaporation mainly occurs in topsoil layers, resulting in a gradual decline in heavy isotope values of soil water with increasing soil depth [47,48]. This vertical isotopic gradient induced by soil evaporation has been repeatedly documented in *Eucalyptus* plantation ecosystems with site-specific characteristics. Relevant research on *E. urophylla* plantations in China's Leizhou Peninsula confirms that surface evaporation induces significant $\delta^{18}O$ enrichment in 0–40 cm soil water. The $\delta^{18}O$ values within this soil layer gradually become more negative with soil depth, indicating pronounced isotopic fractionation effects [33]. Studies of mixed *Eucalyptus* forests in Hincks Conservation Park verify that surface soil water evaporation induces intense isotopic fractionation, with higher stable isotope enrichment in topsoil than in deep soil layers. Moreover, temperature variations and seasonal shifts substantially modulate the evaporation-induced isotopic fractionation process [49]. Existing studies show marked isotopic enrichment driven by soil water evaporation in the surface layer (above 15 cm) of mixed *Eucalyptus*–oak forest ecosystems. Soil texture, especially high clay content, exerts a considerable influence on evaporation-induced isotopic enrichment in soil water [34]. Critically, soil water heterogeneity arising from texture, pore structure and microtopography creates spatially variable evaporative fractionation patterns, which may lead to biased upscaling of isotopic observations from discrete soil sampling points to the whole Eucalyptus stand.

3.3. Plant Water Transport

3.3.1. Sources of Plant Water

Plants mainly acquire soil water via root water uptake to sustain physiological metabolism, rendering root water uptake a core process that characterizes water transport across the soil–plant interface (Figure 2) [50,51]. Soil water is transported upward to leaves and multiple tissues through xylem vessels and tracheids, and eventually lost via canopy transpiration or used to support fundamental physiological processes including respiration and photosynthesis [52,53]. Numerous studies have demonstrated that, with the exception of a small number of halophytic and xerophytic species, no detectable isotopic fractionation occurs during root water uptake and subsequent xylem water transport from roots to branches [54–56]. Accordingly, comparing the hydrogen and oxygen isotopic signatures of xylem water with those of potential water sources enables quantitative estimation of the proportional contributions of different water sources to plant water use and clarifies plant

water utilization patterns [2,57]. Such isotopic tracing principles for plant water sourcing have been widely applied to reveal water-use strategies in *Eucalyptus* species. In a recent study conducted in the Great Artesian Basin of Queensland, Australia, Jones et al. analyzed the stable hydrogen and oxygen isotopic signatures of soil water, *E. camaldulensis* xylem water, and groundwater. The results indicated that *E. camaldulensis* predominantly utilizes shallow soil water above the groundwater aquifer [58].

Currently, stable hydrogen and oxygen isotopes have been widely applied to quantify plant water sources, with mainstream approaches including direct comparison [59,60], water uptake depth models [61], binary and ternary linear mixing models [62,63], the multi-source IsoSource linear mixing model [64], and Bayesian stable isotope mixing models (Table 1) [65–67]. Direct comparison and water uptake depth models can only identify the dominant soil depth of plant water absorption, while other models allow for the quantitative estimation of the proportions of plant water use from diverse potential water sources. Notably, Bayesian stable isotope mixing models (e.g., MixSIR, SIAR, and MixSIAR) comprehensively account for uncertainties in source isotopic signatures, mixture isotopic signatures, and overparameterization effects, thereby achieving high accuracy in quantifying the proportional contributions of plant water sources [68–70]. Zuo et al. (2025) integrated stable hydrogen isotopes with the MixSIAR model to analyze seasonal water use strategies of *Eucalyptus* plantations in southern subtropical China and identified distinct age-dependent differences in water use between the dry and wet seasons [71]. Model partitioning results are highly sensitive to endmember definition and discretization, and spatiotemporal variation in source isotopic composition may introduce additional uncertainties, especially when potential water sources exhibit poor isotopic separation. Nevertheless, these mixing models are constrained by the equifinality problem and require clear isotopic separation among endmembers; overlapping isotopic signals of soil water at different depths in *Eucalyptus* plantations may increase uncertainty in source apportionment, and model outputs often rely on predefined source classification rather than capturing dynamic, depth-varying water availability.

