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# From salmon to salmonberry: The effects of salmon-derived nutrients on the stomatal density of leaves of the nitriphilic shrub *Rubus spectabilis*

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## Abstract

1. Nutrients derived from the carcasses of Pacific salmon have been shown to have wide-ranging effects on riparian systems. These include changes in community species composition and an increase in leaf nitrogen concentration, with the latter effect pronounced in the nitriphilic shrub *Rubus spectabilis* (salmonberry). Experimental work with other species has shown that leaf stomatal density increases in response to nitrogen fertilization.
2. We predicted that the stomatal density of salmonberry leaves would vary directly with the density of spawning salmon in salmonberry leaves collected from 16 streams in the vicinity of Bella Bella, on British Columbia's central coast.
3. We estimated the stomatal density along each stream, and quantified stream characteristics, including the number of spawning salmon (*Oncorhynchus* spp.), canopy cover, stem density and soil moisture.
4. We found that salmon have both direct and indirect effects on stomatal density, the latter mediated by canopy cover and stem density. Salmonberry stomatal density increased by 1.12 stomata per mm<sup>2</sup> (~0.5%) for every kg of salmon per metre of stream. Over the range of salmon densities observed (1.8–49.0 kg per metre of stream), stomatal density increased by almost 45 mm<sup>-2</sup>, or more than 20%.
5. These data confirm that the stomatal density in salmonberry responds positively to the opportunity for greater productivity provided by salmon carcasses. The data provide insight into the physiological and morphological processes supporting nitrogen uptake, which in turn influences plant community composition.

## KEYWORDS

Great Bear Rainforest, nutrient subsidy, *Rubus spectabilis*, salmonberry, salmon-derived nutrients, stomata, stomatal density

## 1 | INTRODUCTION

Nutrient subsidies can have profound effects on ecosystem processes (Anderson & Polis, 1999). Examples of subsidies include guano from seabirds (Anderson & Polis, 1999; Cocks, Balfour, & Stock, 1998), aquatic

insects (Bultman, Hoekman, Dreyer, & Gratton, 2014; Hoekman, Dreyer, Jackson, Townsend, & Gratton, 2011), and reef-derived kelp (Hyndes, Lavery, & Doropoulos, 2012). In each case, this nutrient source is deposited into a different, nearby ecosystem where it alters the quality, abundance, or composition of primary or secondary producers.

Pacific salmon deliver an enormous nutrient addition from the sea to coastal forests of western North America (Mathewson, Hocking, & Reimchen, 2003), and provide a well-studied ecosystem subsidy. Anadromous Pacific salmon are born in freshwater, but acquire 95% or more of their body mass at sea, before returning to natal streams where they spawn and die (Gende, Edwards, Willson, & Wipfli, 2002; Moore et al., 2007; Reimchen, 2000). Salmon carcasses wash up along the banks of spawning streams, and are deposited inland by scavengers and by floods (Ben-David, Hanley, & Schell, 1998). Predators such as bears and wolves also catch live salmon and transport them into the adjacent forests for consumption (Ben-David et al., 1998; Mathewson et al., 2003; Reimchen, Mathewson, Hocking, & Moran, 2003; Traveset, Bermejo, & Willson, 2001). The carcasses as well as the excrement of predators and scavengers enriches soil with marine-derived nutrients (Drake, Smith, & Naiman, 2005), which are subsequently distributed throughout food webs (Bartz & Naiman, 2005; Bates, Mächler, Bolker, & Walker, 2015; Bilby, Beach, Fransen, Walter, & Bisson, 2003; Helfield & Naiman, 2001; Hocking & Reynolds, 2011, 2012; Reimchen et al., 2003). Hocking and Reynolds (2011) demonstrated a variety of effects of salmon-derived nutrients in these riparian systems, and also showed experimentally (Hocking & Reynolds, 2012) that salmon carcasses provide nutrients that elevate the nitrogen content of some riparian plants.

Nutrient and mineral availability has many effects on the physiology and morphology of plants. In this paper, we focus on one such effect, namely the density of stomata in the leaves. The carbon dioxide required for photosynthesis is taken up through stomata, while minerals and nutrients acquired in the root system are distributed by the vascular system via capillary action as water is lost through stomata by transpiration (Carlson, Holsinger, & Prunier, 2011; Shabala, 2013). Stomatal density is positively correlated with soil fertility (Frey, Scheidegger, Gunthardt-Goerg, & Matyssek, 1996; Körner, Bannister, & Mark, 1986; Siegwolf et al., 2001), and with light intensity (Pazourek, 1970; Sáez, Bravo, Latsague, Sánchez, & Ríos, 2012), probably because CO<sub>2</sub> uptake rather than light or nutrient availability limits growth. Stomatal density thus mediates a trade-off between carbon gain and water loss, and plants adjust stomatal density and aperture (Manzoni, Vico, Palmroth, Porporato, & Katul, 2013) in response to the availability of CO<sub>2</sub>, nutrients, light and water—all of which affect the balance between allowable water loss and potential photosynthetic gain (Abrams, 1990; Kolodziejek & Michlewska, 2015; Manzoni et al., 2013; Xu & Zhou, 2008).

