6-7-2023

Updating the Dual C and O Isotope—Gas-exchange Model: A Concept to Understand Plant Responses to the Environment and Its Implications for Tree Rings

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Comments

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REVIEW

Plant, Cell & Environment PCE WILEY

Updating the dual C and O isotope—Gas-exchange model: A concept to understand plant responses to the environment and its implications for tree rings

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Abstract

The combined study of carbon (C) and oxygen (O) isotopes in plant organic matter has emerged as a powerful tool for understanding plant functional responses to environmental change. The approach relies on established relationships between leaf gas exchange and isotopic fractionation to derive a series of model scenarios that can be used to infer changes in photosynthetic assimilation and stomatal conductance driven by changes in environmental parameters (CO_2, water availability, air humidity, temperature, nutrients). We review the mechanistic basis for a conceptual model, in light of recently published research, and discuss where isotopic observations do not match our current understanding of plant physiological response to the environment. We demonstrate that (1) the model was applied successfully in many, but not all studies; (2) although originally conceived for leaf isotopes, the model has been applied extensively to tree-ring isotopes in the context of tree physiology and dendrochronology. Where isotopic observations deviate from physiologically plausible conclusions, this mismatch between gas exchange and isotope response provides valuable insights into underlying physiological processes. Overall, we found that isotope responses can be grouped into situations of increasing resource limitation versus higher resource availability. The dual-isotope model helps to interpret plant responses to a multitude of environmental factors.

KEYWORDS
CO_2, drought, dual C and O isotope model, environmental changes, environmental pollution, H_2O gas exchange, isotopic fractionation, photosynthetic carbon uptake, water relations

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1 | INTRODUCTION

Plants are subject to a wide range of environmental impacts and even withstand environmental extremes, for example, in temperature (e.g., −60°C to +45°C in boreal forests), water availability (e.g., prolonged months of drought in arid regions), nutrient availability (e.g., low nutrients in mountainous regions) and disturbance (e.g., storm damage, fires or land use change). Their ability to acclimate is, therefore, critical for their survival. To describe the nature of responses and acclimatization, detailed and comprehensive plant physiological measurements are necessary; however, the intensive nature of these measurements often limits the extent to which they can be applied across space and time. Moreover, numerous physiological measurements of plant responses to the environment (e.g., classical approaches such as plant gas exchange, light interception, sap flux measurements, etc.) cannot be applied retrospectively. Here, the analysis of stable isotope ratios of plant organic matter can provide additional insights by effectively tracing and integrating physiological processes over both time and space (Dawson & Siegwolf, 2007).

The use of stable isotopes as a proxy for understanding the relationships between environment and plant function relies on a mechanistic understanding of the drivers of isotopic fractionation (Dawson et al., 2002). For instance, changes in environmental conditions and related effects on photosynthesis and transpiration result in predictable carbon (C) and oxygen (O) isotope ratios that are imprinted on photosynthetic assimilates (e.g., carbohydrates). Although there may be further possible isotope fractionations between primary and secondary metabolites, these isotopic 'fingerprints' are ultimately reflected in the biomass. The isotopic ratio of assimilates can thus be used as a record of the interaction between the plant and its environment over the lifetime of the specific tissue (e.g., leaf, roots or wood).

In the last four decades, the application of stable C and O isotope ratios ($^{13}C/^{12}C$ and $^{18}O/^{16}O$) in plant ecophysiological research has become common for studies of short- and long-term effects of environmental changes on vegetation (Dawson & Siegwolf, 2007; Ehleringer et al., 2000; Farquhar et al., 1982; Griffiths et al., 1997). As each isotope ratio reflects particular physiological responses caused by specific environmental changes, the use of both C and O isotope ratios provides highly complementary information that improves the potential for interpreting cause and effect. Consequently, the two isotope ratios have been combined and linked with leaf-level CO_2 and H_2O gas exchange in a formal conceptual model that facilitates inferences about changes in assimilation and stomatal conductance (g_s) in response to the environment (Scheidegger et al., 2000).

The dual C and O isotope model formally proposed by Scheidegger et al. (2000), hereafter described as the DI-model, has been subject to a diversity of applications. With these applications have come new insights into the strengths and weaknesses of the model that have led to advances in our understanding of plant function. To highlight these insights, we carried out a quantitative review of studies that have engaged the DI-model to date.

Specifically, we aimed to (1) review the mechanistic basis of the model, (2) determine the nature and extent of its application to date in different fields and (3) review studies that provide data on the mechanistic relationships between plant physiology and C/O isotopes to strengthen our ability to interpret the model (Table 2).

Finally, we would like the readers to keep in mind that although the DI-model looks simple and straightforward at first sight, caution is needed and a solid knowledge of the physiology of the plants under investigation and their environment is crucial. In the following sections, we refer to the points to watch out for.

2 | THE DI-MODEL FOR C₃ PLANTS

The purpose of the DI-model is to make inferences regarding the responses of net assimilation (A_{net}; Larcher, 2003) and g_s to environmental variables based on a dual-isotope (carbon and oxygen) response. Since (1) both carbon and plant oxygen isotope ratios are modified during CO_2 and H_2O gas exchange in the same leaf, but by different processes (photosynthesis and transpiration), and (2) both carbon and oxygen are incorporated into organic matter equally, it provides a means by which to distinguish whether a change in isotope ratios is the result of a change in photosynthesis or in g_s. The current theory on C and O isotope fractionations is summarized in Boxes 1 and 2.

In general, two conditions are compared so that it is possible to draw an arrow from condition A to condition B both with respect to the isotope and gas-exchange observations (Figure 1). Conditions A and B may represent two treatments in a controlled experiment, two functional groups (e.g., compare the responses of grasses and herbs or broad-leaved plants with conifers, when exposed to the same environmental changes or treatment, see Section 3) in the same ecosystem, leaf or stem samples from two positions in a canopy, one species compared between two sites, the same plant at two time points (temporal development) and so on. Note that the red arrows in Figure 1 refer to a range of plant responses, indicating the plasticity of plants responding to environmental impacts within the indicated range. A difficulty with long-term isotope data records is the variability of relative humidity (RH), the $\delta^{18}O$ values of the source water and the water vapour. Therefore, a multiproxy approach, for example, ice core data or sediments, is recommended (Churakova (Sidorova) et al., 2019; Sidorova et al., 2008). Furthermore, there are also new tools based on machine learning to estimate reliably past variations in $\delta^{18}O$ of precipitation (Nelson et al., 2021), or calculate RH (Wu et al., 2023) and in relation to soil water, isotope variations (Graf et al., 2019).

First, the carbon and oxygen isotope ratios need to be measured in an organic compound (e.g., bulk leaf, cellulose, sugars, wood or root material) of the plants under investigation. Proper selection of the plant and compound is important, as it has consequences for the temporal scale at which the physiological processes are integrated. For instance, the time integrated by different compounds is usually longest for cellulose, intermediate

BOX 1.

C isotopes: Figure 4 shows a situation with increasing environmental stress from left to right, for example, increasing drought. The variability of the $^{13}C/^{12}C$ isotope ratio in organic matter is predominantly determined by the extent of CO_2 and H_2O gas exchange during photosynthesis (Farquhar et al., 1982, 1989; Vogel, 1980). The degree of the stomatal opening regulates the water loss (i.e., transpiration). This leads to a concomitant decrease in the diffusion of CO_2 along the gradient between the ambient air (c_a) and the leaf intercellular spaces (c_i). Thus, depending on the stomatal conductance (g_s) and the drawdown of $[CO_2]$ by photosynthesis (net assimilation [A_{net}]), c_i will decrease along with the c_i/c_a ratio (see Figure 4). Farquhar et al. (1982, 1989) showed that a reduction in c_i/c_a, decreases the fractionation between ^{13}C and ^{12}C ($\Delta^{13}C$). This results in a higher $^{13}C/^{12}C$ ratio because the partial pressure of $^{12}CO_2$ decreases faster, as a consequence of the isotopic effect (Bigeleisen, 1965), than that of the $^{13}CO_2$, resulting in an increase of the $^{13}CO_2$ relative to the $^{12}CO_2$ partial pressure. This higher $^{13}CO_2$ partial pressure ratio leads to a higher incorporation rate of ^{13}C, expressed in decreased $\Delta^{13}C$ (or less negative $\delta^{13}C$) values of organic matter synthesized under the respective conditions. Consequently, $\delta^{13}C$ values reflect the interplay between g_s and A_{net}, which is controlled by environmental variations in water supply and demand, as well as photosynthetically active radiation, temperature or CO_2 concentration. This isotope ratio is transferred to sugars, the primary photosynthetic product, and used for metabolic processes and biomass synthesis (see also Cernusak et al., 2013).
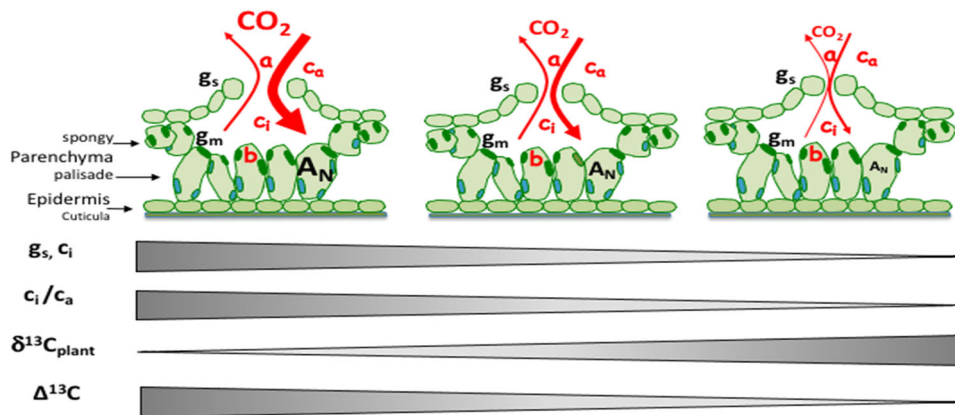

FIGURE 4 Mechanistic linkages between plant physiology and isotopes of carbon at the leaf level. c_a and c_i stand for ambient and intercellular CO_2 mol fractions, respectively, g_s and g_m for stomatal and mesophyll conductance; a is the fractionation of CO_2 in the air and through stomata, b the fractionation during carboxylation and A_N is the net photosynthesis. Note that in most cases the stomata are on the bottom side of the leaf. [Color figure can be viewed at wileyonlinelibrary.com]