Table 1. Comparison of different methods for identifying plant water sources via stable isotopes.

Methods and Models	Properties	Application Advantages	Reference
Direct comparison	Qualitative	Qualitatively identify plant root water uptake from soil water at specific depths	[60]
Water uptake depth model	Semiquantitative	Determine the average depth of plant water uptake from soil	[61]
Two- or three-compartment linear mixing models	Quantitative	Quantify the relative contributions of multiple water sources to plant water use	[63]
Multisource linear mixing model: IsoSource	Quantitative	Suitable for quantifying plant water sources under multi-source mixing conditions	[64]
Bayesian stable isotope mixing models: MixSIR, SIAR, MixSIAR	Quantitative	Quantify the proportional contributions of plant water sources; MixSIAR generally provides the highest analytical accuracy	[65]

Plant water source allocation and root water uptake patterns are tightly regulated by environmental factors, including regional climate conditions, root distribution characteristics, and soil water regimes. In recent decades, altered precipitation patterns have resulted in spatiotemporal precipitation heterogeneity in tropical and subtropical zones, triggering frequent seasonal droughts that severely constrain the growth of local *Eucalyptus* plantations [72,73]. In response to frequent seasonal droughts in *Eucalyptus* planting regions,

Eucalyptus trees can flexibly adjust their root water uptake strategies to adapt to changing environmental conditions, shifting water uptake among soil water from different depths and thus inducing pronounced seasonal variations in water source allocation. Using stable isotope techniques, Wang et al. (2023) found that *E. urophylla* × *E. grandis* plantations in China's Leizhou Peninsula adopt flexible water use strategies under variable hydrological conditions (Table 2) [33]. During the rainy season, the trees mainly utilize soil water from the 0–100 cm layer, with a proportional contribution of 52.3%. In contrast, during the dry season, they predominantly depend on deep soil water (150–200 cm) and groundwater, with corresponding proportional contributions of 29.9% and 22.6%, respectively.

Table 2. Potential water source utilization ratios of *Eucalyptus urophylla* × *E. grandis* plantations in rainy and dry seasons.

Potential Water Source	Rainy Season (%)	Dry Season (%)
Soil water (0–100 cm)	52.3	27.8
Soil water (100–200 cm)	33.0	48.6
Groundwater	14.7	22.6

Furthermore, the water source allocation of *E. urophylla* × *E. grandis* in karst regions of Southwest China is strongly modulated by slope position and seasonal variations in precipitation and soil water content. In the rainy season, *E. urophylla* × *E. grandis* predominantly absorb shallow soil water on upper slopes but substantially increase deep soil water utilization at slope bottoms. During the dry season, the trees mainly depend on deep soil water at upper slopes and shallow soil water at slope bottoms [32]. These findings demonstrate the prominent advantages of stable isotope techniques in exploring *Eucalyptus* water sources, including minimal environmental disturbance, high detection accuracy, and high analytical efficiency. Isotope-based approaches can quantitatively elucidate the water sources, utilization patterns, and adaptive strategies of *Eucalyptus* trees, thereby advancing the mechanistic understanding of how *Eucalyptus* regulates water use processes in response to shifting precipitation regimes.

3.3.2. Soil–Plant Hydraulic Redistribution

Soil–plant hydraulic redistribution (SPHR) is defined as the passive root-mediated water transport across heterogeneous soil layers, driven by water potential gradients at the root–soil interface [74–76]. Specifically, root-mediated water movement occurs vertically between shallow and deep soil layers [77], horizontally along slopes or soil profiles [78], and downward from surface soil to subsoil layers [79]. Burgess et al. (2001) reported that following rainfall-induced surface soil wetting, roots of Australian *Eucalyptus camaldulensis* (river red gum) and *Eucalyptus platypus* (coastal moort) transported considerable volumes of water downward into dry subsoil, verifying root-driven hydraulic redistribution [80]. This process serves as a critical physiological adaptation formed through long-term natural selection, enabling plants to cope with the spatial heterogeneity of soil moisture. It not only promotes soil water and nutrient redistribution but also modulates interspecific water use competition, further enhancing the stability and productivity of terrestrial ecosystems.