In many species, stomatal density has fallen over the past century, as expected due to the increasing partial pressure of CO<sub>2</sub> in the atmosphere (Beerling & Kelly, 1997; Frey et al., 1996; Luomala, Laitinen, Sutinen, Kellomäki, & Vapaavuori, 2005; Mao, Wang, Wang, & Voronin, 2005; Morison, 2001; Woodward, 1987). A decline in stomatal density allows plants to reduce water loss while maintaining photosynthetic performance (Liao, Chang, & Wand, 2005; Rico, Pittermann, Polley, Aspinwall, & Fay, 2013; Schuepp, 1993). Longer records demonstrate changes in stomatal density in step with atmospheric CO<sub>2</sub> over historical time (Beerling & Chaloner, 1993) and even over the recent glacial–interglacial cycle (Van de Water et al., 1994).

Experimental studies (Beerling & Chaloner, 1992) and altitudinal comparisons show that stomatal density can increase under reduced CO<sub>2</sub> availability, as in plants at high elevations compared to lower-elevation conspecifics (Kouwenberg, Kürschner, & McElwain, 2007).

These changes in stomatal density are likely composed both of microevolutionary change as a result of natural selection, as well as of evolved phenotypic plasticity in response to local conditions. Some plant species exhibit a high degree of phenotypic plasticity in stomatal density (Nicotra et al., 2010), as well as other traits. Most stomata are initiated early in leaf development (Fanourakis, Heuvelink, & Carvalho, 2015; Sun, Yan, Cui, & Liu, 2014). Plants adjust to conditions with more light or nutrients by producing leaves with elevated stomatal density (Broadley, Escobar-Gutiérrez, Burns, & Burns, 2001; Evans & Seemann, 1989) to acquire more CO<sub>2</sub> (Hepworth, Doheny-Adams, Hunt, Cameron, & Gray, 2015; Uscola, Villar-Salvador, Oliet, & Warren, 2014), so increasing photosynthetic potential.

Salmonberry (*Rubus spectabilis*) is a common shrub in coastal forests of the Pacific northwest. This nitrophilic plant seems especially well-adapted to exploiting salmon-derived nutrients (Hobbie, Macko, & Williams, 2000) as its abundance and foliar nitrogen are both elevated along streams with larger spawning runs of salmon (Hocking & Reynolds, 2011). Here, we report a study of salmonberry plants alongside 16 streams in the Great Bear Rainforest on the central coast of British Columbia, Canada. These streams support a wide range of densities of naturally spawning pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon. We predict a positive correlation between the density of salmon and the stomatal density of salmonberry. We test for an effect of soil moisture, but as our sites are all riparian systems in a wet temperate forest we do not expect water to be limiting and that any effect would be weak. Finally, as salmon-derived nutrients can lead to alterations in the structure of riparian vegetation (Hocking & Reynolds, 2011) that affect the light level, and as stomatal density changes positively with light level, we predict an effect on stomatal density by this indirect pathway.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

Between September 15 and October 22, 2016, we visited 16 streams in the Heiltsuk First Nation territory around Bella Bella, British Columbia, Canada. The streams are located in the Coastal Western Red Cedar–Hemlock Biogeoclimatic Zone, with high precipitation (>3,300 mm/year; Pojar, Klinka, & Meidinger, 1987), and are accessible only by boat. This region is remote, and the study streams and adjacent riparian zones have minimal recent impact by humans. All streams are nutrient poor (Reimchen et al., 2003), surrounded by low productivity forests, and all except one (Lee Creek) are precipitation driven. Some are narrow, with steep and high banks, while others have wide riparian areas. Stream characteristics are summarized in Table 1.

