



COMMENTARY

10.1029/2020JG006043

Key Points:

- Stable isotope data can be used to quantify “branchpoints” in the flows of water and carbon through ecosystems
- Key branchpoints are transpiration vs. evaporation, photosynthetic water-use efficiency, and efficiency of carbon conversion to biomass
- The branchpoint data can be compared to eddy covariance and biometric measures, as well as model predictions

Correspondence to:

J. D. Marshall,
john.marshall@slu.se

Citation:

Marshall, J. D., Laudon, H., Mäkelä, A., Peichl, M., Hasselquist, N., & Näsholm, T. (2021). Isotopic branchpoints: Linkages and efficiencies in carbon and water budgets. *Journal of Geophysical Research: Biogeosciences*, 126, e2020JG006043. <https://doi.org/10.1029/2020JG006043>

Received 30 AUG 2020
 Accepted 16 DEC 2020

Author Contributions:

Conceptualization: John D. Marshall, Hjalmar Laudon, Annikki Mäkelä, Matthias Peichl, Torgny Näsholm
Methodology: Matthias Peichl
Project Administration: Hjalmar Laudon, Torgny Näsholm
Software: Annikki Mäkelä
Writing – original draft: John D. Marshall
Writing – review & editing: John D. Marshall, Hjalmar Laudon, Annikki Mäkelä, Matthias Peichl, Torgny Näsholm

Isotopic Branchpoints: Linkages and Efficiencies in Carbon and Water Budgets

John D. Marshall¹ , Hjalmar Laudon¹ , Annikki Mäkelä², Matthias Peichl¹, Niles Hasselquist¹, and Torgny Näsholm¹

¹Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, Umeå, Sweden,

²Department of Forest Sciences, University of Helsinki, Helsinki, Finland

Abstract Forests pass water and carbon through while converting portions to streamflow, soil organic matter, wood production, and other ecosystem services. The efficiencies of these transfers are but poorly quantified. New theory and new instruments have made it possible to use stable isotope composition to provide this quantification of efficiencies wherever there is a measurable difference between the branches of a branchpoint. We present a linked conceptual model that relies on isotopes of hydrogen, carbon, and oxygen to describe these branchpoints along the pathway from precipitation to soil and biomass carbon sequestration and illustrate how it can be tested and generalized.

Plain Language Summary The way a forest works can be described in terms of carbon and water budgets, which describe the ways that carbon and water flow through the forest. The paths of such flows are frequently branched and the branches are often different in their stable isotope composition. This means that stable isotopes can be used to describe the branching events. We present isotopic methods of quantifying several such events, then link them in a chain that begins with the evaporation of water and ends with biomass production.

Mechanistic understanding of carbon and water fluxes is central for our ability to predict consequences and feedbacks of forests to a changing climate. In spite of recent progress, critical unknowns remain. For example, although rates of evapotranspiration (ET) are well quantified, the proportion of transpiration as part of ET is less well constrained. Likewise, the exchange rate of CO₂ uptake to water vapor loss is not well quantified although this exchange is the central link between ecosystem carbon and water fluxes. Furthermore, the rate at which photosynthetic production is converted to biomass, the carbon-use efficiency, is a subject of heated debate. These unknowns make it difficult to predict and manage ecosystem responses, which complicate rational decision-making about the ecosystem services provided by these carbon and water flows.

Stable isotopes are frequently used at convergences, where isotopically distinct flows mix, but they are also useful at branchpoints, where isotopically distinct flows split (Kirchner & Allen, 2020). In mixing processes, the isotopic signatures are conserved, e.g., when water derived from melted snow is mixed with summer rainwater as tree roots take up water from the soil. In splitting processes, the isotopic distinction arises from physical or biological processes on one leg of the split. For example, when water evaporates from leaf surfaces, the evaporated water is depleted (contains less) of the heavy isotope than the water that remains behind. Several splitting processes are described by remarkable bodies of theory based on the physics and biology of isotopic discrimination (Busch et al., 2020; Farquhar et al., 1982; Hayes, 2001). At the same time, a new generation of field-portable instruments has increased precision and flexibility of application (Penna et al., 2018; Stangl et al., 2019).

