Severe declines in hydraulic capacity and associated carbon starvation drive mortality in seawater exposed Sitka-spruce (Picea sitchensis) trees

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Abstract

Sea-level rise is causing widespread tree mortality of coastal forests, with large consequences on the Earth system as a result of these forests' importance in carbon and nutrient export. The mechanisms of mortality under these conditions are, however, poorly tested. We used wood anatomy traits, wood δ13C, and tree radial growth to retrospectively assess the physiological process of seawater effects on whole tree xylem hydraulic capacity, gas exchange, and radial growth. During the latter stages of mortality (2018–2019), we directly measured metrics of water use and carbon metabolism across trees having crowns ranging from fully foliated to completely defoliated to investigate mortality processes at the sub-annual scale. Upon seawater exposure, soil salinity increased and allocation to hydraulic function declined, resulting in a dramatic reduction in water supply to the crown, increased crown-level water stress, and subsequent crown foliage loss. Simultaneously, leaf-level photosynthetic capacity declined steeply with increasing salinity. The combined loss of crown foliage area and photosynthetic rates per unit leaf area promoted carbon starvation, while no evidence of hydraulic failure was observed. These results elucidate mechanisms of coastal forest death under seawater exposure, enabling more accurate modeling in the future.
Introduction

Coastal forests play an important role in hydrological, energy, and biogeochemical cycles and provide numerous ecosystem services such as mitigating the impacts of storms and erosion (IPCC, 2019). Risks to coastal forests’ structure and function under accelerating sea-level rise are increasing, making these ecosystems highly vulnerable (Lovelock et al. 2015, Thorne et al. 2018), as freshwater ecosystems transition to seawater-exposed ecosystems and accelerate non-halophytic (e.g. non-mangrove) tree mortality (Lovelock et al. 2015, Kirwan and Gedan, 2019, Wang et al. 2019). Indeed, observations reveal that sea level driven land conversion creates widespread ‘ghost forest’ formation in North America (Kirwan and Gedan, 2019). Such mortality events can transform coastal land cover and affect coastal interface net primary production, ecohydrological cycling, and biodiversity (IPCC, 2019). However, our understanding of non-halophytic coastal forests’ physiological processes during seawater-induced tree mortality is limited, hindering our ability to model dynamic exchanges of energy and matter at much of the global coastal interface (Ward et al. 2020).

The physiological mechanisms of tree mortality have received significant attention in the past decade, with much research focused on drought-induced tree mortality (McDowell et al. 2011, Anderegg et al. 2016, Adams et al. 2017, Hartmann et al. 2018). This drought-related research has provided strong evidence for hydraulic failure leading to mortality, in which failure to meet water demand results in runaway embolism (Adams et al. 2017, Choat et al. 2018, Arend et al. 2021). Frequent observations of the occurrence of the carbon starvation process as evidenced by depleted carbohydrate reserves have also been observed, but complete carbohydrate exhaustion has never been reported during drought-induced mortality (Adams et al. 2017). However, drought and seawater exposure are very distinct drivers, and the mechanisms of tree mortality may differ.

Physiological mechanisms of seawater-induced tree mortality in mature non-halophytic trees have not been well tested compared to the extensive work that has been done to understand salinity impacts on plant growth in halophytes, crops, and non-halophyte seedlings (Snapp and Shennan, 1992, Kozlowski, 1997, Negrão et al. 2017, Munns et al. 2020). We can build upon this extensive literature to develop hypotheses regarding the mechanisms of mortality under seawater exposure (Kozlowski, 1997). In an increasingly saline environment, the osmotic imbalance between soil porewater and roots results in a decrease in the soil-to-root water potential gradient, thus constraining water uptake (Boursiac et al. 2005). The soil-to-root decline in conductance forces subsequent declines in hydraulic and stomatal conductance, with the latter exacerbated by the need to maintain foliar osmotic pressure (Conner and Askew, 1993, Ewers et al. 2004, Sutka et al. 2011, Stavridou et al. 2017). Salinity damage may also contribute to the accumulation of foliar ions to toxic concentrations, decreasing hydraulic function and carbohydrate storage (Munns and Tester, 2008, Negrão et al. 2017). Thus, it is expected that decreases in whole tree hydraulic conductance lead to subsequent reductions in photosynthesis and tree growth (Plaut et al. 2012, Hubbard et al. 2013), ultimately resulting in tree mortality.