**TABLE 1** Characteristics of the 16 study streams near Bella Bella, British Columbia, Canada

STREAM	Stomatal density ( $\text{mm}^{-2}$ )	St. Dev. stomatal ( $\text{mm}^{-2}$ )	Chum biomass (kg)	Pink biomass (kg)	Salmon density ( $\text{kg}/\text{m}$ )	Canopy cover (%)	Stem density (1.5 m radius)	Soil moisture (%)	Spawning reach (length, m)	Stream width (m)	Leaf area ( $\text{cm}^2$ )
Ada	222.8	45.8	2,145	155.3	19.5	71	15.2	35.6	350	11.1	28.7
Beales Left	236.1	61.8	399.7	1,443.7	11.7	66	40.2	39.7	235	10.9	31.9
Beales Right	203	57.9	70.7	133	1.8	65	26.5	29	200	9.6	32.9
Bullock Main	227.2	44.8	4,026.3	2,524.3	28	59	31.1	36	540	10.9	32.3
Clatse	232.4	32.1	3,888.3	27,500	49	63	40	25.8	835	22.8	35.3
Fancy Right	207.8	44.7	703.3	69	6.9	63	20.7	40.5	325	4.8	26.3
Fannie	195.7	44.1	6,269.7	8,068.7	20.3	71	17.1	30.3	1,375	12.8	22.8
Fell	197.3	53	136	63	2.6	64	10.8	31.5	190	10.9	31.6
Goatbushu	199.6	43.4	161.3	1,131.3	7	71	39.9	28.4	240	7.5	26.2
Hooknose	206	48.8	1889	3,791.7	5.5	70	19.1	22.3	1800	16.9	22.3
Jane	165.9	36.2	3.7	0	0	68	15.9	51.5	300	4.6	33
Kill	246.1	68.9	1831.3	315	14.3	66	32.3	59.1	420	3.5	32.7
Kunsoot	229.6	62.9	1998.7	13,173.3	15.6	56	29.3	35.2	1,280	13.1	25.9
Lee	280	66.8	5,766.7	365.7	22.8	49	26.3	22.3	800	12.4	34.4
Troupe South	195.9	47.3	36.7	35.7	0.8	59	7.5	43.7	195	4.1	32.1
Troupe North	189.8	42.9	3.7	46	0.3	65	5.3	30.5	18	4.4	23.4

## 2.2 | Salmon density

Chum and pink salmon in these streams have been counted in every year since 2007 by JDR's research group, in conjunction with personnel from Fisheries and Oceans Canada as well as fisheries biologists from the Heiltsuk First Nation. To make a count, each stream was traversed from the estuary mouth up to a distinct barrier (e.g., waterfall), a lake, or until salmon were no longer observed. Streams were visited up to three times in a season. In those visited three times, the "area-under-the-curve" method was used to estimate the total number of salmon that returned (Hocking & Reynolds, 2011). When only one or two counts were available, the "Peak Live +Dead" method was used (Hocking & Reynolds, 2011). The methods give similar estimates (Hocking & Reynolds, 2011). Annual returns varied from 0 to 31,388 fish per stream. To estimate salmon nutrient input, we used the method developed by Hocking and Reynolds (2011). We averaged for each stream the number of salmon over the years 2012–2014, and multiplied by the average weight of each species (Chum 3.1 kg, Pink 1.05 kg). This total was divided by the length of the spawning area to derive an estimate of the salmon density (kg salmon/m).

## 2.3 | Salmonberry sampling

On each stream, we collected two leaves from each of 15 salmonberry plants. Both leaves originated from the same stem. Plants were selected opportunistically along both banks along the length of the spawning reach (range 100–1800 m) within 3 m of the stream bank, though we avoided plants that had their roots in the stream. We collected healthy, fully expanded mature leaves. As there is likely intra-plant variation in stomatal density depending on height and leaf location leaves from each plant were collected from the same position within a plant (chest height, outer stem). Salmonberry is clonal, with clones in coniferous forests similar to ours typically <5 m in diameter (Tappeiner, Zasada, Ryan, & Newton, 1991). To ensure that leaves were collected from different clones, sample sites were separated by at least 10 m. Each leaf was pressed and dried for at least 3 days before storage.

We measured the soil volumetric moisture at the base of each stem using a soil moisture metre with a 12 cm probe (HydroSense CD620; Campbell Scientific Inc.). We estimated plant density as the number of salmonberry stems within a radius of 1.5 m around this point. Tappeiner et al. (1991) found two to six clones on 4 m<sup>2</sup> plots, so more than one clone may have been involved in the counting of the stems. We took a canopy measure (% cover) at each collection site, using a densiometer on which 42 points were selected, and converted this to percentage cover.