However, the observed (net) fractionation ($\Delta^{13}C_{observed} \approx \Delta^{13}C_{net}$) is a composite of numerous, complex biochemical and physical processes, and can be summarized as $\Delta^{13}C_{observed} = \Delta_b - \Delta_{g_s} - \Delta_{g_m} - \Delta_e - \Delta_f$, where each fractionation term is flux rate weighted (Ubierna et al., 2018). A range of fractionation values is provided by Ubierna and Farquhar (2014). Δ_b is the fractionation associated with the carboxylation of CO_2 via RuBisCo, in the absence of any respiratory fractionation. Δ_{g_s} is the fractionation associated with the diffusion of CO_2 through the boundary layer and the stomata. Δ_{g_m} is the fractionation associated with mesophyll conductance g_m. Δ_e reflects fractionations associated with the day-respiration rate R_d (cytoplasmatic decarboxylation, mitochondrial metabolism, C-remobilization and respiration of the heterotrophic tissues; Tcherkez et al., 2017). Since Δ_b and Δ_{g_s} are linked to the largest C-flux they reflect the largest part of the observed fractionation in the biomass, while Δ_e and Δ_f are associated with the small respiratory C-fluxes and are often neglected. Thus, the $\Delta^{13}C_{observed}$ can be described by the simplified equation[1] (Cernusak et al., 2013; Ubierna & Farquhar, 2014). However, for plants operating at low light conditions (e.g., understorey plants) the respiratory fractionations might become more relevant (Barbour, Ryazanova et al., 2017; Busch et al., 2020; Liu et al., 2021). A comprehensive overview of day respiration and its impact on e is given in Tcherkez et al. (2017). Moreover, after entering the intercellular spaces, the CO_2 molecules traverse the mesophyll cell walls and chloroplast membranes with their diffusional resistance r_{mb} and r_c respectively, which is summarized as $g_m = 1/(r_{mb} + r_c)$. As g_m can contribute significantly to the limitation of photosynthesis by regulating the chloroplast CO_2 concentration (c_c), it impacts the ^{13}C isotope fractionation (Δ_{g_m}) accordingly (Evans 2021; Evans & von Caemmerer, 2013; Sharkey, 2012). Yet, its experimental determination is challenging and often not possible; therefore, mostly a model-based estimation.

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[1]The simplified net isotope fractionation for C_3 plant is described as $\Delta^{13}C_{observed} = a + (b - a) \gamma c_i/c_a$, with a, the fractionation of CO_2 in the air and through stomata, b the fractionation during carboxylation and c_i and c_a are the mol fractions for the intercellular and ambient CO_2.

What are the implications of Δ_{g_m}, Δ_e and Δ_f with regard to the use of the dual C and O isotope approach? Mostly, the observed fractionation, $\Delta^{13}C_{observed}$ or $\delta^{13}C_{observed}$, is used, which is driven by the most dominating C-flux that is controlled by A_{net} and g_s with its associated fractionation Δ_b and Δ_{g_s}. Therefore, Δ_{g_m}, Δ_e and Δ_f can be neglected. However, as soon as any of these components are separately studied, or derivatives of the fractionation model, for example, intrinsic water-use efficiency (Gimeno et al., 2020; Ma et al., 2021), it is essential that the respective fractionation components are taken into account accordingly.

for bulk, smaller for water-extracted compounds and shortest for isolated sucrose (Lehmann et al., 2017). Second, the 'input scheme' of the DI-model, a plot of $\delta^{18}O$ data versus $\delta^{13}C$ data comparing the conditions of interest, can be drawn. Alternatively, the oxygen enrichment in the organic compound relative to source water ($\Delta^{18}O$) can be presented. This is advantageous in cases where the $\delta^{18}O$ of source water differs between treatment condition A compared to condition B; given that this is not a leaf-level physiological effect, it could ultimately result in erroneous model interpretations (Roden & Siegwolf, 2012). Similarly, it is also possible to present the carbon isotope discrimination ($\Delta^{13}C$) instead of original $\delta^{13}C$ data, particularly in cases where the $\delta^{13}C$ of source CO_2 is not constant (e.g., atmospheric CO_2 enrichment studies, or retrospective analyses of organic matter, tree ring, peat bogs). Note that in the case of $\Delta^{13}C$, the ^{13}C arrows in the dual-isotope plot will be pointing to inverse inclination as compared to using (δ) original data, whereas for $\Delta^{18}O$, the direction of the arrows remains unchanged. Here, we assume the presentation of $\delta^{18}O$ or $\Delta^{18}O$ versus $\delta^{13}C$ (Figure 1).

Deducing the A_{net}–g_s response from the isotope pattern can be explained as a three-step process: (1) a change in c_i/c_a (the ratio of leaf internal to ambient CO_2) is deduced from a change in the $\delta^{13}C$, (2) a change, for example, in the RH or vapour pressure deficit (VPD) is causing a change in g_s, which is deduced from a change in the $\delta^{18}O$, and (3) the estimated c_i/c_a change is reinterpreted as a change in A_{net} and g_s based on the information derived in the previous steps. The first step is relatively straightforward based on the theory of C isotope fractionation during photosynthesis (Box 1) (Farquhar et al., 1982, 1989). Thus, a significant increase in $\delta^{13}C$ (scenarios #1, #2 and #8) will be interpreted as a decrease in c_i/c_a, a decrease in $\delta^{13}C$ (scenarios #4–6) as an increase in c_i/c_a and no change in $\delta^{13}C$ (scenarios #3 and #7) as no change in c_i/c_a. In the second step, a change in $\delta^{18}O$ can be interpreted as a response of g_s to humidity (Box 2). This is also relatively straightforward, given the established theory on O isotope fractionation. In particular, enrichment is strongly driven by drier conditions (low RH or high VPD affecting g_s, resulting in a low leaf external to internal partial vapour pressure ratio (e_a/e_i; see Box 2) and leading to higher $H_2^{18}O$ enrichment, as described by the Péclet modified Craig and Gordon and Dongmann (CG & D) model (Cernusak et al., 2002; Craig & Gordon, 1965; Dongmann et al., 1974; Farquhar & Lloyd, 1993; Kahmen et al., 2008). The third step is the most critical one, resulting in

the qualitative nature of the model. As an example, we discuss scenarios #4 and #6, which both indicate increasing c_i/c_a that can be caused by either increasing g_s or decreasing A_{net}. In scenario #4, we additionally know that there is higher $\delta^{18}O$ ('drier conditions'); therefore, the case of increasing g_s seems physiologically implausible and the other possibility, decreasing A_{net}, is selected. In contrast, for scenario #6, we observe lower $\delta^{18}O$ at increasing c_i/c_a and thus increasing g_s is more likely than decreasing A_{net}. This kind of reasoning directly results in the proposed A/g_s scenarios shown in the bottom row of Figure 1.

The use of the original DI-model is applicable to a large number of studies. However, care must be taken when plants respond differently to specific conditions, for example, to increasing CO_2, air pollutants, changes in nutrient supply or extreme drought. This can lead to model outputs that suggest plant responses that contradict our current physiological understanding because such additional and potentially uncontrollable impacts are not considered by current C or O isotope fractionation models. Consequently, the DI-model will produce physiologically nonplausible results as it is strictly based on the C and O isotope fractionation and gas-exchange principles (see Sections 3, 5 and 6). Such discrepancies then need to be resolved, by considering the changes in fractionation mechanisms or different timing of leaf- and stem-level processes.

An example of such discrepancies is cases of extreme and ongoing drought. Under such conditions, the isotopic signal of the leaves could not be found in the stems or other heterotrophic tissue. Since water became so limited, no growth was possible and the assimilates were barely sufficient to maintain the metabolism. Therefore, no isotope signal representing these hot and dry conditions was evident in the wood (Pflug et al., 2015; Sarris et al., 2013).

Slightly deviating responses are possible and shown with the dashed red arrows in the model outputs, which reflect a certain range of physiological responses rather than a singular direction (Figure 1), as suggested also by (Grams et al., 2007). Overall, the strength of the approach remains obvious: plant functional response to environmental impacts can generally be deduced using isotopes in the absence of detailed physiological measurements. Or, in the case of nonplausible results, the model facilitates the discovery of previously overlooked plant responses through a new lens.

BOX 2.

Oxygen isotopes: We must keep in mind that major ^{18}O fractionation processes occur on two different levels: first processes, which alter the isotope ratio before water is taken up by plants, and second changes, which are a result of ^{18}O fractionation processes during H_2O and CO_2 gas exchange and assimilate incorporation into plant organic matter.

1. The isotope ratio of precipitation water varies depending on (i) its *origin*, that is, water vapour from warm regions like the Mediterranean is more enriched in $H_2^{18}O$ than northern vapour masses (Rozanski et al., 1993). Furthermore, continental (Bowen et al., 2005, 2014) or altitudinal (Poage & Chamberlain, 2001) effects change $\delta^{18}O$ of precipitation significantly. (ii) *Seasonality* of the precipitation is another source of variation in $\delta^{18}O$ of precipitation water. That, however, is closely linked to the temperature dependence of $\delta^{18}O$ resulting in an annual sinusoid pattern (Dansgaard, 1964, Gat et al., 2001). Further fractionations occur during atmospheric transport, rainfall, canopy throughfall and infiltration in the soil. Depending on the soil structure, season and climate, $\delta^{18}O$ of soil water shows a great vertical variability (Sprenger et al., 2016), with typically higher $\delta^{18}O$ values in upper soil layers due to evaporative effects. (iii) As plant roots seem to take up water from any *soil depth*, where its accessibility enables its uptake under a minimum of energy, roots potentially have a large range in soil depths from where they absorb water. Brinkmann et al. (2018), Allen et al. (2019) and Goldsmith et al. (2022) found that considerable amounts of absorbed soil water originate from winter water, resulting in temporal shifts (described as temporal origin) of soil $\delta^{18}O$. Accordingly, this leads to a strongly dampened variability of the source water isotope signal (xylem water), the isotopic basis for leaf water $H_2^{18}O$ enrichment in leaves.

