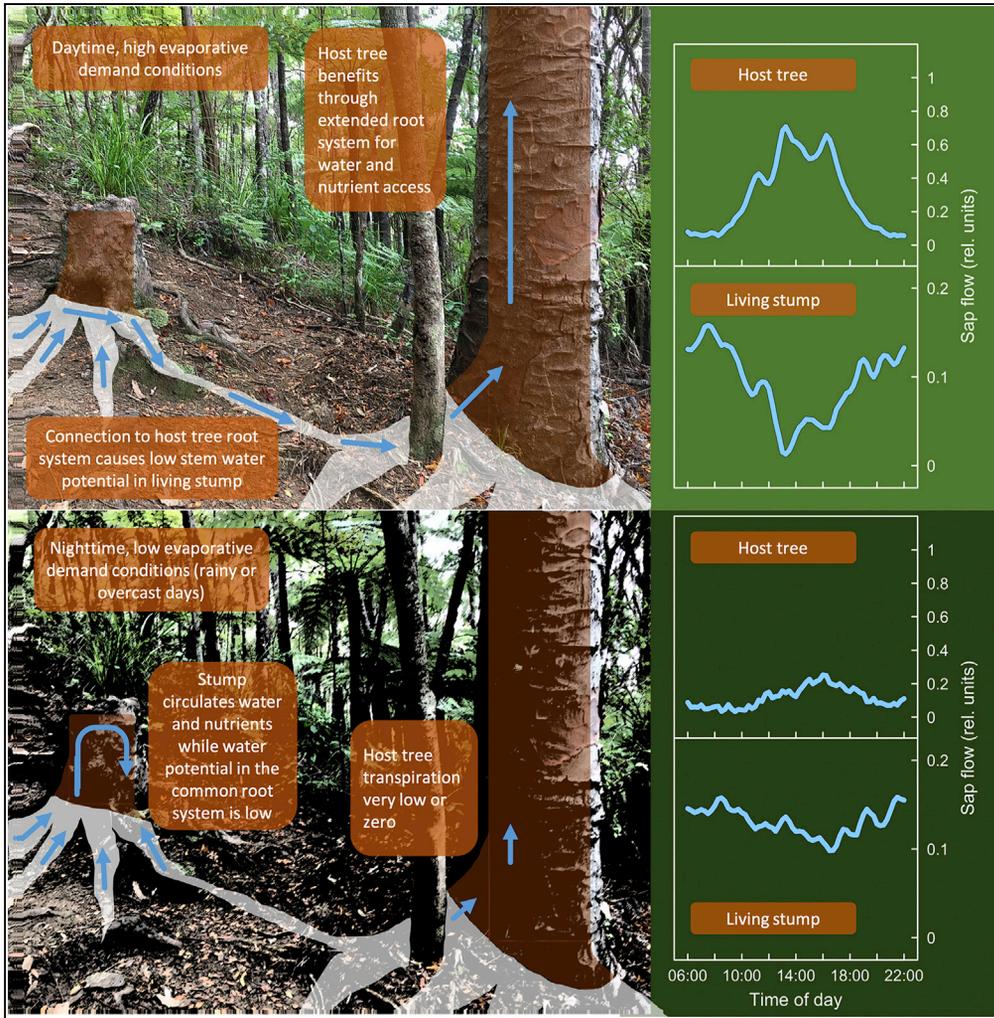


Article

Hydraulic Coupling of a Leafless Kauri Tree Remnant to Conspecific Hosts



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HIGHLIGHTS

A living kauri tree stump is physiologically tightly linked to conspecific neighbors