Stable isotope tracing approaches typically use deuterium-enriched heavy water to irrigate plant root zones, followed by monitoring variations in hydrogen and oxygen isotopic signatures in rhizosphere soil water and xylem water. Stable isotope tracers have been deployed to quantify this root-mediated hydraulic redistribution in *Eucalyptus* plantation ecosystems. Comparative analysis of isotopic fractionation enables identification and quantitative evaluation of hydraulic redistribution (HR) intensity [81,82]. Further field investigations by Asensio et al. (2020) documented year-round persistent hydraulic redis-

tribution in Brazilian *Eucalyptus grandis* plantations, which remained detectable even in the rainy season with a groundwater depth of 17 m [83]. In a tracer experiment, Brooksbank et al. (2011) injected deuterium-labeled water into the groundwater of five-year-old *Eucalyptus kochii* subsp. *borealis* (C. Gardner) D. Nicolle plantations [84]. Two weeks after injection, the deuterium concentrations in surface soil water and xylem water were 30% higher than those in the control plots. These results strongly demonstrate that *E. kochii* subsp. *borealis* can absorb deep groundwater via roots and release it into shallow soil layers, realizing effective hydraulic lift. This finding highlights that the integration of high-precision soil water monitoring and isotope tracing approaches allows for the quantitative assessment of HR effects on rhizosphere and landscape-scale water balance [85]. However, most current isotope-based interpretations of preferential water uptake assume homogeneous root water extraction and neglect non-linear root foraging behavior under fluctuating soil water availability, which may oversimplify the actual water-use plasticity of Eucalyptus roots under heterogeneous field conditions.

3.4. Plant Transpiration

Plant transpiration and soil evaporation constitute the main vapor conversion pathways within the forest SPAC, and transpiration exerts a dominant influence on forest evapotranspiration dynamics [61,73]. Stable hydrogen and oxygen isotopic approaches enable quantitative separation of water sources contributing to transpiration and soil evaporation, serving as a powerful tool for partitioning water flux in terrestrial ecosystems [86]. Isotopic fractionation occurs during soil water evaporation, leading to heavy isotope enrichment in surface soil water. In contrast, soil water vapor produced by evaporation exhibits lower heavy isotope abundance relative to bulk soil water [61]. Transpiration vapor derives from foliar water loss. As water escapes from leaves, leaf water becomes enriched in heavy isotopes relative to stem xylem water [87]. Transpiration vapor shares similar isotopic signatures with xylem water only when transpiration reaches an isotopic steady state (ISS) [88]. Accordingly, evapotranspiration source partitioning can be achieved by identifying isotopic differences between transpiration vapor and soil evaporation vapor. The Keeling plot method is widely adopted to calculate evapotranspiration vapor isotopic composition, which describes the linear relationship between isotopic signatures of atmospheric water vapor and the reciprocal of vapor concentration [89]. Furthermore, the flux-gradient and eddy covariance methods are also applicable for analyzing isotopic characteristics of evapotranspiration vapor [90].

These isotope-based partitioning frameworks for evapotranspiration have been applied to disentangle water flux components in *Eucalyptus* plantation ecosystems. Using the capability of stable isotopes to differentiate evapotranspiration vapor sources, many studies have examined transpiration and evapotranspiration processes in *Eucalyptus* ecosystems. Walker and Brunel (1990) used stable isotopic techniques to examine transpiration and evapotranspiration dynamics in semi-arid mixed *Eucalyptus* forests [49]. Their results reveal that foliar transpiration induces pronounced heavy isotope enrichment in leaf water relative to stem water, and plant transpiration substantially modulates community-scale evaporation. Spatially heterogeneous transpiration rates across leaf tissues cause variable isotopic enrichment within individual leaves. For instance, *E. pauciflora* exhibits lower transpiration rates at leaf bases than leaf tips under both dry and wet conditions, corresponding to depleted ΔD values at leaf bases [91]. Environmental factors strongly modulate *Eucalyptus* transpiration and leaf water isotopic signatures. High atmospheric humidity reduces transpiration intensity and alters leaf water isotopic signatures in *E. citriodora*, further altering vegetation evapotranspiration characteristics [92]. Critically, the conventional evapotranspiration partitioning framework generally assumes complete mixing of