2. The variability of the $\delta^{18}O$ values in leaf organic matter is predominantly determined by the extent of leaf H_2O gas exchange during photosynthesis. Cernusak et al. (2016) and Song et al. (2022) with literature therein provide a summarizing overview of the various aspects of leaf water enrichment. Therefore, we cover only briefly the most important aspects of leaf water enrichment. While leaf water transpires out of the stomata, the lighter $H_2^{16}O$ evaporates more readily than the heavier $H_2^{18}O$ molecules, leaving the remaining leaf water enriched in $H_2^{18}O$ (Craig & Gordon, 1965; Dongmann et al., 1974) (see Figure 5). These authors (Craig and Gordon and Dongmann [CG & D]) described leaf water enrichment ($\delta^{18}O_{LW}$) at the location of evaporation as a function of the ratio of the ambient versus the leaf intercellular water vapour mole fraction, e_a/e_i (≈relative humidity [RH], the $\delta^{18}O$ of source (xylem) water ($\delta^{18}O_s$) and $\delta^{18}O$ of ambient water vapour ($\delta^{18}O_V$) under steady-state conditions. The extent to which leaf water is enriched in $H_2^{18}O$ is inversely proportional to the ratio of e_a/e_i as indicated in Figure 5. However, the calculated values according to CG & D tend to overestimate $\delta^{18}O_{LW}$ relative to observed values. As a consequence, Farquhar and Lloyd (1993) took the transpiration rate into account by (i) introducing the *Péclet effect*. It describes the diffusional flux of enriched water from the location of enrichment into the leaf water body as opposed to a convective water flux of unenriched water molecules from the xylem into the leaf, replenishing the transpired water loss (E). Thus, with increasing E, a decrease in $\delta^{18}O_{LW}$ is observed (see Figure 5), which results for numerous species in a negative relationship between stomatal conductance (g_s) versus $\delta^{18}O_{LW}$ (see Barbour et al., 2021). A so-called path length or path length-related parameter (L), is defined as $L = k \times l$, where k is a scaling factor and l is the actual within-leaf water transport length. In addition, 'k' can be further expressed as the ratio of leaf area to the cross-sectional area of the transpiration stream perpendicular to the flow (Barbour & Farquhar, 2003; Song et al., 2013). This definition partly explains the variation of L by orders of magnitudes as often found in the literature. The applicability of the Péclet approach is likely tied to the hydraulic leaf design (Zwieniecki et al., 2007) for which three designs 'with different pathways for water movements and levels of mesophyll connectedness were defined' (Barbour et al., 2021). However, the existence of a negative g_s–$\delta^{18}O_{lw}$ relationship based on the Peclet effect alone should not be taken for granted for all different types of species (see Barbour et al., 2021), and so we may need to examine the validity/suitability of the DI model on a species-by-species basis. Another approach was the application of (ii) a two-pool model (Song, Loucos, et al., 2015; Yakir et al., 1990), yielding better estimates for some species, in particular, for plants where the leaf was strongly compartmented into a pool with nonenriched and a pool linked to the location of enrichment. Roden et al. (2015) found improved estimates for coniferous plants when the two-pool model was combined with the Péclet correction. Based on a study of 27 species, Barbour et al. (2021) made recommendations on how to consider the hydraulic design for the Peclet correction or the two-pool model or the combination of both. In this context, it is also useful to consider the spatial patterns of isotopic composition within leaves, as the isotopic composition tends to be more enriched towards the tip of the leaf or at a greater distance from the mid-vein (Cernusak et al., 2016). Farquhar and Gan (2003) considered such effects by expanding the one-dimensional Péclet model, separating convection-diffusion effects in the leaf xylem and lamina.
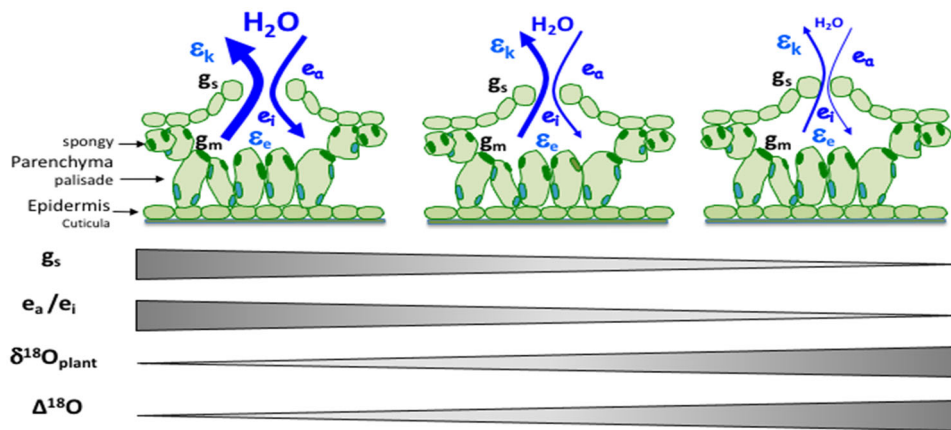
FIGURE 5 Mechanistic linkages between plant physiology and isotopes of oxygen at the leaf level. e_a and e_i stand for ambient and intercellular water vapour mol fractions, respectively, and g_s and g_m for stomatal and mesophyll conductance. ε_e and ε_k are the equilibrium and kinetic fractionation factors, respectively. Note that in most cases the stomata are on the bottom side of the leaf. [Color figure can be viewed at wileyonlinelibrary.com]

Another process that impacts $\delta^{18}O_{LW}$ is (iii) the diffusion of ambient water vapour into the substomatal cavities (Farquhar et al., 2021; Seibt et al., 2006). Depending on g_s, the ambient water vapour mole fraction (e_a) and its isotopic value ($\delta^{18}O_V$, which is not isotopically in equilibrium with the source water) will alter the $\delta^{18}O_{LW}$. Particularly, high ambient humidity conditions and open stomata (mostly low E) facilitate a high vapour exchange rate between substomatal cavities and ambient air (Kagawa, 2022; Lehmann et al., 2018, 2020), impacting $\delta^{18}O_{LW}$ considerably and thus $\delta^{18}O_{organic}$. The influx of ^{18}O-depleted water vapour ($\delta^{18}O_V < \delta^{18}O_{LW}$) from the ambient air into the leaf intercellular spaces and its mixing with leaf water diminish the $\delta^{18}O_{LW}$ enrichment (Farquhar et al., 2021; Lehmann et al., 2020); similar to what we observe with source water replenishing the transpirative water loss (E), g_s *modulates* both processes (replenishment and air to substomatal cavity exchange rate), resulting in a negative g_s–$\delta^{18}O_{LW}$ relationship. This gaseous vapour influx is often overseen, probably due to the assumption that the isotopic values of source water and ambient water vapour are in equilibrium, which is rarely the case in the field (Bögelein et al., 2019).

(iv) Mostly, for the prediction of $\delta^{18}O_{LW}$ isotopic steady-state (ISS) conditions are assumed, yielding often good agreements between observed and predicted values. Changes in ISS/NSS (nonsteady state) occur when the transpired water has an isotopic composition differing from that of source water (Farquhar & Cernusak, 2005). As the diurnal dynamics of environmental drivers (RH, T_{leaf}, PaR) is considerable, the impact on $\delta^{18}O_{LW}$ is accordingly, as the prevalent conditions are isotopic NSS. Therefore, the observed $\delta^{18}O_{organic}$ values are the result of fractionation processes under NSS conditions. For temporal short-term analyses, various studies found remarkable improvements when comparing observed with predicted $\delta^{18}O_{LW}$ values applying the NSS approach (Dubbert et al., 2014; Farquhar & Cernusak, 2005; Seibt et al., 2006). In recent years, the combined use of gas-exchange systems coupled to isotope laser spectrometers allowed for an online monitoring of the isotopic fractionation of $\delta^{18}O_{LW}$ under NSS. Song, Simonin et al. (2015) extended the NSS approach of Farquhar and Cernusak (2005), considering the mixing of the transpired water with the prevalent cuvette vapour with their respective isotope values. In the next step, Dubbert et al. (2017) included leaf traits such as g_s, stomatal density and leaf water content. Farquhar et al. (2021) provide a good discussion on the latest developments on ISS and NSS leaf water enrichment.

(v) In contrast to leaf water enrichment, the ISS assumption yields suitable results for organic matter, since the transport of sugars in the phloem and the synthesis of organic material, that is, cellulose are much slower processes than leaf water enrichment. Note that the organic assimilates ($\delta^{18}O_{organic}$) are enriched in ^{18}O relative to the leaf water by 27‰ due to the biochemical fractionation ε_{bio} (da Silveira Lobo O'Reilly Sternberg & DeNiro et al., 1983; Lehmann et al., 2017). The assimilates are a mixture of carbohydrates formed under various environmental conditions, representing an integration of the short-term isotope signal variation within a time frame of hours to days (Andreu-Hayles et al., 2022). Furthermore, reserve carbohydrates mix with freshly formed assimilates (Gessler et al., 2014). The mechanistic coherence between leaf water enrichment and sucrose synthesis is summarized in Barbour and Farquhar (2000) and the mathematical description of the oxygen exchange between the carbonyl group and the source water during cellulose synthesis and its application is given in Barbour, Roden et al. (2004) and Barbour (2007). Hirl et al. (2021) described the climate sensitivity of these exchange processes and ε_{bio} and its significance for the interpretation of climate reconstructions and physiological processes.
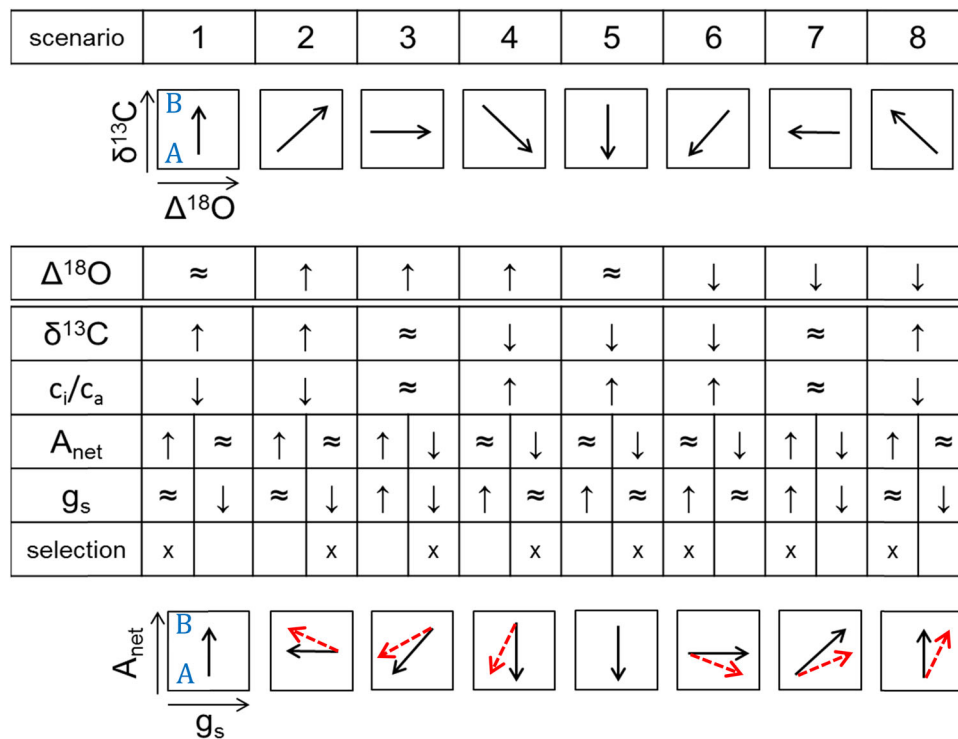
FIGURE 1 The updated dual C–O isotope model according to Scheidegger et al. (2000). Arrows compare 'two varying situations: A and B' as explained in the text. The symbol '≈' indicates no significant change. Arrows pointing up (↑) or down (↓) stand for an increase/decrease in the respective values. $\Delta^{18}O$ is the isotopic fractionation for ^{18}O versus ^{16}O; $\delta^{13}C$ is the value for the stable carbon isotope; c_i/c_a is the ratio between the intercellular versus ambient CO_2 mole fractions; A_{net} is the net photosynthesis (sensu Larcher, 2003); g_s the stomatal conductance. Red dotted arrows show intermediate A_{net}–g_s responses not covered in the original model. [Color figure can be viewed at wileyonlinelibrary.com]