## 2.4 | Stomatal density measurement

Stomatal density of each leaf was measured using the "nail varnish impression" method described by Van Den Dries, Gianni, Czerednik, Krens, and Klerk (2013), Geisler, Nadeau, and Sack (2000), and Kolodziejek and Michlewska (2015). As there may be intra-leaf variation in *SD*, the position of the impression on each leaf was standardized. Clear nail varnish was applied to the bottom (abaxial) side of a leaf not more than 1 cm from the midvein, dried for at least 20 min, and removed using clear tape. The impression was made at the widest portion on the middle of the three leaflets, between the second and fifth lateral secondary veins on each side of and directly next to the main central vein. Photographs of the impressions were taken with a Canon 5D Mark II camera mounted on a Nikon Eclipse 600 microscope equipped with a Nikon plan fluor 20× objective and a Nikon 2.5× phototube lens using brightfield illumination, recording an image area of 0.1944 mm<sup>2</sup>. Multiple focal planes were photographed to generate focused images of the majority of stomata. Images of focal planes were imported into Adobe Photoshop CC, and a black circle was placed on each stoma with each circle on a separate layer. The number of layers with circles represents the number of stomata.

## 2.5 | Statistics

The stomatal density estimate for each stream was made as follows: We made a total of 960 impressions (16 streams, 15 plants per stream, 2 leaves per plant, and 2 impressions per leaf). Repeat

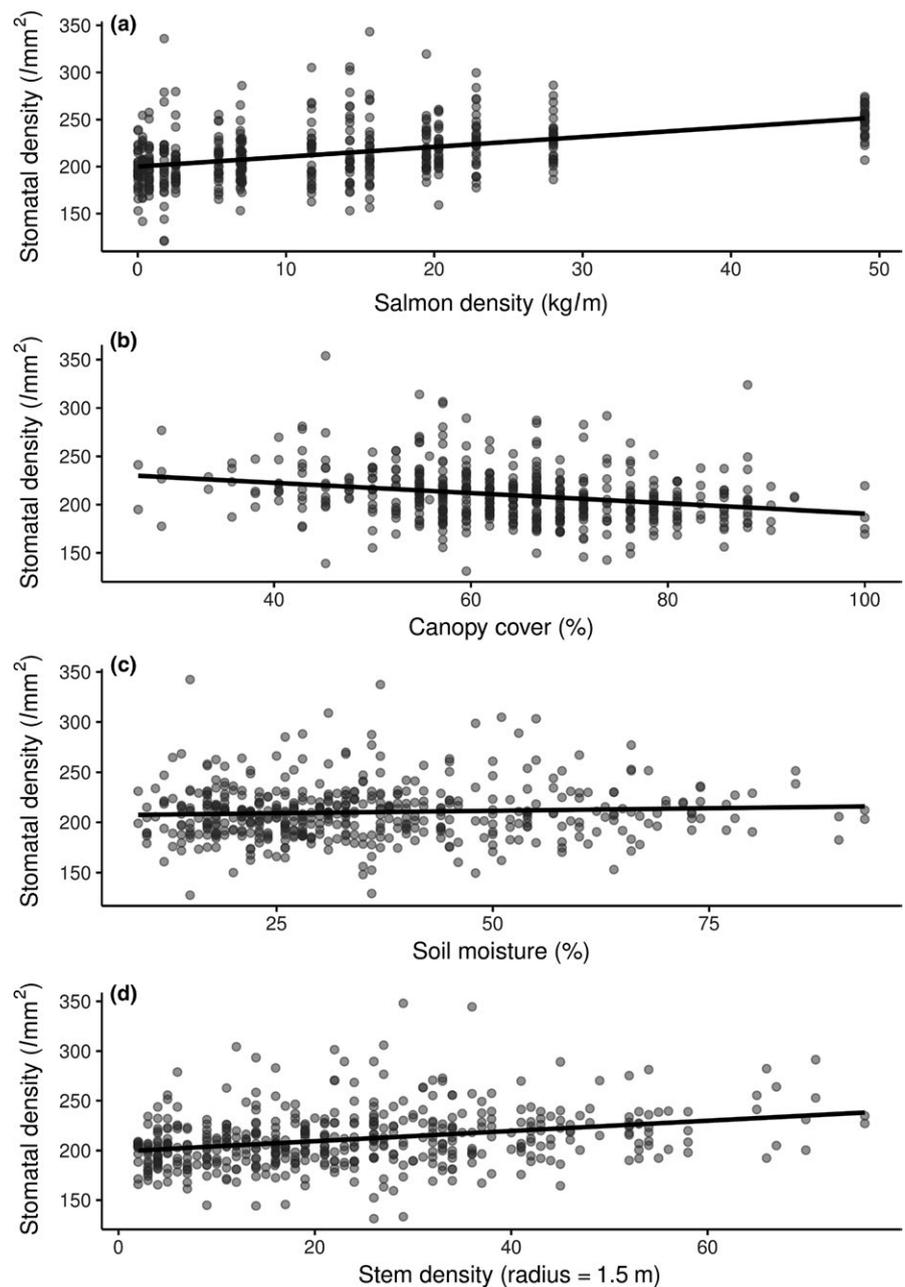
**TABLE 2** Correlation matrix (Pearson's *r* values) between the variables measured on the 16 study streams. Significant correlations in boldface ( $p < 0.05$ )