Here, we examined the physiological process underlying tree death from seawater exposure, focusing on the key predictive mechanisms underlying the death process. We hypothesized that seawater exposure resulted in xylem anatomy changes (Acosta-Motos et al. 2017, Zhang et al. 2021a), and that these changes should lead to decreases in xylem hydraulic capacity, whole tree gas exchange, tree radial growth, and transpiration preceding mortality. We further predict that these processes lead to both hydraulic failure through increasing embolism, and carbon starvation through depletion of carbon reserves without replacement with new photosynthate. We tested our hypotheses in a mature Sitka-spruce (Picea sitchensis)-dominated floodplain as it transitioned from freshwater to seawater-dominated hydrology in a unique cause-and-effect, ecosystem-scale study of seawater impacts. We used wood anatomy traits, wood δ13C, and tree radial growth to retrospectively assess seawater effects on whole tree xylem hydraulic capacity, gas exchange, and radial growth since the culverts were removed. This allowed an annually resolved investigation into the long-term shifts in the trees’ hydraulic function foliar gas exchange upon exposure to seawater. During the latter stages of mortality (2018–2019), we directly measured metrics of water use and carbon metabolism across trees having crowns ranging from fully foliated to completely defoliated to investigate mortality processes at the sub-annual scale.

Materials and methods

Study area
The study site is located in the Pacific coast of Washington State, USA (46° 54’ 25.2’’ N, 123° 58’ 33.6’’ W), where the climate is dominated by warm, dry summers and cool, wet winters. Over the last three decades (1990–2019), mean annual temperature was 10.4 °C, with December the coldest month (5.7 °C) and August the warmest month (15.7 °C). The annual mean precipitation was 1,932 mm during the period 1990–2019. We performed the research in a mature Sitka-spruce (Picea sitchensis) forest growing on a floodplain along Beaver Creek, a first-order tidal creek that drains into the Johns River and subsequently Grays Harbor, Washington, USA. Sitka-spruce, also known as
tideland spruce and coast spruce, is the largest of the world’s spruces and is one of the most prominent forest trees in stands along the northwest coast of North America. It is usually distributed not far from tidewater, where moist maritime air and summer fogs contribute to maintain humid conditions necessary for growth. The age of the Sitka-spruce trees that we sampled was approximately 120 years old based on dendrochronology at breast height (~1.3 m). Until 2014, there were two culverts around the river mouth that prevented seawater inundation into the floodplain but did allow freshwater to drain out of Beaver Creek (WDFW, 2019). In November 2014, the culverts were removed by the state government to restore tidal influx further upstream to promote salmon spawning habitat. Thus, the previously freshwater environment was thereafter exposed to frequent seawater intrusion (Yabusaki et al 2020), which resulted in the death of ~75% of mature Sitka-spruce trees by July 2018 (Wang et al 2019). The soil salinity varied seasonally, with lower salinity occurring in winter and higher in summer. We monitored soil porewater salinity since 2018 with values ranging from 6.1 PSU (PSU-practical salinity unit) in May to 14.4 PSU in September (Yabusaki et al 2020).

**Experimental design**

Our aim was to understand the physiological mechanisms of seawater-induced tree mortality in this unique cause-and-effect ecosystem manipulation. We selected 28 trees throughout the floodplain by maximizing the range of crown foliar greenness (Gaylord et al 2013, Poyatos et al 2013), which we term the percentage live foliated crown (PLFC) in order to space the trees along a gradient of proximity to death. We monitored PLFC changes throughout the 2019 growing season. We used tree ring proxies (width, δ13C, and wood anatomy) to retrospectively assess how seawater exposure influenced tree radial growth, gas exchange and specific xylem hydraulic conductivity through comparing these ecophysiological processes before and after seawater exposure. We measured sap flow from 16 trees within our PLFC gradient to detect how seawater induced transpiration changes before tree mortality. Finally, we conducted measurements of non-structural carbohydrate (NSC) dynamics of stems using the approach of (Zhang et al 2021b) and percentage loss of conductance (PLC) via the approach of (Zhang et al 2021a) to assess the role of carbon starvation and hydraulic failure in the process of seawater-induced tree mortality.