This suggests root grafting between live trees and living stumps

Stump water status responds inversely to changes in host tree physiology

The hydraulic coupling challenges our views on drought and pathogen impacts

Article

Hydraulic Coupling of a Leafless Kauri Tree Remnant to Conspecific Hosts

M.K.-F. Bader¹ and S. Leuzinger^{1,2,*}

SUMMARY

Trees are commonly regarded as distinct entities, but the roots of many species fuse to form natural root grafts allowing the exchange of water, carbon, mineral nutrients, and microorganisms between individuals. Exploiting the phenomenon of leafless (photosynthetically inactive) tree remnants being kept alive by conspecifics, we show tight physiological coupling of a living kauri (*Agathis australis*) stump to conspecific neighbors. The trunk remnant displayed greatly reduced, inverted daily sap flow patterns compared with intact kauri trees. Its stem water potential showed strong diel variation with minima during daytime and maxima at night, coinciding with peak and minimal sap flow rates in neighbors, respectively. Sudden atmospherically driven changes in water relations in adjacent kauri trees were very rapidly and inversely mirrored in the living stump's water status. Such intimate hydrological coupling suggests a "communal physiology" among (conspecific) trees with far-reaching implications for our understanding of forest functioning, particularly under water shortage.

INTRODUCTION

Aboveground most trees appear as individuals, but they are often intricately connected belowground through mycorrhizal networks and also through natural root grafts, facilitating the exchange of carbon, nitrogen, and other mineral nutrients (Bormann, 1966; Stone and Stone, 1975; Tarroux, 2011). While mycorrhiza-mediated nutrient transfer between trees has attracted considerable interest in recent decades (Brownlee et al., 1983; Vogt, 1991; Courty et al., 2010; Klein et al., 2016), the role of natural root grafts has received little attention over the past half century, despite some 150 woody angio- and gymnosperm species (Beddie, 1941; Bormann, 1966) reported to show the phenomenon globally, and accounts of more than 60% grafted individuals within a population (Graham and Bormann, 1966; Basnet et al., 1993). It is important to distinguish between three fundamentally different types of root connections: those within an individual (self-grafting, Baret, 2011), which are common to most, possibly all trees (Graham and Bormann, 1966); those between genetically different individuals of the same species (intraspecific grafting, Fraser et al., 2006); and those between different species (interspecific grafting, La Rue, 1934; Beddie, 1941). While self-grafting is little surprising and its adaptive value is easily explained (e.g., increased stability and transport of water and nutrients within individuals), intra- and interspecific grafting raise important questions as to their evolutionary advantage (Callaway and Mahall, 2007; Keeley, 1988). Several hypotheses trying to explain this trait have been put forth, ranging from evolutionary neutrality, increased resistance to windthrow, improved water and nutrient exploitation, all the way to a parasitic nature of the phenomenon (Loehle and Jones, 1990; Lev-Yadun, 2011). However, there is no consensus, and natural root grafting may have evolved several times for different reasons. The question as to the adaptive value of intraspecific root grafting becomes more challenging yet when one of the grafted individuals is a leafless tree stump, a phenomenon that was first reported in 1833 for European silver fir (*Abies alba*) and several times since (Dutrochet, 1833; Eis, 1972; Graham and Bormann, 1966), including in the species we investigate here (Ecroyd, 1982). A "living stump" without foliage, provided an intact root system, needs to receive at least carbohydrates from neighboring trees. Assuming that the grafting was in place before the tree became a leafless stump, it is far from obvious what causes conspecifics to continue the provisioning of carbohydrates to a photosynthetically inactive individual. It has been argued that the host tree may benefit from mechanical stability through enhanced soil anchorage (Rigg and Harrar, 1931; Keeley, 1988) or through improved access to soil resources via the extended root system provided by the stump (Bormann, 1966), but no increased growth was observed in trees that were artificially grafted to living stumps (Holmsgaard and Scharff, 1963). Because carbohydrates are transported in solution, and because the transpirational pull is absent in living stumps, the question of what physiological processes orchestrate such intriguing symbioses is eminent, yet has not been addressed to date.