3 | C AND O ISOTOPIC SIGNALS DURING CARBOHYDRATE TRANSFER FROM LEAF TO WHOLE PLANT (POSTPHOTOSYNTHETIC FRACTIONATION)

The DI-model was originally derived from leaf isotope and gas-exchange data. This raises the question as to whether or not, and to what degree, its application to other tissues (e.g., tree rings, roots, etc.) is possible? As the isotope signals of the newly formed assimilates are dampened by postphotosynthetic processes, a careful evaluation is needed to understand whether the patterns are consistent among tissues. Given the available literature (Cernusak et al., 2013, 2016 Gessler et al., 2014, and literature therein), we focus on those factors that have the most prominent postphotosynthetic impact on the isotope signals during the carbohydrate transfer processes between leaves and whole plant.

3.1 | Carbon isotopes

Postphotosynthetic fractionations begin immediately following the sugar formation and before phloem export. For carbon, kinetic and equilibrium isotope fractionations related to aldolase-catalysed reactions can cause that transitory starch and remobilized sugars at night-time are more ^{13}C-enriched than daytime sucrose (Gleixner et al., 1998; Tcherkez et al., 2004, 2011). However, starch-related ^{13}C fractionations can be very small (Maunoury-Danger et al., 2009) and their significance can vary among species and are under debate (Bögelein et al., 2019, Lehmann et al., 2019). Moreover, $\delta^{13}C$ differences among individual sugars in leaf and phloem have been observed (Churakova et al., 2018; Rinne et al., 2015), with sucrose being often the most ^{13}C-enriched soluble sugar. The ^{13}C fractionations have been related to the invertase reaction (Mauve et al., 2009). In addition, tree tissues, leaves or needles often have high abundances of sugar alcohols or cyclitols, which can be isotopically different from primary sugars (Lehmann et al., 2017; Rinne et al., 2015). The low turnover rate of sugar alcohols can strongly dampen the $\delta^{13}C$ responses of bulk sugar fractions to changes in physiology and climate (Galiano Pérez et al., 2017). Additional isotope fractionations may occur during the export of leaf sugars into the phloem and during their translocation to sink tissues (Gessler, Brandes, et al., 2009). Only recently it has been observed that phloem exudates can be more enriched than leaf assimilates and that the ^{13}C enrichment increased with canopy height (Bögelein et al., 2019). The isotope fractionation was explained by the compartmentalization of leaf sugars in the mesophyll, causing designated export sugar sucrose is more ^{13}C-enriched in the cytosol than stored sucrose in the vacuole. As a result of these fractionation

processes, leaf $\delta^{13}C$ values are on average often 2‰–3‰ lower than tree branches or other sink tissues (Badeck et al., 2005; Chevillat et al., 2005). Subsequent C isotope fractionation associated with branch respiration could also contribute to a shift in isotope ratios (Ghashghaie et al., 2003). Furthermore, mixing between old, stored and new freshly assimilated carbohydrates occurs during phloem transport (Bögelein et al., 2019), which is partly affected by the formation of fresh carbohydrates via stem photosynthesis (Brügge-mann et al., 2011).

During early wood formation in spring, trees (particularly broadleaves) utilize stored assimilates from previous years, then gradually switch to fresh more ^{13}C-depleted assimilates (Helle & Schleser, 2004). These stored carbohydrate compounds are generally enriched in ^{13}C (Jäggi et al., 2002) compared to fresh assimilates, especially for starch and its derivatives (Gleixner & Schmidt, 1997).

Also, when considering longer time periods, the impact of industrialization (starting ca. 1850) on the C isotope ratio in atmospheric CO_2 becomes visible as $\delta^{13}C$ continuously decreases (Suess effect) resulting from fossil fuel burning and land use change. For the evaluation of time series (tree rings or comparing conserved plant material), this effect needs to be considered (McCarroll & Loader, 2004).

3.2 | Oxygen isotopes

For $\delta^{18}O$ in organic matter, the isotope ratio of source water is key. In early studies, the $\delta^{18}O$ values of precipitation were used as a surrogate for source water. However, plants acquire water from a range of lateral and vertical distances with considerable $\delta^{18}O$ (δ^2H) gradients depending on the soil structure (Goldsmith et al., 2019; Mueller et al., 2014; Sprenger et al., 2016), resulting in a strongly dampened amplitude of the $\delta^{18}O$ variability of the source water compared to that of precipitation. Thus, the xylem water represents a mixture of water bodies extracted from the soil, with the highest $\delta^{18}O$ values generally at the soil surface and the lowest at the deepest rooting depth (Barbeta et al., 2020). Further issues arise with seasonal changes in $\delta^{18}O$ of source water (Saurer et al., 2016). Brinkmann et al. (2018) and Allen et al. (2019) showed that the seasonal origin of the isotope signature of xylem water in summer originated in large part from winter precipitation. Thus, source water varies as a function of both time and space. This temporal shift in the isotope signal needs critical consideration: The source water $\delta^{18}O$ signal represents a delayed value depending on its seasonal origin relative to $\delta^{18}O$ in precipitation and the given climatic conditions (Allen et al., 2019; Brinkmann et al., 2018). On the other hand, the effect of the seasonal origin could be balanced under continuous humid climate conditions, as the impact of $\delta^{18}O$ of water vapour diffusing from the ambient air into the leaf intercellular spaces could override the isotopic signal of the source water (Lehmann et al., 2019; Roden et al., 2005).

While no significant isotope fractionation is assumed during H_2O uptake and transport in the xylem along a tree trunk (Barbeta

et al., 2020), leaf water is generally enriched in ^{18}O in the leaves during transpiration with its magnitude depending on the atmospheric evaporative demand (VPD) and the $\delta^{18}O$ of ambient water vapour (Kahmen et al., 2011; Lehmann et al., 2018; Box 2). During photosynthesis, an exchange between the oxygen atoms originating from CO_2 and that of H_2O takes place imprinting the $\delta^{18}O$ values of the leaf water on the assimilates because the amount of oxygen atoms of water is much larger than that of assimilated CO_2 (Barbour, 2007; Barbour & Farquhar, 2000; Lehmann et al., 2017). Thus, freshly assimilated carbohydrates always reflect both the isotopic variations of source and leaf water. Biosynthetic fractionation causes sugars to be about 27‰ more enriched compared to leaf water or, more precisely, the water used for photosynthesis (da Silveira Lobo O'Reilly Sternberg & DeNiro, 1983; Yakir & DeNiro, 1990). During carbohydrate transport via phloem and concurrent cellulose synthesis, a partial oxygen exchange with unenriched xylem water occurs. This exchange, resulting in a ca. 40% oxygen exchange, dampens the leaf water $\delta^{18}O$ signal (Barbour, 2007; Roden & Ehleringer, 1999), likely the largest modification of the $\delta^{18}O$ signal in assimilates between the leaves and tree rings. Finally, the observed $\delta^{18}O_{organic}$ values reflect a considerable bandwith of varying temperatures, RH (VPD) and $\delta^{18}O_{vapour}$ values, environmental factors that impact the isotopic O fractionation of water molecules. As a consequence, $\delta^{18}O_{organic}$ is a result of O-fractionation processes under mostly nonsteady state (NSS) conditions.

To summarize: For the $\delta^{13}C$ values, the mixing of old stored carbohydrates (starch and sugar synthesis and remobilization) likely has the strongest dampening effect, probably stronger than phloem loading and (heterotrophic) stem and root respiration. Therefore, we recommend for tree-ring analyses to distinguish between early and latewood wherever possible to minimize the inclusion of isotopic signals from old carbohydrates from previous years. For the $\delta^{18}O$ values, the oxygen exchange during carbohydrate transport in the phloem and during tree-ring cellulose synthesis probably impacts the oxygen isotope signal the strongest, which might also be amplified by the seasonal origin of the source water (Allen et al., 2019; Brinkmann et al., 2018). To minimize the postphotosynthetic fingerprints on C and O isotope ratios, it is recommended to use late wood in tree rings whenever possible. However, in spite of these modifications and dampening of the C and O isotope values during the carbohydrate transport from leaves to whole plant and cellulose synthesis, the leaf-level isotope signal is largely maintained in the wood (Saurer et al., 1997; Song et al., 2011).

4 | OVERVIEW OF RESEARCH ENGAGING THE MODEL

We identified 261 studies citing Scheidegger et al. (2000) (ISI Web of Knowledge, September 2018). Of these studies, we excluded review and commentary articles and identified 184 containing new plant carbon and oxygen isotope data (see overview in Table 1). Studies

TABLE 1 Summary of research studies containing plant ^{13}C and ^{18}O measurements that cite and apply the DI-model of Scheidegger et al. (2000).

Plant functional type	No. of studies	Wood isotopes only	Leaf isotopes only	Leaves and wood or phloem isotopes	Plant gas-exchange measured	Temperate	Arid	Tropical	Arctic	Controlled conditions
Gymnosperms	78	59	15	4	7	47	16	4	10	1
Angiosperms	79	30	43	6	26	35	16	13	4	11
Both	27	13	11	3	5	21	3	0	0	3

Note: Information listed contains the type of material analysed, the availability of gas-exchange data and the region where the study was carried out. Controlled conditions indicate greenhouse or growth chamber studies.