Here, we focus primarily on the exchange of water for carbon at the leaf-atmosphere interface and the branchpoints upstream and downstream of that exchange. This exchange, termed the water-use efficiency (WUE), is central to processes ranging from leaves to canopies, ecosystems, and catchments. WUE is the central link of the water cycle to the carbon cycle. It has been acted on by natural selection, generating a complex tapestry of species-specific traits and responses to environmental conditions. But the complexity can be simplified by measuring the integrated exchange over a whole forest using stable isotope ratios. Here, we propose a new conceptual framework where we connect WUE to several branchpoints in a sequence,

© 2020. The Authors.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial License](https://creativecommons.org/licenses/by/4.0/), which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

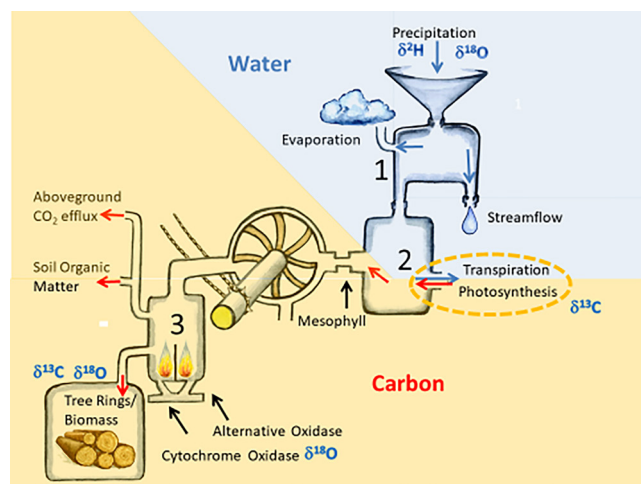


Figure 1. Diagram of the coupled fluxes of water and carbon in a forest ecosystem. The blue arrows in the blue section describe water fluxes and the red arrows in the brown section describe carbon fluxes. The numbers refer to the branchpoints below. The exchange of water for carbon is shown inside the orange dashed circle. The carbon-water exchange and the branchpoints in this diagram leave an isotopic signature, which can be used to quantify each step in the conversion of water to biomass.

where the flow from one delivers substrate to the next (Figure 1). The sequence begins with water gain as precipitation and, via WUE, ends with biomass production. Using novel isotopic measurement techniques, our approach aims to quantify the transfers at each branchpoint along the way.

1. Branchpoint 1: Evaporation vs. Transpiration

To couple the carbon and water cycles, the evapotranspiration flux must be partitioned into its components: surface evaporation and transpiration by plants (Figure 1, top right). This is necessary because transpiration from leaves is closely tied to photosynthesis, but evaporation from leaf and soil surfaces is almost irrelevant to the carbon cycle. This splitting can be treated as a branchpoint because the two water flux pathways have such distinct isotopic compositions (Williams et al., 2004). Partitioning between the two has so far been poorly constrained, where global transpiration estimates range between 20% and 65% of the combined ET losses (Jasechko et al., 2013). The isotopic composition of transpiration can be mechanistically modeled, but we often assume steady-state conditions to simplify the problem. Although steady state may be rare on an hourly basis (Dubbart et al., 2014), it is more commonly observed on the daily integration. Such simplifications, based on solid theory and empirical evidence, increase the feasibility of the isotopic methods.

These transpiration losses are also related to the “two water-worlds hypothesis,” which proposes that plants transpire different water than that passed on to streams (Brooks et al., 2010). Accurate quantification of the isotopic compositions of transpired vs. stream water is necessary if one is to state that plants tap a different source of water from that in the streams (McDonnell, 2014). Earlier models proposed that the water in soil was sampled without isotopic preference, and that stream and transpired water came from a common well-mixed pool (Sklash & Farvolden, 1979). These models assumed simple displacement of the pore water in soils as new precipitation fell. There is considerable evidence that controverts the simple model, but it is only now being replaced by various multiple-pool/multiple-flow path alternatives (Dubbart et al., 2019; Penna et al., 2018). Parameterizing these new models will be essential as we improve models of streamflow and the carbon-water linkage.