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<https://doi.org/10.1016/j.isci.2019.05.009>

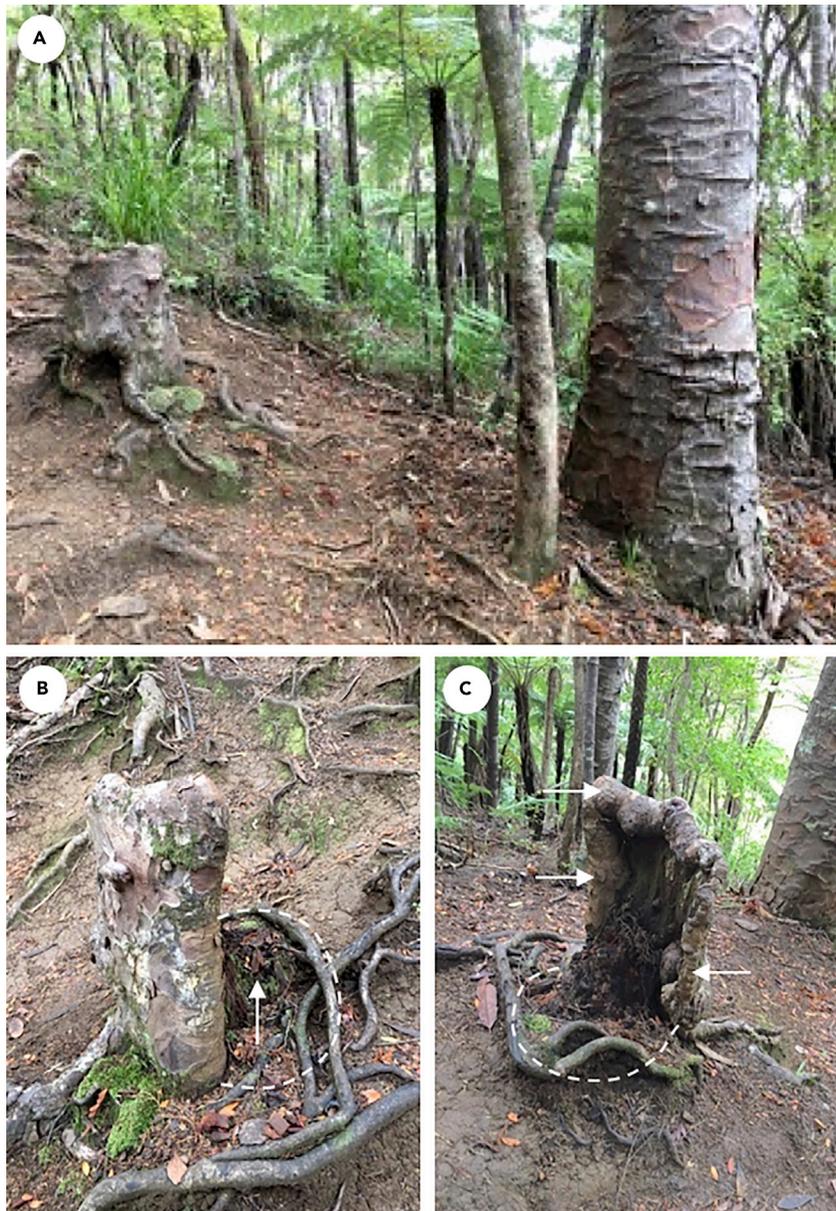


Figure 1. Living, Leafless Stump of the Southern Conifer *Agathis australis* (Kauri)

Frontal view of the tree trunk remnant from the forest track (A). Oblique lateral view showing the typical gray-brown kauri bark with distinctive hammer mark pattern and decaying wood behind it (arrow) indicating the original trunk perimeter (dashed line) (B). Rear view showing a thick callus lining along the edge (arrows). The dashed line indicates the approximate original trunk perimeter (C).

RESULTS AND DISCUSSION

In living, leafless stumps, the lack of foliar transpiration implies a cyclic flow of water requiring an extensive rearrangement of water transport pathways, which prompted us to examine vertical and horizontal sap flow patterns simultaneously (Figure 1). The amplitude of the normalized diel sap flow velocity (hereafter simply referred to as sap flow) in the trunk remnant was about two times larger in the vertical compared with the horizontal direction (Figures 2C and 2D). The stump's vertical sap flow maxima were more than five times smaller relative to the surrounding intact conspecifics (Figures 2A and 2B), whereas its stem water potential exhibited a pronounced diel cycle ranging from values close to 0 MPa at night to -3.7 MPa around midday (Figure 2E). Interestingly, the stump's sap flow and stem water potential were both