Abbreviation: DI-model, dual carbon and oxygen isotope model.

after this date were considered in the text. These studies covered a broad range of plant life forms, although the majority (89%) comprised woody (tree or shrub) species. Most studies were performed in temperate regions, but a few were from tropical (dry or moist), polar, and/or dry regions. Gymnosperms and angiosperms from evergreen and deciduous species were similarly represented. Notably, although the DI-model was originally applied to leaf isotope ratios, studies applying the model to wood are more numerous. Only a few studies combined leaf gas-exchange and wood measurements (Supporting Information: Appendix S1).

Paired measurements of leaf gas exchange (i.e., A_{net} and g_s) and isotopes ($\delta^{13}C$, $\delta/\Delta^{18}O$) serve as the basis for validating the DI-model to interpret plant functional response to the environment; we identified 17 studies with such paired measurements (Supporting Information: Table S1). The majority of these studies comprised broadleaf and coniferous trees of temperate and Mediterranean climate regions. Additionally, three studies were performed on grasses and one on herbs. Most of the measurements were carried out at the leaf level on field-grown, mature plants, while some studies were performed under controlled conditions on saplings. Four studies compared leaf gas exchange with the isotope pattern of tree stem tissue.

Within the 17 studies with isotopes and leaf gas exchange, we identified 29 different conditions for which the model scenarios had been evaluated (Supporting Information: Table S1 and Figure 2). In 18 of the 29 cases (62%), the gas-exchange measurements were fully consistent with the observed isotope patterns, clearly validating the DI-model. In a few cases (21%), the scenarios of gas exchange and isotope patterns did clearly not match (e.g., under the influence of air pollutants, extreme droughts and various nitrogen regimes, see also Section 5). However, when grouping the responses into situations of improved versus reduced resource availability, rather than individual scenarios, a consistent pattern emerged (Figure 2): Studies that resulted in improved plant resource availability (e.g., light, water, nutrients and CO_2) clustered around scenarios #6 and #7, both with respect to isotopes and gas exchange (Figure 2a), consistent with an increase in photosynthesis (A_{net}) and/or g_s (Figure 2b). In contrast, a limitation or reduction of resource availability (e.g., increased competition, reduced water/nutrient supply, or temperature stress) commonly reflected in scenarios #2–4 (Figure 2a). Following the model, plants show a decrease in A_{net} and/or g_s (Figure 2b). For both groups, these model interpretations are generally well-confirmed by gas-exchange measurements. As a consequence, this means that an isotope pattern can give reliable information about resource limitation also in the absence of gas-exchange data.

Nevertheless, the DI-model cannot resolve all cases, as it is based on generally accepted plant physiological gas-exchange responses to the environment and the well-accepted isotope fractionation models. As indicated by the grey arrow in Figure 2b, the original DI-model does not predict all A_{net}/g_s cases, in particular, not a negative relationship between A_{net} and g_s. The two 'missing' A_{net}/g_s scenarios are only observed in rare situations; for instance, a change from cold and wet to moderate and dry conditions, scenario #2 (Figure 1) might cause a decrease in g_s and increase in A_{net}. A similar pattern is observed for increasing ambient CO_2, where A_{net} is stimulated with a constant or concomitant decline in g_s,
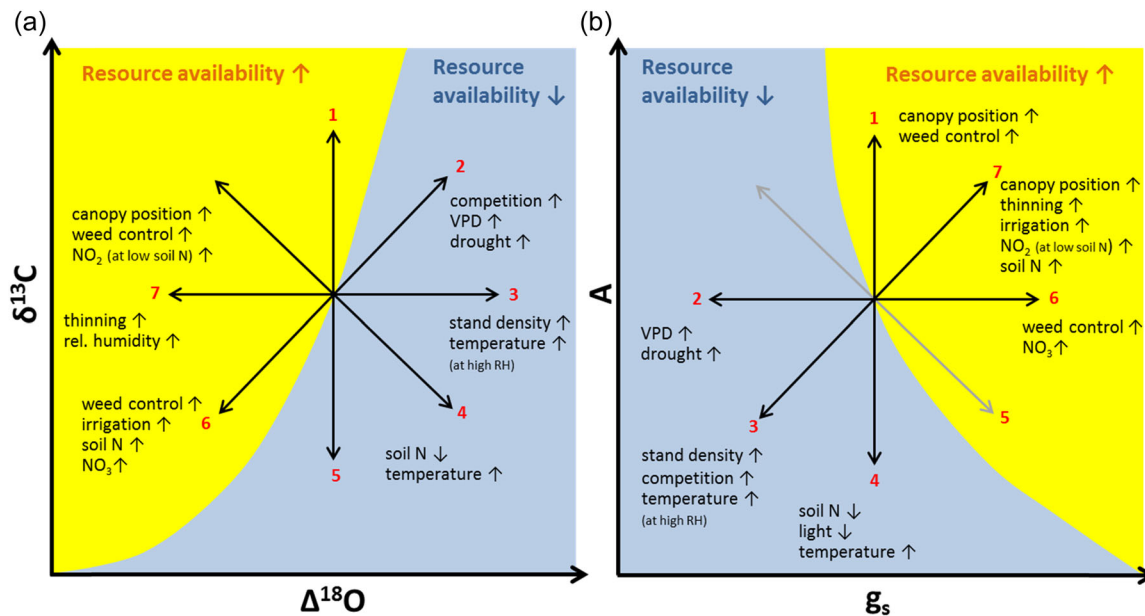
FIGURE 2 Stable C/O isotope and net assimilation/stomatal conductance (A_{net}/g_s) scenarios and their environmental drivers as observed from experimental studies, including (a) isotope patterns reflecting dual carbon and oxygen isotope model (DI-model) scenario #1–8 and (b) corresponding observed gas-exchange scenarios #1–8. The yellow and grey areas reflect scenarios with generally increasing and decreasing resource availability, respectively. [Color figure can be viewed at wileyonlinelibrary.com]

corresponding to scenario #8 (Figures 1 and 2). Scenario #5 can be found for plants growing under very humid conditions and (opened stomata) decreasing light (decrease in A_{net}, for example, understorey plants in the tropics). The DI-model can also yield nonplausible results for extreme stress conditions such as drought and air pollution. Under such conditions, the assumptions of the isotope fractionation models are violated (i.e., see Sections 5.1 and 5.5), as the metabolic changes are not considered relative to known fractionation and gas-exchange principles.

5 | MODEL APPLICATIONS— ENVIRONMENTAL EFFECTS ON PLANT CARBON AND OXYGEN ISOTOPES

Here we describe how the DI-model has been applied to infer plant functional responses to common environmental factors over time and space. We explain, which isotope responses would be expected for a certain dominant driving variable, like drought, and review the most common applications of the DI-model as found in the literature, both at the scale of the leaf and that of the whole plant, with some representative examples. A summary of the physiological responses to these environmental drivers, as reflected in the C and O isotope ratios, is given in Table 2.

5.1 | Water supply and demand

Drought stress primarily affects plants through diminished soil water availability and increased atmospheric moisture demand, that is, VPD

(Grossiord et al., 2020), which increases nonlinearly with rising temperatures (Breshears et al., 2013). Generally, stomata close with increasing drought stress to mitigate leaf turgor loss or stem hydraulic failure and desiccation. This often leads to reduced photosynthetic rates due to limited CO_2 diffusion (Flexas et al., 2008). The strong stomatal response mostly results in increased $\delta^{13}C$ (Farquhar et al., 1989). Similarly, $\delta^{18}O$ is expected to increase, particularly when the air is getting dry. The increase in VPD (decrease of RH) also causes stomatal closure (reduced g_s) diminishing transpiration. Thus, the Péclet effect is reduced due to a reduced replenishment with $H_2^{18}O$-depleted xylem water minimizing the dilution of the $H_2^{18}O$-enriched leaf water (Cernusak et al., 2016; Farquhar & Lloyd, 1993). Furthermore, the back diffusion of ambient ^{18}O-depleted water vapour via stomata into the leaf intercellular spaces is reduced (Lehmann et al., 2018), corresponding to scenario #2 for the isotopes. However, when both A_{net} and g_s are reduced to similar degrees, this may result in a constant c_i/c_a (Ehleringer & Cerling, 1995; Saurer, Siegwolf, et al., 2004) and no change in $\delta^{13}C$, as reflected in scenario #3 (Table 2).

Leaf-level isotope and gas-exchange studies focused on the functional response to water supply and demand have frequently observed scenario #2, including for black poplar plants under various VPD regimes (Rasheed et al., 2015), for conifer species along a wet-dry gradient in Australia (Brodribb et al., 2013) and for leaves and phloem sap in beech trees along a climatic gradient in Europe (Keitel et al., 2006). Therefore, the DI-model generally works for explaining drought-related physiological responses.

However, an isotopic decoupling between leaf-level and tree rings can sometimes result in implausible isotope patterns. During hot

TABLE 2 Impact of the dominant environmental drivers on plant physiological parameters and stable C and O isotope fractionation: The theoretical responses might deviate from field observations, since other environmental factors impact gas exchange and isotopic fractionation besides the dominant drivers, which does not always correspond with all responses from field observations.