2. Branchpoint 2: Water-Use Efficiency

This is where the hydrologic and carbon cycles meet and where isotopic measurements can be used to convert the transpiration rates from branchpoint 1 to photosynthesis. Plants lose water vapor to the atmosphere in exchange for the CO₂ they acquire through photosynthesis. This exchange ratio, WUE, can be determined from stable carbon isotope ratios ($\delta^{13}\text{C}$) of photosynthates (Busch et al., 2020; Farquhar et al., 1989). In fact, the parameter derived from $\delta^{13}\text{C}$ is termed the intrinsic water-use efficiency (iWUE), but the conversion to WUE is straightforward given estimates of leaf temperature and atmospheric humidity (Cernusak et al., 2013). WUE depends on environmental conditions and the physiology of the plant. It is well described by optimality arguments, which state that the plant should adjust the water “price” it is willing to pay for photosynthesis as a function of water availability (Cowan & Farquhar, 1977; Dewar et al., 2018; Mäkelä et al., 1996; Medlyn et al., 2011). There is a branchpoint hidden in the process at the point where CO₂ molecules are either taken up via photosynthesis or they diffuse, with a changed isotopic composition, back into the atmosphere. We present it as a special case because it is fundamental to the coupling of the carbon and water cycles. Although this application of $\delta^{13}\text{C}$ to parameterize carbon-water exchange has been available for decades (Farquhar et al., 1989), it has been criticized for requiring an uncertain empirical adjustment (Keenan et al., 2013). Recent developments in theory (Busch et al., 2020) and instrumentation (Stangl et al., 2019) have provided mechanistic descriptions of the adjustment, strengthening the case for using

isotopic data to describe the exchange. In addition, there is evidence that $\delta^{13}\text{C}$ can vary as photosynthetic sugars move down from the leaves to the stem (Bögelein et al., 2019; Offermann et al., 2011). Such variation would interfere with the inference of carbon-water exchange ratios from phloem contents or wood, but it can be corrected for empirically (Ubierna & Marshall, 2011; Wei et al., 2013). These corrections allow us to use tree-ring $\delta^{13}\text{C}$ to infer iWUE in the past (Marshall & Monserud, 1996; Voelker et al., 2016).

3. Branchpoint 3: Carbon-Use Efficiency

The efficiency with which photosynthetic carbohydrates are converted into biomass is referred to as the carbon-use efficiency (CUE). One definition of CUE is the ratio NPP:GPP, where NPP is the total biomass production by photosynthetic organisms and GPP is the total ecosystem photosynthesis. By this definition, any carbon lost to respiration reduces the efficiency of biomass conversion. Forest stands may display large variations in CUE, ranging from 0.23 to 0.83 (Collalti & Prentice, 2019; DeLucia et al., 2007; Mäkelä & Valentine, 2001), although some still maintain that it is nearly constant (Landsberg et al., 2020; Waring et al., 1998, 2016). The disagreement can be attributed in part to differences in methods and scale, especially related to uncertainty in estimates of belowground carbon fluxes. If CUE is not homeostatically maintained, then mechanisms that would give rise to variation need to be identified.

We propose a branchpoint in ecosystem carbon flow that could help to explain the differences in CUE reported in the literature. Plants and microbes display two competing respiratory pathways. The first, the cytochrome oxidase (COP) pathway, efficiently stores chemical energy (in the form of ATP) as carbohydrates are oxidized to CO_2 . The resulting chemical energy can be used to power the synthesis of new bonds in biomass. The second pathway, the alternative oxidase pathway (AOP), is much less efficient in the storage of chemical energy as carbohydrates are oxidized to CO_2 (Vanlerberghe, 2013). Thus AOP “wastes” photosynthetic carbon and reduces carbon-use efficiency (Sieger et al., 2005), releasing the residual energy as heat (Lambers & Ribas-Carbo, 2005). The pathways differ in their isotopic fractionation against ^{18}O , leaving a quantitative signal of their proportional rates in the residual pool of molecular oxygen (O_2) as respiration consumes it. Although oxygen is so well-mixed that these effects cannot be observed in most of the ecosystem, they can be measured where the mixing is restricted, e.g., inside tree stems, in wet soils, and in closed cuvettes. A range in the proportional activity of the AOP/COP could well underlie the reported differences in CUE (Hansen et al., 2001). Research on the contribution of AOP to ecosystem respiration has been hampered by a lack of field-compatible, efficient methods for assessing its activity. However, a field-compatible $\delta^{18}\text{O}$ method has recently been proposed to assess activity of the AOX pathway (Henriksson et al., 2019), providing a new opportunity to quantify this branchpoint over whole ecosystems.