Percentage of live foliated crown

The PLFC (%) of each tree was determined through visual estimation of how much green foliage was left in the branched canopy (e.g. not including the lower, unbranched and therefore unfoliated stem). We defined a tree as 100% PLFC if it had only green foliage throughout the branched length of the crown, and as 0% PLFC if the entire branched crown contained only brown (no green) foliage. This method of tree mortality measurement has been successfully used in previous studies (Galiano et al 2011, Anderegg et al 2012, Gaylord et al 2013, Poyatos et al 2013), though we acknowledge that visual estimation of PLFC risks subjectivity and may differ across observers and observation months. To make the PLFC estimation consistent, we used the same three observers on each date. All three observers examined each tree from all directions surrounding the tree to make independent PLFC estimates, and then we averaged the three measurements into one PLFC estimate for each tree and date. In total, we measured 28 mature Sitka-spruce trees during our six campaigns in 2019. There was a gradient of PLFC from 95% to 7% at our first campaign in March 2019. By October 2019, 12 of the 28 trees had reached 0% PLFC, while another four trees reached below 5%. In this study, we defined PLFC ≤5% as a dead tree.

**Modeled porewater salinity**

A three-dimensional variably saturated flow and salinity transport model calibrated and validated at Beaver Creek was used to estimate porewater salinity for each tree (Yabusaki et al 2020). The model utilizes the PFLOTRAN Richards equation simulator (Hammond et al 2014). We used continuous measurements (April 2018–April 2019) of pressure and electrical conductivity in the stream and floodplain groundwater to dynamically drive the floodplain water levels and salinities in the model. We repeated the one-year (April 2018 to April 2019) dataset to model the progressive and topographically controlled salinization of the floodplain subsurface from 2014 to July 2019. Time histories of pore water content and salinity at 3 m lateral and 0.1 m depth resolution were generated, and assessed for tree-specific locations. For each tree location, we selected output at 5 cm, 15 cm, 25 cm, 35 cm and 45 cm depths corresponding to the time of monthly maximum water level in the floodplain, and the immediately following local minimum (average 7.5 h later). The model validated well against the magnitude and timing of monitored water level and salinity dynamics observed at our site (Yabusaki et al 2020).
Sap flow measurement

We selected 16 trees across the PLFC gradient of 28 monitored trees to measure sap flow. In March 2019, 3.5 cm-long thermal dissipation sap flow probes were installed into the sapwood of each tree. We mounted the sensors with waterproof silicone and covered by reflective insulation to reduce the influence of the external environment. Each sensor was supplied with 0.2 W of constant power. Sapflow data was recorded every 30 min with a CR1000 data logger (Campbell Scientific Inc.). In this study, we used the sap flow data from March 22rd to October 30th 2019, including the whole growing season. During this time period, 8 of the 16 trees that were installed with sap flow sensors’ trees died.

Sap flux density \( J_s \) \( \text{L m}^{-2} \text{h}^{-1} \) was calculated according to the equation proposed by (Granier et al 1996):

\[
J_s = 4.28 \times \left( \frac{\Delta T_m}{\Delta T} - 1 \right)^{1.23}
\]

where \( \Delta T \) \( ^\circ \text{C} \) represents the temperature difference between the two probes. \( \Delta T_m \) \( ^\circ \text{C} \) represents the maximum \( \Delta T \) at nighttime. Data processing was conducted using the software Baseline 4.0 (Oishi et al 2016).

We used Baseline to automatically estimate baseline nighttime flow based on a joint set of conditions, including nighttime hours (characterized by near-zero radiation), stable temperature differential between probes (estimated using coefficient of variation) and low vapor pressure deficit (VPD, see Oishi et al 2016) for more details.