Dominant driving variables	g_s	A_{net}	c_i/c_a	$\delta^{13}C/\Delta^{13}C$	e_a/e_i	$\delta^{18}O/\Delta^{18}O$	DI-model scenario
VPD	Decreasing with increasing VPD. The degree of decrease is species dependent	Initially constant, then decreasing with increasing VPD	Progressively decreasing with increasing VPD	$\delta^{13}C$: Increases / $\Delta^{13}C$: Decreases with increasing VPD	Decreasing with increasing VPD	Both increase with increasing VPD	2, 3 / 7, 8 (isotopic decoupling Section 5.1)
Temperature	High at low temperatures, ±constant over the temperature range between 15°C and 40°C, given that VPD stays constantly low (VPD ≤ 0.5 kPa) Decreasing with increasing temperature, mostly due to increasing VPD	Follows an optimum curve increasing from low to higher temperatures, and decreasing at higher temperatures with an average optimal range between 15°C –30°C. Temperature compensation points on average at 3°C and 40°C	Follows an inverse curve of A_{net}, with increasing temperature, the ratio decreases to remain constant for the optimal range of A_{net}, and then increases with decreasing A_{net}	$\delta^{13}C$: Follows an optimum curve, and is decreasing in the low and high temperature ranges. $\Delta^{13}C$: Follows an inverse curve of A_N, with increases in the low and high temperature ranges	Decreasing trend with increasing temperature, as a result of a higher leaf than ambient temperature (increasing trend of LAVPD)	$\delta^{18}O$ and $\Delta^{18}O$: Increasing trend due to decreasing e_a/e_i caused by increasing leaf temperature, resulting in increasing LAVPD	1 and 5
PAR	Increasing, following a saturation curve	Increasing, following a saturation curve; light saturation is species and light exposition (sunlit shade)-dependent	Declines logarithmically (x-axis mirrors the saturation curve) depending on the A_{net}/g_s ratio	$\delta^{13}C$: Increases, following the A_{net} curve / $\Delta^{13}C$: Decreases, following an inverse curve of A_{net}	Constant or decrease with increasing PAR	Both constant or decrease with increasing PAR	7 or 8
CO₂	*Lab conditions:* Decreasing with increasing CO₂ *Field studies (FACE)* minimal decrease to no response (see Section 5.4)	Increasing, following a saturation curve, the degree of CO₂ saturation is species- and nutrient-specific	*Lab conditions:* Decreasing with increasing CO₂ *Field studies:* (a) decreasing if c_i constant (b) c_i/c_a constant (c) increasing if c_a–c_i constant	*Lab conditions:* $\delta^{13}C$ increasing $\Delta^{13}C$ decreasing *Field studies:* (a) $\delta^{13}C$ increasing $\Delta^{13}C$ decreasing (b) $\delta^{13}C$ decreasing $\Delta^{13}C$ constant (c) $\delta^{13}C$ decreasing $\Delta^{13}C$ increasing	*Lab conditions:* Decreasing with increasing CO₂ *Field studies (FACE)* constant or slight decrease	$\delta^{18}O$ and $\Delta^{18}O$: *Lab conditions:* Increasing with increasing CO₂ $\delta^{18}O$ and $\Delta^{18}O$: *Field studies (FACE)* constant or slight increase	(a) 1 or 2 (b, c) 4, 5 1, (2) (see Section 5.4)

(Continues)

TABLE 2 (Continued)

Dominant driving variables	g_s	A_{net}	c_i/c_a	$\delta^{13}C/\Delta^{13}C$	e_a/e_i	$\delta^{18}O/\Delta^{18}O$	DI-model scenario
Ozone	Initially decreases, but with increasing duration stomata become nonfunctional	Decreases with a progressive duration of exposure (depending on the concentration)	Increases with a progressive duration of exposure (depending on the O_3 concentration)	$\delta^{13}C$: increases while $\Delta^{13}C$ decreases as a consequence of increased PEP carboxylase activity	Increases with a progressive duration of exposure (depending on the O_3 concentration)	Decreases with a progressive duration of exposure (depending on the O_3 concentration)	1 and 2 (PEP carboxylase-driven isotope patterns) 3 (supported by gas exchange measurements)
SO_2	Initially decreases, but with increasing duration stomata become nonfunctional	Decreases with a progressive duration of exposure (depending on the SO_2 concentration)	Increases with a progressive duration of exposure (depending on the SO_2 concentration)	$\delta^{13}C$: increases $\Delta^{13}C$: decreases; possibly as a consequence of increased PEP carboxylase activity and other detox. mechanisms	Constant or increasing with progressive duration of exposure (depending on the SO_2 concentration)	Constant or decreasing with a progressive duration of exposure (depending on the SO_2 concentration)	1 and 2 (biochemically driven isotope patterns) 3 and 4 (based on gas exchange measurements)
NO_2	Increases for nontoxic concentrations	Increases for nontoxic concentrations	Decreases for nontoxic concentrations	$\delta^{13}C$: increases $\Delta^{13}C$: decreases	Decreases	Decreases	8
Soil N supply NO_3^- and NH_4^+ Highly variable responses, strongly plant species- and habitat-dependent (see Section 5.6)	(a) Increasing (b) Decreasing (c) Constant (d) Increasing (e) Constant to decreasing	(a) Increasing (b) Constant (c) Increasing (d) Increasing (e) Increasing	(a) Increasing when A_{net}/g_s ratio is in favour of g_{st} (b) Decreasing (c) Decreasing (d) Constant (e) Decreasing	(a) Constant $\delta^{13}C$ and decreasing $\Delta^{13}C$ (b) $\delta^{13}C$ constant to slightly decreasing and $\Delta^{13}C$ constant (c) $\delta^{13}C$ increasing, $\Delta^{13}C$ decreasing (d) $\delta^{13}C$ and $\Delta^{13}C$ constant (e) $\delta^{13}C$ increasing, $\Delta^{13}C$ decreasing	(a) Increasing (b) Decreasing (c) Constant (d) Increasing (e) Increasing	(a) $\delta^{18}O$ and $\Delta^{18}O$ decreasing (b) $\delta^{18}O$ and $\Delta^{18}O$ increasing (c) $\delta^{18}O$ and $\Delta^{18}O$ constant (d) $\delta^{18}O$ and $\Delta^{18}O$ decreasing (e) $\delta^{18}O$ and $\Delta^{18}O$ decreasing	(a) 6 (b) 2 (c) 1 (d) 7 (e) 8

Note: DI-model scenario corresponds with the scenario numbers in Figure 1. The red scenario numbers indicate nonplausible gas-exchange responses, as derived from the C and O isotope values.

Abbreviations: A_{net}, net photosynthesis; c_i/c_a, ratio between leaf intercellular versus ambient CO_2 mole fractions; e_a/e_i, ratio between ambient and substomatal partial water vapour molfractions; FACE, free air CO_2 exposure; g_s, stomatal conductance; LAVPD, leaf-to-air vapour pressure deficit; VPD, vapour pressure deficit; $\Delta^{13}C$, net carbon isotope fractionation; $\Delta^{18}O$, oxygen isotope fractionation.
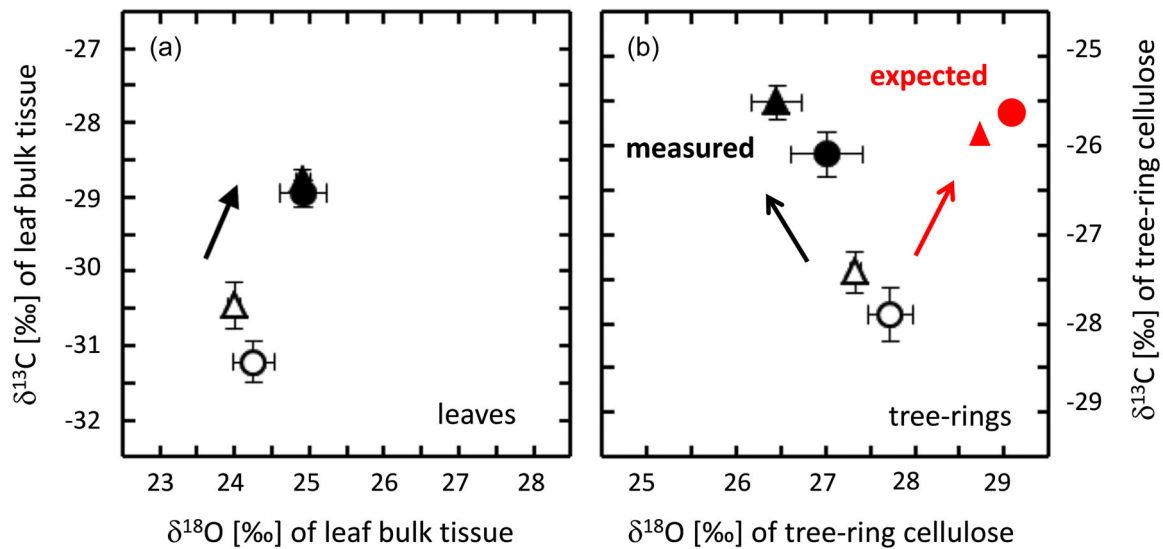
FIGURE 3 Pattern of carbon and oxygen isotopes in (a) leaves and (b) tree-ring cellulose of *Quercus robur*. Black arrows indicate the directional trend in isotopic patterns due to drought treatments. Open circles represent irrigated plants (control) and open triangles irrigated and warmed plants. Filled circles represent drought treatment and filled triangles stand for drought treatments and warmed plants. Data are means ± SE, $n = 8$, an experiment from 2009 (Pflug et al., 2015). The red arrow and symbols represent the expected values, according to the isotopic pattern in the leaves, whereas the black arrow and symbols are measured data. [Color figure can be viewed at wileyonlinelibrary.com]

and dry summers with low precipitation, water availability is often insufficient for plant growth. Although some CO_2 assimilation is still measurable, no tree growth occurs during these dry, but climatically relevant periods. Thus, there is a mismatch between the period of interest and the period in which the tree-ring tissue of interest was formed. Therefore, the DI-model often demonstrates an increase in A_{net} and g_s (scenarios #7 and #8; Table 2), which is not a plausible response for hot and dry conditions (high VPD, low soil moisture; Sarris et al., 2013). What is observed in the tree rings is actually the $\delta^{18}O$ signal from cooler, more humid springtime periods, with lower $\delta^{18}O$ values of precipitation (thus mimicking high g_s, see Figure 3). Since most growth occurs during springtime, the remaining tree-ring signal is not representative of a hot summer period with little or no growth (Sarris et al., 2013). This has also been observed in a controlled drought experiment with *Quercus robur* and *Quercus petraea* seedlings (Pflug et al., 2015).

In contrast, conditions with high air humidity (RH ≥ 90%) and high g_s allow for a high water vapour exchange rate from leaf intercellular cavities to the ambient atmosphere and vice versa (Goldsmith et al., 2017; Lehmann et al., 2018). As a result, the isotopic leaf water enrichment is very small and often not detectable. Thus, we can assume that the $\delta^{18}O$ of leaf water is close to that of the source water (Barbour et al., 2004; Roden et al., 2005). This is mostly the case under long-lasting rain and fog conditions, often found in tropical rain forests or given orographic precipitation and persistent fog at the mountainous tree line. Under such conditions, the variability in $\delta^{18}O$ of leaf water and consequently in cellulose tends towards zero and limits any conclusions regarding g_s (Roden & Siegwolf, 2012).

To summarize: While there may be considerable utility at the leaf scale, the application of the DI-model to tree rings for scenarios

involving considerable drought stress or conditions with high humidity must be addressed carefully. Ultimately, changes in water availability and demand are generally well reflected in isotope patterns (scenarios #2 and #3; Table 2), as long as plants operate under nonextreme conditions, that is, severe drought, near saturated air humidity, or at temperature extremes (see Section 5.2 below).