3.1. Model Parameterization and Testing

Ecological models of forest ecosystems predict ecosystem function from processes that regulate the component fluxes of carbon and water, but their parameterization to date has relied on very uncertain data sources (Franklin et al., 2020). While leaf-level and other fine scale measurements allow for the identification of each component flux separately, their scaling up to ecosystem level necessarily introduces considerable uncertainty (Carnieli et al., 2016; Peichl et al., 2010). On the other hand, ecosystem scale measurements, such as net growth, soil carbon accumulation (Lim et al., 2015) net ecosystem exchange (Chi et al., 2019), and evapotranspiration (Kozii et al., 2020), can also be derived from the sums of component fluxes. If instead the isotopic signatures are used to split the aggregate fluxes into their process-related components, we could decisively improve the ability of our models to make reliable predictions over larger areas and longer time spans. In addition, models actually representing the pathways of isotopic fractionation in trees (e.g., Ogée et al., 2009; Ulrich et al., 2019; Wei et al., 2013) could allow us to relate observed isotopic signatures to process rates inversely. The isotopic parameters could thus be used either as model parameters or to provide a *post hoc* test of the model predictions. For example, a recently developed dynamic model of the isotopic composition of phloem contents has been tested against measurements from the European summer drought of 2018 (Schiestl-Aalto et al., 2020). The model succeeded in predicting the pronounced changes in $\delta^{13}\text{C}$ and WUE that occurred during this unusual event.

3.2. Comparison to Standard Methods

Isotopic descriptions of linked branchpoints can be compared against traditional measurements of ecosystem fluxes. These might include, e.g., streamflow over weirs (Laudon et al., 2013), eddy covariance measurements of ecosystem carbon and water fluxes (Baldocchi et al., 2001) as well as forest biomass production and soil carbon accumulation (Lim et al., 2015). Such measurements describe the net accumulation of carbon in the system on time scales from half-hourly (EC) to annual or more (forest and soil inventories). Although the measurements are not conceptually difficult, it is a practical challenge to ensure that they are accurate and precise enough to detect temporal changes against a variable background (Muukkonen et al., 2009; Yanai et al., 2003). Likewise, detecting long-term changes in water yields can be surprisingly tricky because of weir design, changes in recording devices, and establishment of suitable controls. However, these measurements of changing carbon pool sizes and streamflow are as critical as the isotopic measurements in tests of our carbon-balance and water-balance predictions.

The traditional measurements were often developed to measure key ecosystem services. For example, forest biomass production is related to commodity production in managed forests. Both biomass production and SOM accumulation are major sinks for carbon sequestration from the atmosphere (Luyssaert et al., 2018). Eddy covariance has been used as a means of estimating these sequestration rates and their environmental controls at high time resolution (Baldocchi, 2008; Campioli et al., 2016). Water yields determine water availability for human use, but they also predict risks of flooding and the damage it causes. Better descriptions of the mechanistic basis of these ecosystem processes improve our ability to predict effects of future changes in climate and land management on the provision of these services.

4. Conclusions

One key advantage of the isotopic branchpoint approach is that it complements the eddy covariance and traditional biometric methods by providing independent estimates at temporal and spatial scales that the others do not. A second advantage is that it can be applied where the requirements of eddy covariance are not met, as in complex terrain, on small plots, and in heterogeneous canopies (Vernay et al., 2020). A third advantage is that it is relatively easy to “isotopically enable” existing models of carbon and water flux. This enabling can often be done with a few lines of code (Ogé et al., 2009; Risi et al., 2016; Wei et al., 2013). Quantifying key isotopic branchpoints would complement traditional measurements of ecosystem fluxes, leading to improved descriptions and deeper understanding of the efficiencies and the coupling of the carbon and water fluxes.