Tree ring growth and anatomy

From each of the 28 sample trees, we extracted two tree cores using increment corers from south-facing and east-facing aspects at ~1.3 m height in October 2019. Tree cores were mounted on a wooden stave, then air dried in the lab and sanded using increasingly higher grit sandpaper (from 160 to 400 grit size) until a clear view of the cells and annual ring growth boundary was apparent via microscope. We measured whole ring widths using a LINTAB 6 measuring system (http://www.rinntech.de) with a resolution of 0.01 mm. Ring width series were visually cross-dated using recognizable years and the COFECHA program (Holmes, 1983, Yamaguchi, 1991).

Tree radial growth was calculated from basal area increment \( BAI \) \( \text{cm}^2 \text{yr}^{-1} \), \( BAI = \pi(R_n^2 - R_{n-1}^2) \) of each core using the ‘dplR’ package version 3.6.1 in R (Bunn, 2008), where \( R_n \) is the radius of the cumulative ring width of \( n \)-th year of tree-ring formation and \( R_{n-1} \) is the radius of the cumulative ring width of \( n-1 \)-th year of tree-ring formation.

We selected the 16 trees that had installed sap flow sensors to measure wood anatomy from the collected tree cores. Using a rotary microtome (Leica RM 2245; Leica Camera AG, Germany), we cut 12 \( \mu \text{m} \)-thick cross-sections of each tree core. The cross-sections were stained with 1:1 safranin and astra blue solution, rinsed with ethanol solutions of increasing concentration and dried at 60 \( ^\circ \text{C} \) for 48 h. The slides were then scanned with a slide scanner (Zeiss Axioscan.Z1; Carl Zeiss AG, Germany). Tracheid lumen areas were measured in each annual ring (not separating early- and late-wood) over 2009–2018 with the open-source image processing software ImageJ (Schneider et al 2012). Mean tracheid lumen area and tracheid density (number of tracheids divided by the area per annual ring) were calculated per year.

We used lumen areas within the sapwood to estimate potential xylem hydraulic conductance according to Poiseuille’s law (McDowell et al 2002, Fonti et al 2010):

\[
K_s = \frac{\rho \pi \sum d^4}{128\eta A}
\]

where \( \rho \) is the density of water (998.2 kg m\(^{-3} \) at 20 \( ^\circ \text{C} \)); \( d \) is the vessel diameter of each vessel, which we assumed the vessels were circle and calculated from tracheid area of each vessel; \( \eta \) is the viscosity of water \( (1.002 \times 10^{-5} \text{MPa s}^{-1} \text{at } 20 \text{°C}) \); and \( A \) is the total area for tracheid measurement.

Tree-ring records of carbon isotope discrimination

Tree-ring \( \delta^{13} \text{C} \) results from the allocation of carbon to ring growth from the entire crown, weighted by the assimilation rates throughout the crown. For tree-ring \( \delta^{13} \text{C} \), we used data from our previous research at this site (Wang et al 2019). Briefly, we sampled 12 live and 12 dead trees in February 2018, and analyzed the annual whole-wood \( \delta^{13} \text{C} \) during the period 2009–2017. The \( \delta^{13} \text{C} \) values were measured using an elemental analyzer (ECS4010, Costech Analytical, Valencia, California) coupled with an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus XP, Thermo Electron, Bremen, Germany) at the Washington State University Stable Isotope Core Laboratory, Pullman, WA. The analytical error of the isotope measurements (SD) was less than 0.1‰.
Carbon isotope discrimination (\(\Delta\)) is expressed as the difference between the \(\delta^{13}C\) of atmospheric CO\(_2\) (\(\delta^{13}C_a\)) and measured tree-ring \(\delta^{13}C\) (Farquhar et al. 1989):

\[
\Delta = \frac{\delta^{13}C_a - \delta^{13}C}{1 + \delta^{13}C}
\]

The \(\delta^{13}C_a\) data between the period 2009 to 2017 were obtained from direct measurements at the Mauna Loa observatory (https://www.esrl.noaa.gov/gmd/ccgg/trends/data.html). \(\Delta\) is used to quantify whole leaf-scale gas exchange, which reflects the trade-off of photosynthesis rate and stomatal conductance (Farquhar et al. 1989).