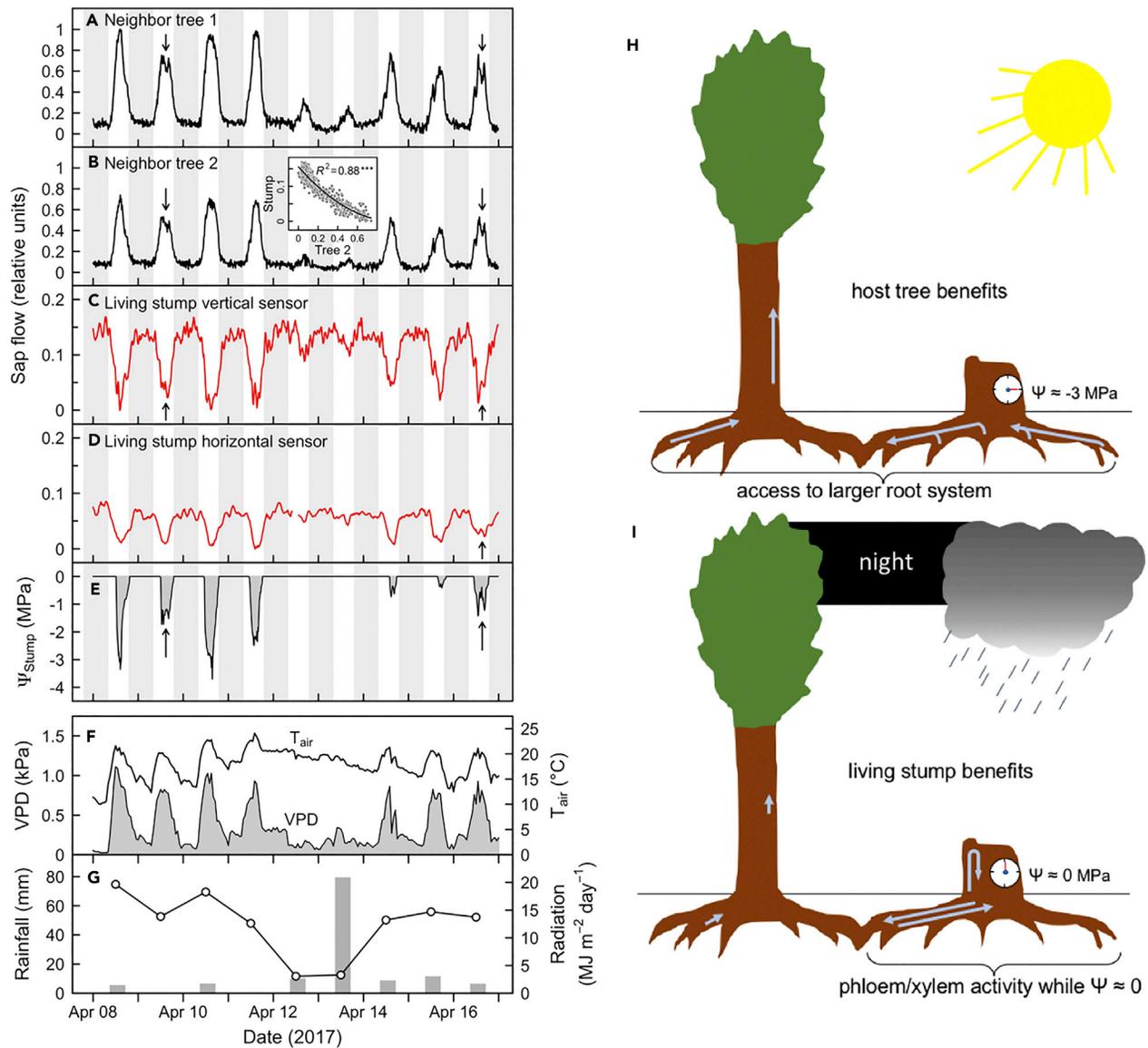


Figure 2. Tight Hydraulic Coupling between a Living, Leafless Stump and Neighboring Trees of the Southern Conifer *Agathis australis* (Kauri)