References

- Baldocchi, D. (2008). ‘Breathing’ of the terrestrial biosphere: Lessons learned from a global network of carbon dioxide flux measurement systems. *Australian Journal of Botany*, 56(1), 1–26. <https://doi.org/10.1071/BT07151>
- Baldocchi, D., Falge, E., Gu, L., Olson, R., Hollinger, D., Running, S., et al. (2001). FLUXNET: A new tool to study the temporal and spatial variability of ecosystem-scale carbon dioxide, water vapor, and energy flux densities. *Bulletin of the American Meteorological Society*, 82(11), 2415–2434. [https://doi.org/10.1175/1520-0477\(2001\)082%3C2415:FANTTS%3E2.3.CO;2](https://doi.org/10.1175/1520-0477(2001)082%3C2415:FANTTS%3E2.3.CO;2)
- Bögelein, R., Lehmann, M. M., & Thomas, F. M. (2019). Differences in carbon isotope leaf-to-phloem fractionation and mixing patterns along a vertical gradient in mature European beech and Douglas fir. *New Phytologist*, 222(4), 1803–1815. <https://doi.org/10.1111/nph.15735>
- Brooks, J. R. J., Barnard, H. R., Coulombe, R., & McDonnell, J. J. (2010). Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nature Geoscience*, 3(2), 100–104. <https://doi.org/10.1038/ngeo722>
- Busch, F. A., Holloway-Phillips, M., Stuart-Williams, H., & Farquhar, G. D. (2020). Revisiting carbon isotope discrimination in C3 plants shows respiration rules when photosynthesis is low. *Nature Plants*, 6(3), 245–258. <https://doi.org/10.1038/s41477-020-0606-6>
- Campioli, M., Malhi, Y., Vicca, S., Luyssaert, S., Papale, D., Peñuelas, J., et al. (2016). Evaluating the convergence between eddy-covariance and biometric methods for assessing carbon budgets of forests. *Nature Communications*, 7, 13717. <https://doi.org/10.1038/ncomms13717>
- Cernusak, L. A., Ubierna, N., Winter, K., Holtum, J. A. M., Marshall, J. D., & Farquhar, G. D. (2013). Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist*, 200(4), 950–965. <https://doi.org/10.1111/nph.12423>
- Chi, J., Nilsson, M. B., Kljun, N., Wallerman, J., Fransson, J. E. S., Laudon, H., et al. (2019). The carbon balance of a managed boreal landscape measured from a tall tower in northern Sweden. *Agricultural and Forest Meteorology*, 274, 29–41. <https://doi.org/10.1016/j.agrformet.2019.04.010>
- Collalti, A., & Prentice, I. C. (2019). Is NPP proportional to GPP? Waring’s hypothesis 20 years on. *Tree Physiology*, 39(8), 1473–1483. <https://doi.org/10.1093/treephys/tpz034>
- Cowan, I. R., & Farquhar, G. D. (1977). Stomatal function in relation to leaf metabolism and environment. *Symposia of the Society for Experimental Biology*, 31, 471–505.