Nonstructural carbohydrates

We collected stem core samples to 3 cm depth for nonstructural carbohydrate analyses (including soluble sugars and starch). Samples were collected using a 5.15 mm increment borer from the 28 sampling trees (the same trees used for ring width measurements). Samples were microwaved at 700 W for 90 s within 5 h after collection, and stored in a portable cooler between sampling and microwaving. Samples were then oven-dried at 65 °C for 72 h, and subsequently ground into a fine powder with a ball mill (SPEx SamplePrep 2010 Geno/Grinder, Metuchen, NJ, USA). NSC extraction and quantification was done following the protocols and enzymatic digestion method of (Landhäusser et al. 2018), with adaptations by (Zhang et al. 2021b) for low NSC concentrations. Soluble sugars i.e. glucose, fructose and sucrose were extracted with 80% ethanol at 90 °C, and then quantified by enzymatic assays photometrically in a 96-well microplate reader (ELx800UV, BioTek Instruments, Winooski, USA) at a wavelength of 340 nm. The ethanol-insoluble pellet was used to determine starch after ethanol evaporation. Starch in the pellet was hydrolyzed with \(\alpha\)-amylase and then digested to glucose via amyl glucosidase. Starch concentrations were calculated by multiplying a reference concentration of glucose by a conversion factor of 0.9. Total NSC was calculated as the sum of starch (% of dry matter) and soluble sugars (% of dry matter). For quality assurance, two inter-lab standards (peach leaves NIST1547 and pinon needles (Dickman et al. 2019)) and one synthetic standard (Landhäusser et al. 2018) were used in the analyses.

Percentage loss of conductance

We measured hydraulic conductivity and vulnerability curves from twelve individual trees in March 2019, while we could only get live branches from eight of the twelve trees in July 2019 due to the rapid loss of crown foliage. We collected long branches (~1.5 m) in the field and cut a stem segment~14 cm in length and 5~8 mm in diameter under water, then measured for native hydraulic conductivity \(K_{h,\text{n}}\) (kg m~\(^{-1}\) MPa~\(^{-1}\)) in the laboratory. \(K_h\) was calculated as:

\[
K_h = \frac{F \times L}{\Delta P}
\]

where \(F\) was the flow of water through the stem (F; kg s~\(^{-1}\)), \(L\) represents the stem length (m), and \(\Delta P\) represents the driving force (MPa). Hydraulic conductivity was also normalized by sapwood area \((A_{sw};\text{m}^2)\) to calculate sapwood-specific hydraulic conductivity \(K_s\) (kg m~\(^{-1}\) s~\(^{-1}\) MPa~\(^{-1}\)). Sapwood area was determined at both ends of the segment.

Maximum hydraulic conductivity \((K_{\text{max}})\) was measured after quantification of native \(K_h\). The segments were submerged in a degassed water solution (20 mM KCl) under vacuum infiltration overnight (>12 h) to remove emboli. \(K_{\text{max}}\) was measured as above for the \(K_h\) measurement. The degree of xylem embolism was estimated using \(K_h\) and \(K_{\text{max}}\) to calculate the native percentage loss of hydraulic conductivity (PLC). PLC was calculated as:

\[
\text{PLC} = 100 \times (1 - (K_h / K_{\text{max}}))
\]

Vulnerability to cavitation was measured using the bench dehydration method (Sperry et al. 1988, Cochard et al. 2010). We allowed the branch samples progressively dry on the bench as measurements were conducted. \(K_h\), \(K_{\text{max}}\) and water potential were measured during slow desiccation of the samples on the bench until the stem lost >90% conductivity or the water potential < −8 MPa. Four to six stem xylem segments were collected from each long branch per individuals. A reparametrized Weibull model was used to generate vulnerability curves by fitting water potential versus PLC data points (Neufeld et al. 1992, Ogle et al. 2009).

Meteorological data and statistical analyses

A weather station was installed in the study site to record half-hourly meteorological data, including air temperature, precipitation, relative humidity and photosynthetically active radiation etc. We used this
meteorological data for 2019 to correlate with sap flow data. In addition, the monthly climate data was extracted from the interpolated PRISM (4 km resolution) gridded climate dataset (Daly et al 2008).