5.2 | Temperature

Temperature plays a critical role in plant function by mediating photosynthesis and postphotosynthetic processes, water use, and ultimately growth. Increases in global temperature have been associated with decreased growth and increased tree mortality in a number of vegetation studies (Allen et al., 2010). Assuming that water supply and VPD are not a limiting factor and thus the stomatal response and $\delta^{18}O$ values remain unchanged, then temperature primarily affects plant photosynthetic activity at the biochemical level. Consequently, when approaching their optimum temperature range, plants get closer to maximum photosynthesis, leading to a decrease in c_i/c_a and less negative $\delta^{13}C$ values, which is consistent with scenario #1. In contrast, low and high (nonoptimal) temperatures cause a decrease in A_{net} and thus an increase in c_i/c_a and lower $\delta^{13}C$ values, reflecting scenario #5 (Table 2).

Outside highly controlled experimental conditions, however, a change in the daily air temperature is probably always accompanied by a change in air humidity and thus VPD. A change in g_s due to a possible change in VPD can therefore not fully be excluded and humidity often masks potential temperature effects on gas exchange and thus on isotope fractionation, particularly under warm and dry conditions (Liu

et al., 2014; Martín-Benito et al., 2010; Moreno-Gutiérrez et al., 2012). In the field, as temperature increases VPD also increases, which affects g_s much more than temperature (Grossiord et al., 2020). Thus, what is often described as temperature effects is in fact a VPD effect. This may explain the highly equivocal results with both gas exchange and stable isotopes in leaf-level and tree-ring studies; most studies on the effect of temperature did not show any significant response. What further complicates the evaluation of the temperature response of the isotope fractionation is the ability of plants for photosynthetic acclimatization, that is, plants shift their photosynthetic temperature optimum with increasing/decreasing temperature.

Studies at tree lines may be useful for assessing temperature effects on plant isotope ratios in the field (Streit et al., 2013), as water availability is not limiting (yearly precipitation > 1000 mm) and atmospheric water demand (VPD) is low. A strong temperature and CO_2-driven relationship were observed between isotope ratios and growth in conifers at the tree line of the Austrian Alps (Wieser et al., 2016), reflecting scenario #6. An often-overlooked condition is low temperatures (0–7°C). Although water is hardly limiting under such conditions, the fact that growth at low temperatures is very slow (Körner, 2015) results in small tree rings. Consequently, the proportion of tree-ring biomass that would contain an isotopic low-temperature signal is very small compared to that of the remaining tree-ring mass, which was formed under warmer conditions. Therefore, the low-temperature signal is hardly visible. So far, only a few studies on low-temperature effects to date have matched the predicted scenarios #1 and #5 (Table 2).

To summarize: Since changes in temperature are mostly accompanied by changes in VPD, but also light intensities, seasonality and nutrient availability, a single factorial assessment with regard to temperature is only possible in controlled experiments. Evaluating the impact of temperature must always include the effect of other factors. Nevertheless, there seems to be a temperature-driven impact on isotopic fractionation above 15°C and below 40°C on average (see von Caemmerer & Evans, 2015).

5.3 | Light

Light drives the variations of c_i/c_a by mediating A_{net} and for variations in e_a/e_i by mediating g_s directly, and indirectly via leaf energy balance, changing the leaf temperature, particularly with the given variability of responses under nonlight saturated conditions (e.g., shaded plants). The theoretical $\delta^{13}C$ response is rather straightforward given the strong A_{net} effects on c_i/c_a and thus on $\delta^{13}C$ values (Ehleringer & Cerling, 1995). Therefore, the $\delta^{13}C$ would follow the light response curve for A_{net}. However, experimental evidence is somewhat controversial because light effects on carbon isotope discrimination have often been inferred from canopy height gradient studies where other factors may interfere, like VPD gradients and hydraulic constraints for tall trees (Farquhar et al., 1989; Waring & Silvester, 1994). Moreover, the apparent ^{13}C fractionation increases considerably in the low light range (near light compensation point;

Barbour, Ryazanova, et al., 2017; Busch et al., 2020; Liu et al., 2021; see also Box 1). Yet, a large part of carbon acquisition occurs mostly under high light and biomass production under nonlimiting growth conditions (Körner, 2015; Zweifel et al., 2021). Therefore, except for understorey plants, the low light impact on ^{13}C fractionation is hardly visible in tree rings. In comparison to $\delta^{13}C$, changes in $\delta^{18}O$ might be rather low or follow the inverse g_s direction of the A_{net} response given constant water supply and VPD conditions.

Studies with both gas exchange and dual-isotope data that considered the influence of light are rare. Bögelein et al. (2012) and Roden and Farquhar (2012) show that 'pure' light effects may be faithfully described by the DI-model scenario #7 or #8 (Table 2), but that there are often overlapping abiotic effects. Studies focused on understanding the effect of thinning (Giuggiola et al., 2016) using tree rings showed that changes in soil water $\delta^{18}O$ due to evaporation or changes in transpiration may complicate the application of the model, as soil water availability may become the dominant driver and lead to scenario #1 or most likely scenario#2 (Table 2).

To summarize: What might appear straightforward at first sight turns out to be more complex, as water, temperature or nutrient regimes can confound interpretations of light as a sole driver of plant physiological response. The strongest impact of light on isotopic fractionation is found under nonsaturated light conditions where A_{net} and g_s show its greatest variability (i.e., in the understorey); with decreasing light intensity, the fractionation of photorespiration and day respiration has an increasing impact on the net fractionation (Busch et al., 2020; Tcherkez et al., 2017), which is in support of generally lower $\delta^{13}C$ values of understorey or shaded leaves in contrast to sunlit leaves (see Box 1).

5.4 | CO_2

Understanding the influence of increasing CO_2 concentration on plant gas exchange and carbon allocation is essential for a correct interpretation of the isotope ratios and for improving carbon cycle models (Schimel et al., 2001). Tree-ring isotope studies, particularly when applied in large spatial networks, can provide unique information on how forests have responded to increases in CO_2 since the start of industrialization (Frank et al., 2015; Voelker et al., 2016). Based on $\delta^{13}C$ trends over the last 100 years, presumably caused by increasing CO_2, three types of gas-exchange responses caused by a variable g_s and A_{net} interaction have been inferred (Saurer, Siegwolf, et al., 2004): (a) c_i = constant, that is, the supply function (g_s) decreases and demand functions (A_{net}) stays constant; (b) c_i/c_a = constant, g_s and A_{net} change proportionally; (c) $c_a − c_i$ = constant, g_s stays constant and A_{net} increases. If we assume that $\delta^{18}O$ remains constant (no g_s response) or increases (g_s decreases), we can deduce the following from the DI-model: for case (a), we find either scenario #1 or #2, and for cases (b) and (c), we find scenarios #4 or #5 (Table 2). However, these theoretical assumptions and applications of the DI-model to CO_2 responses are relatively rare, particularly in combination with leaf gas-exchange measurements. A 9-year study at an alpine site with a free air CO_2 enrichment (FACE) experiment observed an

increase in A_{net} in two conifer species, but no stomatal response to CO_2 (no changes in g_s and $\delta^{18}O$, in contrast to a high VPD sensitivity), which is consistent with scenario #1 (Streit et al., 2014). At three FACE sites, Battipaglia et al. (2013) found changes in intrinsic water-use efficiency between tree species growing at ambient atmospheric (control) and elevated CO_2 treatments. An increase in $\delta^{13}C$ of tree-ring cellulose for all species at all CO_2 elevated study sites was observed, but elevated CO_2 resulted in only a slight increase in $\delta^{18}O$ for some species during the experiment (scenario #1 or #2; Table 2).

Natural springs emitting geological CO_2 can be used as long-term CO_2 fertilization experiment with a source isotope signal similar to atmospheric air, thus facilitating the application of the DI-model. Oak trees in a Mediterranean ecosystem in Italy exposed to ca. 800 ppm CO_2 during their lifetime demonstrated increased $\Delta^{13}C$ values (decreased $\delta^{13}C$) and unchanged $\delta^{18}O$ values (scenario #1), indicating a down-regulation of A_{net} and constant g_s for these rather harsh conditions in a dry and nutrient-limited environment (Saurer et al., 2003).

To summarize: The wide variety of isotopic patterns in response to CO_2 changes reflects the variability of species-specific responses to elevated CO_2. This includes (a) stimulation of A_{net} under high CO_2 levels (Bader et al., 2013; Streit et al., 2014), (b) down-regulation of photosynthesis (Grams et al., 2007; Sharma & Williams, 2009), (c) reduction in g_s or (d) no stomatal response to changes in CO_2 (Bader et al., 2013; Keel et al., 2007; Klein et al., 2016; Streit et al., 2014) as summarized in Table 2. Therefore, each isotope data set should be analysed for its response to increasing CO_2 before drawing any further conclusion.

5.5 | Atmospheric pollution

5.5.1 | Ozone (O_3)

Atmospheric pollution has been shown to affect plants on local and global scales (Omsa et al., 2005; Mills et al., 2018). At the regional scale, O_3 is the most relevant phytotoxic air pollutant (Ainsworth et al., 2012; Ashmore, 2005). Species respond differently to elevated O_3 depending on the uptake, mesophyll exposure, detoxification capacity and plant age (Fuhrer & Booker, 2003; Matyssek & Sandermann, 2003; Paoletti et al., 2020). A unique DI-model study observed that young, but not adult, beech trees were affected by elevated O_3 concentrations (Grams et al., 2007), with an increase in $\delta^{18}O$ and $\delta^{13}C$ in leaf cellulose (scenario #2). Increasing $\delta^{18}O$ values in response to O_3 exposure have also been observed in other studies with various species; this was explained by reduced g_s values (Gessler, Loew, et al., 2009; Grams & Matyssek, 2010). However, the response of $\delta^{13}C$ and thus in A_{net} was more variable (scenarios #2 and #3). A_{net} responses to O_3 might potentially be biased by the increased enzymatic activity of phospho-enol pyruvate carboxylase (PEPC) (Doubnerová & Ryšlavá, 2011; Saurer et al., 1995). In contrast to RuBisCo, the net PEPC isotope effect results in a net enrichment of ^{13}C, as PEPC uses bicarbonate as substrate rather than CO_2

(Vogel, 1993). Since the equilibrium effect between bicarbonate and CO_2 is larger than the relatively small isotope effect associated with PEP carboxylation, the plant tissue is increased in $\delta^{13}C$ relative to the RuBisCo fractionation. This observation would intuitively be the result of a reduced c_i/c_a ratio, thus suggesting an increased A_{net}. However, this is in contrast to observed, reduced leaf gas-exchange fluxes with higher c_i/c_a ratios in plants exposed to O_3, which should result in more negative $\delta^{13}C$ values (Farquhar et al., 1989). The impact of O_3 on C isotope fractionation demonstrates that once the traditional fractionation model for C_3 plants is no longer applicable, the model will yield physiologically implausible results.