- DeLucia, E. H., Drake, J. E., Thomas, R. B., & Gonzalez-Meler, M. (2007). Forest carbon use efficiency: Is respiration a constant fraction of gross primary production? *Global Change Biology*, *13*(6), 1157–1167. <https://doi.org/10.1111/j.1365-2486.2007.01365.x>
- Dewar, R., Mauranen, A., Mäkelä, A., Hölttä, T., Medlyn, B., & Vesala, T. (2018). New insights into the covariation of stomatal, mesophyll and hydraulic conductances from optimization models incorporating nonstomatal limitations to photosynthesis. *New Phytologist*, *217*(2), 571–585. <https://doi.org/10.1111/nph.14848>
- Dubbert, M., Caldeira, M. C., Dubbert, D., & Werner, C. (2019). A pool-weighted perspective on the two-water-worlds hypothesis. *New Phytologist*, *222*(3), 1271–1283. <https://doi.org/10.1111/nph.15670>
- Dubbert, M., Cuntz, M., Piayda, A., & Werner, C. (2014). Oxygen isotope signatures of transpired water vapor: The role of isotopic non-steady-state transpiration under natural conditions. *New Phytologist*, *203*(4), 1242–1252. <https://doi.org/10.1111/nph.12878>
- Farquhar, G., O'Leary, M., & Berry, J. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Functional Plant Biology*, *9*(2), 121. <https://doi.org/10.1071/PP9820121>
- Farquhar, G. D., Hubick, K. T., Condon, A. G., & Richards, R. A. (1989). Carbon isotope fractionation and plant water-use efficiency. In P. W. Rundel, J. R. Ehleringer, & K. A. Nagy (Eds.), *Stable isotopes in ecological research* (pp. 21–40). Berlin, Germany: Springer. https://doi.org/10.1007/978-1-4612-3498-2_2
- Franklin, O., Harrison, S. P., Dewar, R., Farrior, C. E., Brännström, Å., Dieckmann, U., et al. (2020). Organizing principles for vegetation dynamics. *Nature Plants*, *6*(5), 444–453. <https://doi.org/10.1038/s41477-020-0655-x>
- Hansen, L. D., Smith, B. N., Criddle, R. S., & Church, J. N. (2001). Responses of plant growth and metabolism to environmental variables predicted from laboratory measurements. In E. D. McArthur, & D. J. Fairbanks (Eds.), *Comps. Shrubland ecosystem genetics and biodiversity: proceedings; 2000 June 13-15, Provo, UT. Proc. RMRS-P-21* (Vol. 21, pp. 259–264). Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.
- Hayes, J. M. (2001). Fractionation of carbon and hydrogen isotopes in biosynthetic processes. *Reviews in Mineralogy and Geochemistry*, *43*(1), 225–277. <https://doi.org/10.2138/gsrmg.43.1.225>
- Henriksson, N., Marshall, J., Lundholm, J., Boily, Å., Boily, J.-F., & Näsholm, T. (2019). Improved in vivo measurement of alternative oxidase respiration in field-collected pine roots. *Physiologia Plantarum*, *167*(1), 34–47. <https://doi.org/10.1111/ppl.12910>
- Jasechko, S., Sharp, Z. D., Gibson, J. J., Birks, S. J., Yi, Y., & Fawcett, P. J. (2013). Terrestrial water fluxes dominated by transpiration. *Nature*, *496*(7445), 347–350.
- Keenan, T. F., Hollinger, D. Y., Bohrer, G., Dragoni, D., Munger, J. W., Schmid, H. P., & Richardson, A. D. (2013). Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature*, *499*(7458), 324–327.
- Kirchner, J. W., & Allen, S. T. (2020). Seasonal partitioning of precipitation between streamflow and evapotranspiration, inferred from end-member splitting analysis. *Hydrology and Earth System Sciences*, *24*(1), 17–39. <https://doi.org/10.5194/hess-24-17-2020>
- Kornfeld, A., Horton, T. W., Yakir, D., Searle, S. Y., Griffin, K. L., Atkin, O. K., et al. (2012). A field-compatible method for measuring alternative respiratory pathway activities in vivo using stable O₂ isotopes. *Plant, Cell and Environment*, *35*(8), 1518–1532. <https://doi.org/10.1111/j.1365-3040.2012.02507.x>
- Kozii, N., Haahti, K., Tor-ngern, P., Chi, J., Hasselquist, E. M., Laudon, H., et al. (2020). Partitioning growing season water balance within a forested boreal catchment using sap flux, eddy covariance, and a process-based model. *Hydrology and Earth System Sciences*, *24*(6), 2999–3014. <https://doi.org/10.5194/hess-24-2999-2020>