We statistically analyzed significant differences in tree growth, $\Delta$ and $K_s$ before and after removal of culverts through one-way ANOVA test. Linear regression model was used to calculate the relationship of $\Delta$ and $K_s$ during the period 2009 to 2017. We also used non-linear regression models to quantify the relationships of tree growth to percentage of live foliated crown and NSC. All the uncertainties were calculated as (SE). Statistical analyses were performed in SPSS 26.0 (IBM Corporation).

Results and discussion

Google Earth imagery from pre and post culverts-removal in 2014 (imagery from 2013 and 2017, respectively) confirmed the widespread mortality of floodplain trees over the four years (figures 1(a), (b)). Other historical photos also show large-scale tree loss during this period (Figure S1 (available online at stacks.iop.org/ERC/4/035005/mmedia)). In 2019 alone, we directly observed mortality of 57% of our 28 sample trees. Tree loss was not due to drought as annual precipitation and VPD during 2015–2018 were near the long-term average values (Figure S2), and no tree mortality was observed at the upland forests neighboring the experimental floodplain. The PLFC was negatively correlated with exposure to modeled soil porewater salinity concentrations (Figure S3). No evidence of insect attack was observed on trees anywhere in the floodplain.

Whole tree hydraulic traits and growth were dramatically impacted by seawater exposure (figure 2). Mean tracheid lumen area and tracheid density per ring area decreased significantly in the years after the culverts were removed (figure 2(a)), which considerably reduced specific xylem hydraulic conductivity of the stem (figure 2(b)). $K_s$ declined by over 80% within three years after seawater exposure was initiated (figure 2(b)). BAI declined by 61.2% in 2015 following the culverts removal (figure 2(c)). Growth declined an additional 47.4% in 2016 compared to 2015 (figure 2(c)). Whole-crown $\Delta$ decreased 1.7‰ in 2015 compared to 2014, suggesting a large decline in stomatal conductance after seawater exposure (figure 2(c)). This large decline in $\Delta$ prior to death appears to be a consistent phenomenon at the regional scale across western Washington (Wang et al 2019). The correlation between $K_s$ and $\Delta$ (figure 3) is consistent with a putative mechanism of declining hydraulic transport capacity driving declines in stomatal conductance (Hubbard et al 2001).