Sap flow in two tall, intact kauri trees in close vicinity to the conspecific living stump (A and B). Vertical and horizontal sap flow in the living kauri stump (C and D). Stem water potential of the living stump (E). Air temperature (T_{air}) and vapor pressure deficit (VPD) (F). Daily rainfall (gray bars) and solar radiation (white circles) (G). Arrows in panels A–E highlight the rapid hydraulic signal propagation between transpiring trees and the connected, non-transpiring stump. During daytime when host trees transpire vigorously, the host tree profits from a larger root system and the stem water potential (Ψ) is low (H). During nighttime and periods of low evaporative demand, the living stump circulates water through its living tissues at Ψ close to zero. In the absence of transpiration, this water movement is likely driven by root pressure or osmolyte accumulation (I).

inversely related to the water flux seen in the tall, surrounding kauri trees (Figures 2A–2E and inset). On sunny days, when neighboring trees were transpiring vigorously, little or no water movement could be detected in the living stump, neither vertically nor horizontally, but its water potential dropped to minimal values (Figures 2E and 2H). At night, however, when transpiration of the surrounding kauri trees was minimal, complete relaxation of the stump’s water potential occurred, and its sap flow reached maximum values (Figures 2C and 2I). In the absence of transpiration in the tree stump, this phenomenon can only be explained by osmotically driven water movement or root pressure (Sperry et al., 1987) (Figures 2H and 2I). During daytime, sudden changes in atmospheric vapor pressure deficit resulting in instantaneous sap flow reductions in adjacent kauri trees were rapidly mirrored by an equivalent, but opposite, pattern in

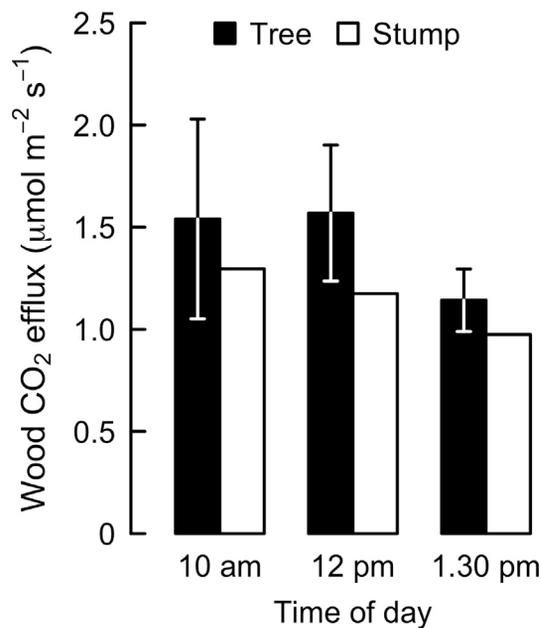


Figure 3. Wood CO₂ Efflux Rates from the Stem of Tall, Intact Trees and a Living, Leafless Stump of the Southern Conifer *Agathis australis* (Kauri)

Measurements were taken at breast height (intact trees) and about 30 cm above the ground (stump) in regular intervals during a sunny day. Intact trees: $n = 3$, stump: $n = 1$. Means \pm SE.

sap flow and immediate relaxation of stem water potential in the living trunk remnant, suggesting intimate hydraulic coupling between the host tree and the stump (see arrows in Figures 2A–2E). Further strong evidence for this tight hydraulic connection came to light under conditions of low evaporative demand. On two consecutive days (April 12 and 13) with either very low vapor pressure deficit or exceedingly high precipitation (ca. 80 mm on April 13), sap flow in the surrounding intact trees dropped to minimal values, whereas in the trunk remnant it stayed continuously high (Figure 2). The strong reduction in transpiration of neighboring trees translated into higher water potentials and increased water availability in the soil and within the joint root system, allowing for sustained daytime sap flow rates in the living stump. The CO₂ release rates from the bark of the living stump and neighboring kauri trees were similar (1–1.5 μmol m⁻² s⁻¹), with almost no diel variation in both host and stump, confirming metabolic activity and thus living tissue in the trunk remnant (Figure 3).