- Lambers, H., & Ribas-Carbo, M. (2005). *Plant Respiration from Cell to Ecosystem*, Netherlands: Springer.
- Landsberg, J. J., Waring, R. H., & Williams, M. (2020). The assessment of NPP/GPP ratio. *Tree Physiology*, *40*(6), 695–699. <https://doi.org/10.1093/treephys/tpaa016>
- Laudon, H., Taberman, I., Ågren, A., Futter, M., Ottosson-Löfvenius, M., & Bishop, K. (2013). The Krycklan Catchment Study—A flagship infrastructure for hydrology, biogeochemistry, and climate research in the boreal landscape. *Water Resources Research*, *49*(10), 7154–7158. <https://doi.org/10.1002/wrcr.20520>
- Lim, H., Oren, R., Palmroth, S., Tor-ngern, P., Mörling, T., Näsholm, T., et al. (2015). Inter-annual variability of precipitation constrains the production response of boreal *Pinus sylvestris* to nitrogen fertilization. *Forest Ecology and Management*, *348*, 31–45. <https://doi.org/10.1016/j.foreco.2015.03.029>
- Luysaert, S., Marie, G., Valade, A., Chen, Y.-Y., Njakou Djomo, S., Ryder, J., et al. (2018). Trade-offs in using European forests to meet climate objectives. *Nature*, *562*(7726), 259–262. <https://doi.org/10.1038/s41586-018-0577-1>
- Mäkelä, A., Berninger, F., & Hari, P. (1996). Optimal control of gas exchange during drought: Theoretical analysis. *Annals of Botany*, *77*(5), 461–468. <https://doi.org/10.1006/anbo.1996.0056>
- Mäkelä, A., & Valentine, H. T. (2001). The ratio of NPP to GPP: Evidence of change over the course of stand development. *Tree Physiology*, *21*(14), 1015–1030. <https://doi.org/10.1093/treephys/21.14.1015>
- McDonnell, J. J. (2014). The two water worlds hypothesis: Ecohydrological separation of water between streams and trees?. *Wiley Interdisciplinary Reviews: Water*, *1*(4), 323–329. <https://doi.org/10.1002/wat2.1027>
- Marshall, J. D., & Monserud, R. A. (1996). Homeostatic gas-exchange parameters inferred from 13C/12C in tree rings of conifers. *Oecologia*, *105*(1), 13–21.
- Medlyn, B. E., Duursma, R. A., Eamus, D., Ellsworth, D. S., Prentice, I. C., Barton, C. V. M., et al. (2011). Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology*, *17*(6), 2134–2144. <https://doi.org/10.1111/j.1365-2486.2010.02375.x>
- Muukkonen, P., Häkkinen, M., & Mäkipää, R. (2009). Spatial variation in soil carbon in the organic layer of managed boreal forest soil—Implications for sampling design. *Environmental Monitoring and Assessment*, *158*(1), 67–76. <https://doi.org/10.1007/s10661-008-0565-2>
- Offermann, C., Ferrio, J. P., Holst, J., Grote, R., Siegwolf, R., Kayler, Z., & Gessler, A. (2011). The long way down—Are carbon and oxygen isotope signals in the tree ring uncoupled from canopy physiological processes? *Tree Physiology*, *31*(10), 1088–1102.
- Ogée, J., Barbour, M. M., Wingate, L., Bert, D., Bosc, A., Stievenard, M., et al. (2009). A single-substrate model to interpret intra-annual stable isotope signals in tree-ring cellulose. *Plant, Cell and Environment*, *32*(8), 1071–1090. <https://doi.org/10.1111/j.1365-3040.2009.01989.x>
- Peichl, M., Brodeur, J. J., Khomik, M., & Arain, M. A. (2010). Biometric and eddy-covariance based estimates of carbon fluxes in an age-sequence of temperate pine forests. *Agricultural and Forest Meteorology*, *150*(7–8), 952–965. <https://doi.org/10.1016/j.agrformet.2010.03.002>
- Penna, D., Hopp, L., Scandellari, F., Allen, S. T., Benettin, P., Beyer, M., et al. (2018). Ideas and perspectives: Tracing terrestrial ecosystem water fluxes using hydrogen and oxygen stable isotopes—Challenges and opportunities from an interdisciplinary perspective. *Biogeosciences*, *15*(21), 6399–6415. <https://doi.org/10.5194/bg-15-6399-2018>
- Risi, C., Ogee, J., Bony, S., Bariac, T., Raz Yaseef, N., Wingate, L., et al. (2016). The water isotopic version of the land-surface model ORCHIDEE: Implementation, evaluation, sensitivity to hydrological parameters. *Hydrology: Current Research*, *7*(4), 1000258. <https://doi.org/10.4172/2157-7587.1000258>