![Figure 1. Floodplain tree mortality. (a) Google Earth image of our sampling site in May 2013, when the culverts let freshwater drain but prevented seawater invasion into the floodplain. (b) Google Earth image of the same place in June 2017, after culvert removal in November 2014. The sampling site is located along Beaver Creek, a first-order tributary of the Johns River that drains into the Grays Harbor estuary, approximately 1.5 km from the area, on the Pacific coast of Washington State, USA (46° 54′ 25.2″ N, 123° 58′ 33.6″ W).](image-url)
Sap flow-based transpiration measurements confirmed that dying Sitka-spruce trees indeed have very low hydraulic transport capacity (figure 4). Despite a PLFC range of 20 to 95%, nearly all sampled trees exhibited near-zero transpiration rates. Only the two trees with the highest PLFC (>80%) exhibited significant transpiration, which showed expected environmental responses to VPD and photosynthetically active radiation (Figure S4). Transpiration was nearly zero under PLFC <80% (figure 4). This extreme decline in transpiration...
suggests that even when trees still had a large fraction of foliage remaining in their crown, the supply of water was small, which should promote both hydraulic failure and carbon starvation. The low transpiration values are consistent with the dramatic decline in hydraulic capacity in the sapwood and associated decline in gas exchange (Zhang et al 2021a). Flooded coastal trees also exhibit reduced sapflow, both in mangroves (Krauss et al 2007) and non-halophytes (Krauss and Duberstein, 2010, Duberstein et al 2020), however, there are no prior studies documenting water use of coastal trees dying from inundation. Our near-zero sap attack-induced tree mortality seawater-induced tree mortality are similar to drought-induced tree mortality (Plaut et al 2012) and beetle attack-induced tree mortality (Hubbard et al 2013), where they also found near-zero sap flow in mature conifer forests before mortality. However, while seawater- and drought-induced mortality may share similar reductions in transpiration during the dying processes, these two drivers differ due to the causal agent (dry versus saline conditions), and the associated mechanisms that co-occur with declining transpiration, hydraulic conductance, and stomatal conductance. Photosynthetic capacity became particularly low (Li et al 2021), due in large part to ion toxicity impacts from elevated foliar sodium (Figure S5) (Munns, 2002, Negrão et al 2017). The low photosynthetic capacity has implications for our interpretation of the large decline in crown-scale $\Delta$ as a measure of declining stomatal conductance (Wang et al 2019). Low photosynthetic capacity confirms that the large decline in $\Delta$ was due to declining crown-scale stomatal conductance rather than due to increasing photosynthetic capacity. This is because $\Delta$ declines due to a reduction in the sub-stomatal concentration of CO₂, which can be driven by either declining stomatal conductance, or due to increased photosynthetic uptake (Farquhar et al 1989). Thus, hydraulic limitations at the stem-scale did transfer to declining gas exchange at the crown scale. Salinity constraints upon xylem hydraulic capacity, whole-tree gas exchange, and radial growth (Kozlowski, 1997, Robert et al 2009, Wang et al 2019) are underlying mechanisms within the mortality process at this site. The dying trees experienced increasing risk of hydraulic failure, indexed as PLC (Zhang et al 2021a). However, PLC rarely exceeded 60%, which is considered a lower threshold for increased risk of mortality from hydraulic failure (Adams et al 2017). Thus, while hydraulic limitations played a large role in the mortality process of these dying trees, hydraulic failure per se does not appear to be the ‘final-straw’ in death from seawater exposure in this ecosystem. However, declining PLC appeared to be an initiating mechanism that resulted in declining xylem hydraulic conductance and PLFC. Using measurements of PLC and PLFC from March and July 2019, we calculate the change in each over these four months (e.g. $\Delta$PLFC and $\Delta$PLC) to assess if the progression of crown loss was associated with a progression of PLC increase. Indeed, there was a strong relationship between $\Delta$PLFC and $\Delta$PLC (figure 5, $R^2 = 0.85, p = 0.01$), showing that crown loss was associated with increasing PLC. This is suggestive that hydraulic failure was likely involved in the mortality process by driving crown loss. Increasing PLFC as trees approached death, combine with the low photosynthetic capacity at the individual leaf-level (Li et al 2021), should result in a decline in photosyntheate production and hence growth and NSC storage. BAI declined strongly with declining PLFC ($R^2 = 0.77, p < 0.001$), such that annual growth approached zero at PLFC < 40% (figure 6(a)). Given that transpiration was near-zero for trees at this PLFC (figure 3), the low growth rates were likely driven by low carbon availability from both a lack of leaf specific gas exchange (as shown by the $\Delta$ results, figure 2(c)) and a reduction in total crown leaf area as PLFC declined (Zhang et al 2021b). In addition, we found that the correlation between BAI and NSC is consistent with the

**Figure 4.** Canopy greenness with relation to whole-tree transpiration. Mean midday sapflux density ($J_s$) across a gradient of percentage of live foliated crown (PLFC) in March 2019. Midday sapflux density was the average from 11 am to 2 pm each day and averaged from March to October 2019.
Regression of the differences between March and July percentage loss of conductance (PLC) versus PLFC. 
ΔPLFC = PLFC_{March} - PLFC_{July}; ΔPLC = PLC_{March} - PLC_{July}. This measures the change in embolism (PLC) as the foliated crown declines. To be exact, in figure 5 we sampled branches from eight trees for PLC measurement. Other trees had branches too high to reach or had totally died by July.

Figure 6. Tree growth relation to percentage of live foliated crown and NSC. (a) Basal area increment (BAI) in 2018 versus percentage of live foliated crown (PLFC) in March 2019. (b) Basal area increment (BAI) in 2018 versus stem non-structural carbohydrate concentration in March 2019. (c) The relationship of number of survival years with percentage of live foliated crown (PLFC). (d) The relationship of the number of survival years with stem nonstructural carbohydrate (NSC) concentration in March 2019. Survival years is the tree ring growth year since seawater exposure at 2015 (e.g. 2015 is the first survival year, 2016 is the second survival year, etc.).
interpretation that growth declined in concert with declining NSC ($R^2 = 0.55$, $p < 0.001$; figure 6(b)). Similarly, NSC declined strongly with PLFC such that NSC values were indistinguishable from 0% per dry weight in the final weeks of survival (Figure S6).