The above evidence for substantial xylem flow in a living stump raises substantial questions on the anatomical, physiological, and evolutionary mechanisms that enable this process, particularly when assuming that the graft was in place before the loss of photosynthetically active tissues of one of the connected trees (Lanner, 1961). With the loss of the foliage, transpiration, and basipetal assimilate transport, the provisioning of living tissues must change considerably. This supply process must be highly efficient, as the here reported stem respiration rates suggest similar metabolic activity between the host and the living stump. The measurable horizontal and substantial vertical sap flow rates suggest that a circular, rather than unidirectional, sap flow pattern may evolve rapidly following the loss of autotrophic tissues, which may explain the 5-fold lower acropetal sap flow rates in the stump compared with the host trees. If no anatomical adaptations take place, the horizontal sap flow presumably occurs via existing vascular rays. The observed sap flow patterns and their associated (likely osmotic) regulation seem to be largely decoupled from the atmosphere, but instead highly dependent on the host trees' physiology. This stands in contrast to the way plants normally function, with both xylem and phloem transport ultimately driven by the highly negative water potential of the atmosphere, which is coupled to the plants via stomata.

Our results strongly suggest intraspecific root grafting in kauri and contribute to our understanding of the evolutionary advantage of root grafts, particularly those between trees and leafless stumps. Intact kauri trees grafted to a closely intertwined root network may adopt the root system of a connected tree that has lost its crown, thereby extending their rooting space and at the same time allowing trunk remnants to persist over long periods of time. The extra resource costs linked to the maintenance of the above-ground part of living trunk remnants may be minor compared with those associated with the suddenly

enlarged root system (Figure 2H). Our findings suggest that the adaptive advantage of the hydraulic coupling may be a lot more important than previously assumed, and may indeed be a means of compensating for the carbohydrates the trunk remnant receives. On the downside, our study also corroborates the notion of facilitated pathogen transmission through root grafts (Graham and Bormann, 1966; Epstein, 1978), which is especially alarming because kauri has recently been classified as threatened owing to the rapid spread of kauri dieback disease, caused by the fungus-like soilborne pathogen *Phytophthora agathidicida* (De Lange et al., 2013).

In conclusion, although observations of living tree stumps have been reported widely (Lanner, 1961), our results on the physiological interactions with host trees indicate that such symbioses may be much more complex than previously assumed: by physiologically exploiting “downtimes” of transpiring trees during the night or rainy days with high water potentials in the root network (Figure 2I), living stumps seem to act partially autonomously, strategically tapping into resources rather than simply becoming part of the neighboring trees’ extended root networks. Although a few studies have suggested that carbon and possibly nutrients are exchanged universally in forests (Simard et al., 1997; Klein et al., 2016), our results indicate that such a “wood-wide web” (Sen, 2000) may in fact extend to the hydraulic system of trees, with far-reaching consequences for drought-related impacts (Allen et al., 2015) and pathogen transfer (Epstein, 1978).

Limitations of the Study

Clearly, only having observed a single living kauri tree stump prevents us from drawing broader conclusions. Although we personally have not yet seen a second occurrence of a living stump belonging to this iconic New Zealand species, from talking to local foresters, we know that this phenomenon has apparently been noticed in the past and the formation of natural root grafts in kauri was already suspected 80 years ago (Beddie, 1941). However, because the reported results are of physiological rather than ecological nature, and through highly consistent temporal replication and reoccurrence of very similar patterns measured with different sensors, we trust our data. Another shortcoming is the lack of direct evidence for root grafting, which can only be achieved with isotope labeling experiments, involving tremendous logistic effort (Klein et al., 2016). In our case, the host tree crowns would need to be labeled with ^{13}C , for example; other options may be phloem labeling or destructive root exposure. However, this would only confirm the stump’s supply with carbohydrates by host trees. To provide further evidence of hydraulic coupling, similar measurements as presented here, possibly adding root sap flow measurements, would be required. Below-ground carbon trading among trees has been shown in the past, but if our admittedly limited data on hydraulic coupling among trees can be confirmed, we might have to revise our general understanding of forest ecosystems as communal “superorganisms.”

METHODS

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.isci.2019.05.009>.

ACKNOWLEDGMENTS

We thank Auckland Council for granting the permit required for this research (WS1074).