soil water, which ignores the core proposition of the two-water world theory; this may underestimate the isotopic offsets between mobile and tightly bound soil water and induce systematic biases in quantifying *Eucalyptus* evapotranspiration sources.

3.5. Water Exchange with Groundwater

Hydrological processes including atmospheric precipitation, soil water movement, root water uptake and groundwater dynamics are closely interconnected within the SPAC of *Eucalyptus* plantations [58,93]. Stable hydrogen and oxygen isotope techniques can effectively trace groundwater sources and migration pathways, and clarify hydrological interactions between groundwater, precipitation, soil water and plant water [94,95]. Intense rainfall first recharges surface soil water during infiltration. With increasing infiltration depth, precipitation gradually replenishes deep soil layers and eventually groundwater aquifers [96,97]. These general isotopic principles for tracing groundwater recharge and plant–groundwater interactions have been validated in many *Eucalyptus* ecosystems. Pettit and Froend (2018) found a strong linear correlation between isotopic signatures of precipitation and groundwater in mixed *Eucalyptus* forests of northern New South Wales, Australia, demonstrating that precipitation serves as an important groundwater recharge source in this region [98]. *Eucalyptus* water use is tightly linked to groundwater availability. When the water table lies within the main root distribution zone, groundwater consumption is largely regulated by plant transpiration. Surface soil moisture declines sharply in the dry seasons and fails to sustain normal tree growth. Under such circumstances, *Eucalyptus* trees absorb water from deep vadose zones and even extract groundwater, which correspondingly reduces groundwater recharge capacity. In contrast, the proportional contribution of groundwater declines remarkably when surface soil water is replenished by seasonal rainfall [99,100].

4. Conclusions

4.1. Existing Problems

Although global studies have made considerable progress in applying stable hydrogen and oxygen isotopes to explore water transport and transformation among multiple water bodies in the *Eucalyptus* plantation SPAC, several limitations remain in the use of isotopic techniques for clarifying interfacial water transport and cyclic mechanisms. These research gaps are summarized as follows.

Current SPAC water transport studies mainly focus on individual hydrological components, including precipitation, soil water, plant water, and groundwater. Nevertheless, insufficient attention has been paid to the interconversion and movement processes among these water components, limiting the comprehensive understanding of hydrological interactions and coupling relationships across the SPAC. Interfacial water transformation processes in the SPAC operate across diverse temporal scales. Accordingly, high-resolution continuous isotopic datasets are essential for capturing the fine-scale spatiotemporal dynamics of water isotopic signatures in the SPAC. Future research should prioritize systematic investigations of water transport in each SPAC component and implement long-term in-situ monitoring of dynamic stable isotopic variations in precipitation, soil water, and plant xylem water. Such strategies can address the limitations of short-term and discontinuous sampling in existing studies, providing a robust approach to clarify water supply and transmission mechanisms within the SPAC.

Distinct discrepancies in δD and δ^{18}O signatures between soil water and xylem water can induce substantial biases in stable isotope-based plant water source partitioning, thereby generating considerable uncertainties in analytical outcomes. Specifically, δD may reflect isotopic fractionation occurring within the plant vascular system, and such frac-