Tree mortality due to seawater exposure may have proceeded due to impacts of reduced hydraulic capacity as crown loss progressed. We calculated a metric of tree survival length from our larger sample population by counting the number of years (tree rings) grown since 2015, after the culverts were removed in November 2014. Trees that had zero growth and ≤5% PLFC were considered dead. The number of survival years was correlated with PLFC (figure 6(c), $R^2 = 0.67$, $p < 0.001$), suggesting the critical importance of maintaining a foliated crown to support survival. The number of survival years was also nonlinearly correlated with NSC (figure 6(d), $R^2 = 0.54$, $p < 0.001$), consistent with the hypothesis that carbohydrates are needed for survival. The particularly low NSC values associated with mortality (<1% total NSC, figure S6) suggests that carbon starvation may have been related to the mortality of these trees, as such low NSC values are rarely observed and are typically associated with death in drought-induced tree mortality studies (Marshall and Waring, 1986, McDowell, 2011, Dickman et al 2015, Hoch, 2015, Hartmann and Trumbore, 2016). While carbon starvation may happen above zero NSC concentrations due to required osmotic and metabolic processes for survival (Sala et al 2010, McDowell, 2011), the consistent measurement of NSC concentrations near zero in trees that died is confirmatory of carbon starvation occurring. These are potentially the lowest NSC values ever measured in dying trees (McDowell, 2011, Adams et al 2017).

To examine the relative dominance of hydraulic failure and carbon starvation during death in these Sitka spruce trees, we employed an integrated hydraulic-carbohydrate framework of (McDowell et al 2008) and (Adams et al 2017) (figure 7). In this framework, measurements of PLC are plotted against measurements of NSC. According to this analysis, most dying trees experienced particularly severe carbon starvation (e.g. a relatively large NSC decline) with relatively modest reductions in PLC (figure 7).

Our results suggest that declining hydraulic capacity, promoted a degree of hydraulic failure and associated carbon starvation through severe crown loss. This decline in NSC may also have been promoted by the (non-significant) decline in photosynthetic capacity with increasing crown loss. We also noted that the relationship of transpiration, NSC, tree growth, survival years and soil salinity response to PLFC decline was nonlinear which might suggest crown loss was rapidly increased due to the dysfunction of tree hydraulic and carbon uptake in tree mortality. Thus, it is important to identify the threshold of crown loss during the process-based tree mortality models. In this research, multiple interpretations deserve mention, as they can lead to improved understanding through future research. First, in this relatively wet location (nearly 2000 mm annual precipitation), extreme hydraulic failure (high PLC) may be unlikely. However, the correlation of increasing PLC with declining PLFC suggests that an increased presence of embolism within the hydraulic network was associated with crown loss. Second, the progression of events in this system may have started with root loss due to anoxia and salinity-induced declines in belowground conductance, along with cell-turgor limitations on the maximum size of tracheids during growth under saline conditions (Woodruff et al 2004, Munns et al 2020).
Detailed assessment of rooting dynamics and belowground conductance are needed to test this hypothesis. Furthermore, the mechanisms of ion toxicity to the photosynthetic machinery is poorly quantified in conifers, and is thus an additional research target for ecosystems such as the Sitka spruce forest we studied. Ultimately, all of these ‘upstream’ mechanisms led to the loss of hydraulic capacity, subsequent reductions in crown-gas exchange through stomatal closure and crown loss, and subsequently, NSC declined to fatal levels. Thus, reduced hydraulic capacity likely drove a degree of hydraulic failure and a subsequent extreme degree of carbon starvation as trees died due to seawater exposure.

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Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Author contributions

N G M, W W, N D W, and V L B, conceived the ideas and designed research; W W, P Z, H Z, S C P, M J N, W L, A L P, R L, N D W, and N G M conducted the field work; W W, C G performed the laboratory work and analyzed the data; W W and N G M led the writing of the manuscript; all the authors reviewed and edited versions of the paper and gave final approval for publication.

Competing interest statement

The authors declare no competing interest.

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