5.5.2 | Sulphur dioxide (SO_2)

Since high ambient SO_2 concentrations have been a rather common problem in industrialized countries, particularly before the mid-1980s, this pollutant may have had a stronger impact on the stable isotope ratios of plant organic matter than previously recognized. Wagner and Wagner (2006) report that the long-term trend in tree-ring $\delta^{13}C$ showed an extraordinary increase between 1945 and 1990 and a rapid decrease after 1990, mirroring trends in atmospheric SO_2 concentrations. Savard et al. (2004, 2020) showed that SO_2 emissions from smelters induced an increase in $\delta^{13}C$ by up to 4‰ in spruce tree rings. This cannot solely be explained by a reduction of g_s. SO_2 exposure impacts $\delta^{18}O$ of leaf water only to a minor degree by reducing stomatal opening (Farquhar & Lloyd, 1993; Sensula & Wilczynski, 2017). Thus, the combined physiological response to high pollution levels is far more pronounced in $\delta^{13}C$ (Martin et al., 1988; Wagner & Wagner, 2006). Based on the reported isotope patterns, we expect scenarios #1 and #2, that is, a strong increase in $\delta^{13}C$ and none-to-moderate change in $\delta^{18}O$. However, gas-exchange responses suggested by the model do not correspond with published CO_2 and H_2O gas-exchange values. Atkinson and Winner (1987), Kropff et al. (1990), Wedler et al. (1995) and Duan et al. (2019) unanimously report a reduction in A_{net} and g_s, which would match scenarios #3 and #4 (Table 2). The impact of enzymatic detoxification mechanisms (Randewig et al., 2012), or a possible enhanced PEPC activity as found for O_3, lead to a considerable ^{13}C enrichment, which outweighs the standing model of C isotope fractionation.

The isotope effects on $\delta^{13}C$ induced by SO_2 detoxification are not taken into account by the C isotope fractionation model for C_3 plants. Thus, the DI-model output does not correspond with the plant's physiological response. It is critical to keep these SO_2 effects in mind when analysing isotopic chronologies from tree rings that originate from regions and periods with large SO_2 emissions (i.e., smelter industries, coal burning, etc.).

5.5.3 | Gaseous nitrogen (NO_2)

We cannot exclude any effects of gaseous NH_X or NO on plant metabolism and C and O isotope fractionation. Since we found no

literature or data describing isotopic effects resulting from these compounds, we discuss only the effects of NO_2 on plant physiology (Sparks, 2009; Wellburn, 1990). Plants demonstrate a proportional increase in $\delta^{13}C$ with increasing NO_2 concentrations (Bukata & Kyser, 2005; Siegwolf et al., 2001). An increase in $\delta^{13}C$ with a concomitant decrease of $\delta^{18}O$, irrespective of the soil nitrogen supply, has also been observed in a growth chamber NO_2 fumigation experiment on *Populus euramericana* (Siegwolf et al., 2001). The interpretation of the C and O isotopic patterns (increase in A_{net} and g_s) corresponded with measured CO_2 and H_2O gas exchange (scenario #8; Table 2). This was subsequently confirmed by field studies (Guerrieri et al., 2009; Saurer, Cherubini, et al., 2004), but the pattern might be changed when drought occurs. Obviously, protection against drought has a higher priority for plant survival, thus the drought response outweighs the influence of NO_2 (Guerrieri et al., 2010).

To summarize: Air pollutants have remarkable effects on isotopic fractionation, particularly O_3 and SO_2. Specific changes in enzyme activities mostly result in altered isotope fractionation, which do not agree and are not plausible in the context of well-accepted isotope fractionation and gas-exchange principles. While NO_2 can have a fertilizing effect, it can also be toxic for plants at higher concentrations.

5.6 | Soil N fertilization (NH_4^+ NO_3^-)

The significance of N depositions and incorporation into the plants is described in Savard et al. (2019) and Etzold et al. (2020). An increase in soil N supply generally reduces $\delta^{13}C$ and $\delta^{18}O$ (scenario #6), even while exposure to atmospheric NO_2 increases $\delta^{13}C$ and reduces $\delta^{18}O$ (Siegwolf et al., 2001). These two different nitrogen sources, NO_2 compared to soil N supply, can have opposite effects on carbon and water relations. One experiment has demonstrated that the addition of N to the soil increases the ratio of A_{net} to g_s in favour of A_{net}, indicating an N-fertilization effect (Guerrieri et al., 2011). This is consistent with the response for resource improvement. However, it has also been observed that a change in the soil N supply to subalpine species resulted in isotopic patterns that were specific to plant functional groups (Bassin et al., 2009). For instance, in the sedge *Carex sempervirens*, both leaf C and O isotope values increased, suggesting a decrease in g_s and a slight increase or constant A_{net} (scenario #2). In contrast, both isotopes declined in forb species, suggesting a constant A_{net} at an increasing g_s (scenario #6). Talhelm et al. (2011) and Marshall et al. (2022) also observed a diversity of isotope responses as a result of soil N supply. In their study, *Acer saccharum* foliage and leaf litter material from four different sites was analysed, resulting in four different DI scenarios (#1, #6, #7 and #8).

To summarize: While plants exposed to atmospheric NO_2 showed a consistent isotope pattern (scenario #8), we find highly diverse isotope responses for soil N-supplied plants (Table 2), with a slight trend towards scenario #6. This high diversity of responses may be a result of variable soil conditions (pH, soil moisture and structure,

acidic or loamy soil, etc.) or it is coupled with other environmental effects. But it also reflects that increased soil N input is not always beneficial, but can shift the competitive balance between species resulting in 'winners' and 'losers' that respond very differently.

6 | RECONCILING THEORY AND REALITY, GENERAL CONCLUSIONS

The theories of CO_2 and H_2O leaf gas exchange are explicitly linked with those of C and O isotope fractionation, as both isotopic patterns occur concurrently in leaves (Cernusak et al., 2016; Dongmann et al., 1974; Farquhar et al., 1982, 1989; Farquhar & Lloyd, 1993). However, when applying the DI-model to real measurements, our review reveals that we sometimes find deviations between measurements and theory.

6.1 | What is the cause for such deviations between measured data and theory/modelling results?

While measured data are mostly analysed given one or maybe two driving variables, a whole spectrum of environmental factors of varying intensities impact gas-exchange processes, plant metabolism, and isotopic fractionation in the field. Often these impacts are difficult to identify or to disentangle, for example, temperature and air humidity (VPD) (see Sections 5.1 and 5.2). With increasing air temperature, VPD also increases. Changes in isotopic ratios in plants may have been attributed solely to temperature, whereas the major impact was in fact VPD. The effect of varying CO_2 is often difficult to detect depending on site conditions, nutrients, species or climate. This is especially true given that studies of CO_2 in this context are generally carried out retrospectively on tree rings. While stomata respond readily to changes in CO_2 in lab experiments, ambiguous results were found for the stomatal response on mature trees where hardly any response to CO_2 was observed in field and FACE studies (Bader et al., 2013; Klein et al., 2016; Körner et al., 2005; Streit et al., 2014), in contrast to Ainsworth and Long (2005, 2021). As for O_3-exposed plants, the C fractionation model for C_3 plants is no longer valid because PEPC activity is increased, resulting in a considerable increase in $\delta^{13}C$ of plant organic matter (Saurer et al., 1995). A similar effect is found for SO_2. Thus, studies focusing on a specific plant–environment interaction may easily overlook the concurrent impact of other factors.

6.2 | Does this invalidate the DI-model?

Whenever the DI-model 'fails to fulfil our expectations', the first conclusion is that the DI-model does not work. However, the mechanistic basis of the conceptual model makes it a powerful diagnostic tool. Analysing isotope data in a physiologically

mechanistic context facilitates the detection of anomalies or nonplausible responses, which otherwise would not be recognized if $\delta^{13}C$ or $\delta^{18}O$ data were studied and interpreted independently from each other. A good example is the tree-ring chronologies from a period and location with heavy SO_2 pollution (Wagner & Wagner, 2006). The anomaly of the carbon isotope values would hardly be detectable by evaluating the $\delta^{13}C$ time series separately. With the application of the DI-model, the physiologically unrealistic interpretation became apparent and demands further exploration (see Section 5.4, SO_2). Yet, for a plausible diagnosis of such data constellations, a thorough analysis of plant functional response to specific environmental impacts is essential. An interesting example for a rigorous discussion, for example, on the complexity and potential problems with oxygen isotopes as a tool to constrain the carbon isotopic variability are given by Lin et al. (2022) and in reply by Guerrieri et al. (2022). Therefore, expecting a 'plug and play tool', which can be blindly applied without the essential physiological background can lead us astray and result in wrong conclusions, as also found in the literature. To counteract such misinterpretations, Roden and Siegwolf (2012) published a list of points to consider when the DI-model is applied. Furthermore, the data analysis with the DI-model can be extended by including additional parameters, such as growth data (e.g., tree-ring width; Gessler et al., 2018), or anatomical parameters (Churakova Sidorova et al., 2019), or adapting the C isotope fractionation model by considering other enzymatic fractionation processes, for example, including the C_4 plant fractionation approach as suggested by Farquhar et al. (1989) and Ubierna et al. (2018).

The use of the DI-model showed that certain isotope patterns can be indicative of an increase or decrease in resource limitation in a wide range of conditions. Based on various studies, we found that scenarios #2–4 (Figure 2) mostly reflect a decrease in resource availability or more stressful conditions that is consistent with gas-exchange results. In this interpretation, there is no need for a perfect match between isotope and gas-exchange scenarios, but rather a range of scenarios is considered. Regarding the application of the DI-model, this reiterates the qualitative nature of the model, but also its wide-ranging application.

To conclude: Ultimately, whether conceptual (qualitative) or mechanistic (quantitative), models are a synthesis of our current knowledge and provide a powerful platform to test and interpret our measured data. Inconsistencies reveal the lack of consideration of a certain mechanism or reveal knowledge gaps in our understanding of metabolic plant processes. Mismatches between models (representing our current understanding) and reality open doors for further research and enhance our understanding of plant–environment interactions. Given these considerations, the DI-model has proven to be a successful and powerful tool in plant ecophysiological research.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data were derived from public domain resources.

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SUPPORTING INFORMATION

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