tionation could serve as the source of these isotopic mismatches. To date, most relevant studies have adopted conventional experimental procedures including field sampling, water extraction, and isotopic measurement to quantify plant water sources. Nevertheless, such conventional approaches are not only destructive to plant and soil samples but also induce prominent uncertainties in the determination of isotopic compositions. Future research should focus on exploring water transport and conversion mechanisms in plant–soil systems across multiple scales, covering microscopic perspectives (e.g., root–soil interface and soil pore scales) and macroscopic regional scales. Such multi-scale investigations can clarify the underlying causes of isotopic deviations and optimize water isotope sample extraction and measurement techniques. Representative applications include the construction of cross-climate zone observation networks covering diverse ecological and vegetation types. Furthermore, these efforts can ultimately elucidate the intrinsic linkages between the dynamic evolution of stable isotopic distribution patterns and hydrological transport heterogeneity across the SPAC, effectively reducing the uncertainties in isotope tracing-based plant water source analysis. In addition, advances in isotope-enabled hydrological modelling, remote sensing and GeoAI offer promising avenues to upscale plot-scale isotope observations; process-based isotope models can reduce equifinality in water-source partitioning, while remote sensing retrievals and GeoAI help map spatial heterogeneity of soil water and plant water use across Eucalyptus plantations, though challenges remain in parameterization and process constraint for these data-driven tools.

Bayesian isotope mixing models, widely adopted to quantify *Eucalyptus* water-source contributions, are particularly sensitive to endmember definition, the degree of isotopic separation among potential water sources, and spatiotemporal variation in source isotopic composition. Weak isotopic differentiation between layered soil water pools and static endmember classification may distort estimates of seasonal shifts in Eucalyptus root water uptake and groundwater reliance. Such methodological uncertainties directly hinder reliable evaluation of plantation water-use strategies and limit our capacity to assess the impacts of Eucalyptus cultivation on regional groundwater balance.

4.2. Suggestions for Future Studies

Constrained by the limitations of current isotopic analytical techniques, existing studies primarily focus on characterizing temporal variations in isotopic datasets, whereas the underlying mechanisms through which plant physiological traits and soil physicochemical properties modulate isotopic fractionation remain poorly understood. Future studies should integrate hydrological principles with plant physiology, soil science, and ecology to advance the microscopic mechanistic understanding of SPAC water transport processes.

Targeting these research gaps, future priorities for *Eucalyptus* plantation SPAC water transport studies are proposed as follows. First, it is essential to strengthen in-situ continuous monitoring of stable isotopic signatures across multiple water sources. This strategy can mitigate the substantial uncertainties in isotope-based water source quantification caused by the inherent temporal dynamics of water isotopes. Long-term and systematic monitoring of isotopic signatures in precipitation, soil water, and groundwater can generate comprehensive datasets of isotopic dynamics, enabling accurate quantification of short-term isotopic fluctuations and providing robust data support for identifying dominant plant water sources.

Second, current methodologies for identifying plant water sources should be optimized. Prior calibration of isotopic observational data is required to eliminate instrument- and environment-induced errors. Combined with the isotopic characteristics of distinct water sources, this approach helps clarify the dominant causes of plant–soil isotopic discrepancies. Such improvements can precisely quantify the proportional contributions of

plant water sources and further facilitate the reliable application of stable isotope mixing models, particularly Bayesian frameworks.

Third, quantitative evapotranspiration partitioning in *Eucalyptus* plantation ecosystems requires further exploration. Isotope tracing techniques should be adopted to quantify seasonal variations in the proportional contributions of soil evaporation and canopy transpiration. Furthermore, a multi-index comprehensive analysis integrating isotopic signals of soil water, xylem water, foliar water, and atmospheric water vapor is recommended to further elucidate the linkages between evapotranspiration ratio dynamics and plant adaptive water use strategies under variable hydrological conditions (e.g., the dry and rainy seasons).

These research directions advance our understanding of water use efficiency (WUE) and sustainable water management for Eucalyptus plantations. Long-term multi-source isotopic monitoring and optimized water source apportionment capture seasonal shifts in plant water uptake, clarify trade-offs between water uptake niche and WUE, and inform stand regulation and safe groundwater exploitation. Isotope-based evapotranspiration partitioning separates unproductive soil evaporation from canopy transpiration, supporting mulching or pruning to boost ecosystem water productivity. Nevertheless, uncertainties persist: field isotopic signals are disturbed by extreme weather and microtopography, Bayesian models rely on prior assumptions, and the long-term stability of plant water-use strategies under progressive aridification remains unconfirmed.

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