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the paper.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

Received: February 26, 2019

Revised: April 9, 2019

Accepted: May 7, 2019

Published: July 25, 2019

REFERENCES

- Allen, C.D., Breshears, D.D., and McDowell, N.G. (2015). On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere* 6, 1–55.
- Baret, M.D.A. (2011). Root connections can trigger physiological responses to defoliation in nondefoliated aspen suckers. *Botany* 89, 753–761.
- Basnet, K., Scatena, F., Likens, G.E., and Lugo, A.E. (1993). Ecological consequences of root grafting in tabonuco (*Dacryodes excelsa*) trees in the Luquillo Experimental Forest, Puerto Rico. *Biotropica* 25, 28–35.
- Beddie, A.D. (1941). Natural root grafts in New Zealand trees. *Transact. Proc. R. Soc. New Zeal.* 71, 199–203.
- Bormann, F. (1966). The structure, function, and ecological significance of root grafts in *Pinus strobus* L. *Ecol. Monogr.* 36, 1–26.
- Brownlee, C., Duddridge, J., Malibari, A., and Read, D. (1983). The Structure and Function of Mycelial Systems of Ectomycorrhizal Roots with Special Reference to Their Role in Forming Interplant Connections and Providing Pathways for Assimilate and Water Transport. *Tree Root Systems and Their Mycorrhizas* (Springer).
- Callaway, R.M., and Mahall, B.E. (2007). Plant ecology: family roots. *Nature* 448, 145–147.
- Courty, P.-E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.-P., Uroz, S., and Garbaye, J. (2010). The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. *Soil Biol. Biochem.* 42, 679–698.
- De Lange, P.J., Rolfe, J.R., Champion, P.D., Courtney, S., Heenan, P.B., Barkla, J.W., Cameron, E.K., Norton, D.A., and Hitchmough, R. (2013). Conservation Status of New Zealand Indigenous Vascular Plants, 2012 (Publishing Team, Department of Conservation).
- Dutrochet, M. (1833). Observations sur la longue persistance de la vie et l'accroissement dans les racines et dans la souche du *Pinus picea* L., après qu'il a été abattu. *Ann. Sci. Nat.* 29, 300–303.
- Ecroyd, C. (1982). Biological flora of New Zealand 8. *Agathis australis* (D. Don) Lindl. (Araucariaceae) Kauri. *New Zeal. J. Bot.* 20, 17–36.
- Eis, S. (1972). Root grafts and their silvicultural implications. *Can. J. For. Res.* 2, 111–120.
- Epstein, A.H. (1978). Root graft transmission of tree pathogens. *Annu. Rev. Phytopathol.* 16, 181–192.
- Fraser, E.C., Lieffers, V.J., and Landhäusser, S.M. (2006). Carbohydrate transfer through root grafts to support shaded trees. *Tree Physiol.* 26, 1019–1023.
- Graham, B., and Bormann, F. (1966). Natural root grafts. *Bot. Rev.* 32, 255–292.
- Holmsgaard, E., and Scharff, O. (1963). Levende stød i rødgranbevoksninger. Summary: living stumps in Norway spruce stands. *Det Forstlige Forsøgsvæsen i Danmark* 28, 97–150.
- Keeley, J.E. (1988). Population variation in root grafting and a hypothesis. *Oikos* 52, 364–366.
- Klein, T., Siegwolf, R.T., and Körner, C. (2016). Belowground carbon trade among tall trees in a temperate forest. *Science* 352, 342–344.
- La Rue, C.D. (1934). Root grafting in trees. *Am. J. Bot.* 21, 121–126.
- Lanner, R.M. (1961). Living stumps in the Sierra Nevada. *Ecology* 42, 170–173.
- Lev-Yadun, S. (2011). Why should trees have natural root grafts? *Tree Physiol.* 31, 575–578.
- Loehle, C., and Jones, R. (1990). Adaptive significance of root grafting in trees. *Funct. Ecol.* 4, 268–271.
- Rigg, G.B., and Harrar, E. (1931). The root systems of trees growing in sphagnum. *Am. J. Bot.* 18, 391–397.
- Sen, R. (2000). Budgeting for the wood-wide web. *New Phytol.* 145, 161–163.
- Simard, S.W., Perry, D.A., Jones, M.D., Myrold, D.D., Durall, D.M., and Molina, R. (1997). Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388, 579–582.
- Sperry, J.S., Holbrook, N.M., Zimmermann, M.H., and Tyree, M.T. (1987). Spring filling of xylem vessels in wild grapevine. *Plant Physiol.* 83, 414–417.
- Stone, J., and Stone, E. (1975). The communal root system of red pine: water conduction through root grafts. *Forest Sci.* 21, 255–261.
- Tarroux, E.D.A. (2011). Effect of natural root grafting on growth response of Jack pine (*Pinus Banksiana*; Pinaceae). *Am. J. Bot.* 98, 967–974.
- Vogt, K. (1991). Carbon budgets of temperate forest ecosystems. *Tree Physiol.* 9, 69–86.