- Schiestl-Aalto, P., Stangl, Z. R., Tarvainen, L., Wallin, G., Marshall, J., & Mäkelä, A. (2020). Linking canopy-scale mesophyll conductance and phloem sugar $\delta^{13}\text{C}$ using empirical and modelling approaches. *New Phytologist*. <https://doi.org/10.1111/nph.17094>
- Sieger, S. M., Kristensen, B. K., Robson, C. A., Amirsadeghi, S., Eng, E. W. Y., Abdel-Mesih, A., et al. (2005). The role of alternative oxidase in modulating carbon use efficiency and growth during macronutrient stress in tobacco cells. *Journal of Experimental Botany*, *56*(416), 1499–1515.
- Sklash, M. G., & Farvolden, R. N. (1979). The role of groundwater in storm runoff. *Journal of Hydrology*, *43*(1), 45–65. [https://doi.org/10.1016/0022-1694\(79\)90164-1](https://doi.org/10.1016/0022-1694(79)90164-1)
- Stangl, Z. R., Tarvainen, L., Wallin, G., Ubierna, N., Röntfors, M., & Marshall, J. D. (2019). Diurnal variation in mesophyll conductance and its influence on modelled water-use efficiency in a mature boreal *Pinus sylvestris* stand. *Photosynthesis Research*, *141*(1), 53–63. <https://doi.org/10.1007/s11120-019-00645-6>
- Ubierna, N., & Marshall, J. D. (2011). Estimation of canopy average mesophyll conductance using $\delta^{13}\text{C}$ of phloem contents. *Plant, Cell and Environment*, *34*(9), 1521–1535.
- Ulrich, D. E. M., Still, C., Brooks, J. R., Kim, Y., & Meinzer, F. C. (2019). Investigating old-growth ponderosa pine physiology using tree-rings, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and a process-based model. *Ecology*, *100*(6), e02656. <https://doi.org/10.1002/ecy.2656>
- Vanlerberghe, G. C. (2013). Alternative oxidase: A mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. *International Journal of Molecular Sciences*, *14*(4), 6805–6847. <https://doi.org/10.3390/ijms14046805>
- Vernay, A., Tian, X., Chi, J., Linder, S., Mäkelä, A., Oren, R., et al. (2020). Estimating canopy gross primary production by combining phloem stable isotopes with canopy and mesophyll conductances. *Plant, Cell and Environment*, *43*(9), 2124–2142. <https://doi.org/10.1111/pce.13835>
- Voelker, S. L., Brooks, J. R., Meinzer, F. C., Anderson, R., Bader, M. K.-F., Battipaglia, G., et al. (2016). A dynamic leaf gas-exchange strategy is conserved in woody plants under changing ambient CO_2 : Evidence from carbon isotope discrimination in paleo and CO_2 enrichment studies. *Global Change Biology*, *22*(2), 889–902. <https://doi.org/10.1111/gcb.13102>
- Waring, R., Landsberg, J., & Linder, S. (2016). Tamm review: Insights gained from light use and leaf growth efficiency indices. *Forest Ecology and Management*, *379*, 232–242. <https://doi.org/10.1016/j.foreco.2016.08.023>
- Waring, R. H., Landsberg, J. J., & Williams, M. (1998). Net primary production of forests: A constant fraction of gross primary production? *Tree Physiology*, *18*(2), 129–134.
- Wei, L., Marshall, J. D., Link, T. E., Kavanagh, K. L., Du, E., Pangle, R. E., et al. (2013). Constraining 3-PG with a new $\delta^{13}\text{C}$ submodel: A test using the $\delta^{13}\text{C}$ of tree rings. *Plant, Cell and Environment*, *37*(1), 82–100. <https://doi.org/10.1111/pce.12133>
- Williams, D. G., Cable, W., Hultine, K., Hoedjes, J. C. B., Yepez, E. A., Simonneau, V., et al. (2004). Evapotranspiration components determined by stable isotope, sap flow and eddy covariance techniques. *Agricultural and Forest Meteorology*, *125*(3), 241–258. <https://doi.org/10.1016/j.agrformet.2004.04.008>
- Yanai, R. D., Stehman, S. V., Arthur, M. A., Prescott, C. E., Friedland, A. J., Siccama, T. G., & Binkley, D. (2003). Detecting change in forest floor carbon. *Soil Science Society of America Journal*, *67*(5), 1583–1593. <https://doi.org/10.2136/sssaj2003.1583>