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Supplemental Information

Hydraulic Coupling of a Leafless Kauri Tree Remnant to Conspecific Hosts

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Transparent Methods

Here we report on the water relations and stem CO₂ release of a living, leafless kauri trunk remnant (*Agathis australis* (D.Don) Loudon) physiologically coupled to intact, conspecific neighboring trees in a native rainforest located in the Waitakere Ranges in the North Island of New Zealand (Fig. 1 A – C).

Tree water relations

Sap flow was recorded using heat ratio method sensors (SFM 1, ICT International, Armidale, New South Wales, Australia). Two sensors were installed at 35 cm (vertical) and 40 cm height (horizontal) in the living stump and one sensor each at breast height in two neighbouring adult trees. Due to the lack of transpirational water loss and the encircling ring of callus restricting acropetal water transport, we anticipated lateral water flow in the living stump, and hence also used a horizontal sensor placement in addition to the usual vertical installation. Stem water potential was measured using stem psychrometers (PSY 1, ICT International, Armidale, New South Wales, Australia). On a ca. 4 × 4 cm area, xylem tissue was exposed using a razor scraper. Sensor heads were sealed onto patches of exposed xylem using silicone grease and fixed with duct tape. During the installation procedure, each of the two neighbouring, intact trees produced copious amounts of resin prohibiting valid measurements. The living stump produced only little resin and exudations dried up quickly allowing reliable water potential readings. Meteorological data was obtained from the closest weather station in Henderson, 15 km away from the research site.

Wood CO₂ efflux

25 CO₂ release from woody tissue was measured using a custom-made static chamber fitted with a diffusion aspirated nondispersive infrared gas analyser (IRGA) and a relative humidity/temperature sensor (GMP343 carbon dioxide probe, HMP75 rH/ T probe; Vaisala, Vantaa, Finland). The water vapour cross-sensitivity of the IRGA was corrected instantaneously by linking it with the relative humidity/temperature sensor.

30 The polypropylene chamber housing had a volume of 5.58 L and featured an internal, battery-driven computer fan providing continuous headspace mixing. During recordings, the chamber opening (5 cm diameter) was attached to the bark using Blu-Tack™ adhesive and elastic straps. Individual recordings lasted 10 min with a 15 s reading interval. The rate of wood CO₂ efflux was derived from the slope of a linear regression

35 applied to the CO₂ increase in the chamber headspace disregarding the first minute of the recordings to avoid flux disturbances involved with chamber placement.

Data handling and statistical analysis

All data processing and statistical analysis were performed using the R software

40 environment. A Hampel filter with window length 10 and the default threshold of 3 (Pearson's rule) was applied to the sap flow data to remove signal spikes caused by short-term voltage fluctuations. The trunk remnant's sap flow data was smoothed using a Gaussian window function with alpha (breadth of the window) set to 1.5, and a window length of 5 and 12 for the vertical and horizontal sap flow, respectively. Feature

45 scaling was applied to the combined sap flow data to normalize all values in the 0 – 1
range, allowing the direct comparison of the daily amplitudes between the
physiologically coupled trees. A nonlinear regression approach using the alternative
form of the quadratic equation [$y = a (x - b)^2$; a = shape parameter, b = symmetry
parameter] was used to model the relationship between sap flow rates recorded in the
50 kauri trunk remnant and a conspecific host tree.