	Stomatal density	Salmon density	Canopy cover	Stem density	Leaf area	Soil moisture	Distance upstream
Distance from bank	-0.019	0.046	0.063	0.014	-0.026	0.003	-0.074
Distance upstream	0.104	0.158	0.010	0.026	0.029	-0.239	
Soil moisture	0.008	-0.142	-0.052	-0.045	0.036		
Leaf area	0.079	0.118	-0.076	0.047			
Stem density	0.250	0.372	-0.069				
Canopy cover	-0.225	-0.106					
Salmon density	0.295						

counts of the same impression were highly correlated ( $n = 960$  pairs,  $r = 0.816$ ,  $p < 0.000$ ) and were averaged. Stomatal densities of the matched impressions on each side of the central vein of each leaf were also highly correlated ( $n = 480$  pairs,  $r = 0.841$ ,  $p < 0.000$ ) and averaged to give an estimate for that leaf. The estimates of the two leaves per plant were highly correlated ( $n = 240$  pairs,  $r = 0.604$ ,  $p < 0.000$ ). We used a linear mixed model to examine the relationship between stomatal density and salmon biomass for each stream, controlling for canopy cover, stem density, soil moisture, leaf area, the distance upstream of stream mouth and the distance from stream bed. We included leaf area (measured using ImageJ) as there is some evidence that leaf size affects stomatal density (England & Attiwill, 2011; Xu & Zhou, 2008). To represent our sampling structure, we used a random intercept model, with plant nested within stream as

a random effect to correct for spatial dependency and the sampling of two leaves per plant (package nlme; (Pinheiro, Bates, DebRoy, & Core Team, 2015; RStudio Team, 2015). No variables were strongly correlated (all  $r < 0.4$ ; see Table 2), so all variables as well as all two-way interactions were included in the initial model. We sequentially removed first unsupported interactions and then unsupported main effects. Significance was assessed using likelihood ratio tests. Order effects were assessed by back-checking the significance of all terms dropped from the model.

We used a piecewise structural equation model (SEM; Lefcheck, 2015) to investigate relationships between the variables linking salmon and stomatal density. SEMs are a form of path analysis, with every path representing a hypothesized causal relationship (Pearl, 2011). The path coefficient measures the strength of the relationship



**FIGURE 1** Stomatal density in salmonberry leaves in relation to environmental characteristics. Measures were made on 16 streams, with 15 plants per stream (average of two leaves per plant) near Bella Bella, British Columbia. Panel (a) salmon density; (b) canopy cover; (c) soil moisture; and (d) stem density. In each case, the line is the prediction of a linear mixed effects model, corrected for the random effect of stream and plant nested in stream and for the other variables and interactions in the model. Points are corrected for the partial residuals. Overlapping points indicated by darker shading. Statistics are summarized in Table 3

**TABLE 3** Summary of the final mixed model. Not shown are the dropped main effects (leaf area, distance from stream bed, distance upstream of stream mouth), and the 19 non-significant pairwise interactions. Likelihood ratio tests for salmon density, soil moisture and stem density alone were not conducted (indicated N/A), as these effects depend on significant interactions

Parameter	Estimate	Loglikelihood-ratio	<i>p</i>
Intercept	20,643.56	60.135	0.000
Salmon density	-27.94	N/A	
Canopy	-5,315.72	6.824	0.009
Soil moisture	54.18	N/A	
Stem density	176.20	N/A	
Salmon density:soil moisture	4.28	5.723	0.017
Stem density:soil moisture	-4.02	11.557	0.000

between two variables, while keeping the other variables constant. To compare path coefficients, explanatory variables are standardized to a mean of zero and a standard deviation of 1. The path coefficients for indirect relationships are calculated as the product of the individual path coefficients.

In the structural network, salmon density, canopy cover, stem density, leaf area, soil moisture and physical location are all hypothesized to have direct relationships with salmonberry stomatal density. Salmon density is also hypothesized to have indirect effects on stomatal density, by influencing stem density, canopy cover and leaf area, each of which may, in turn, affect salmonberry stomatal density.

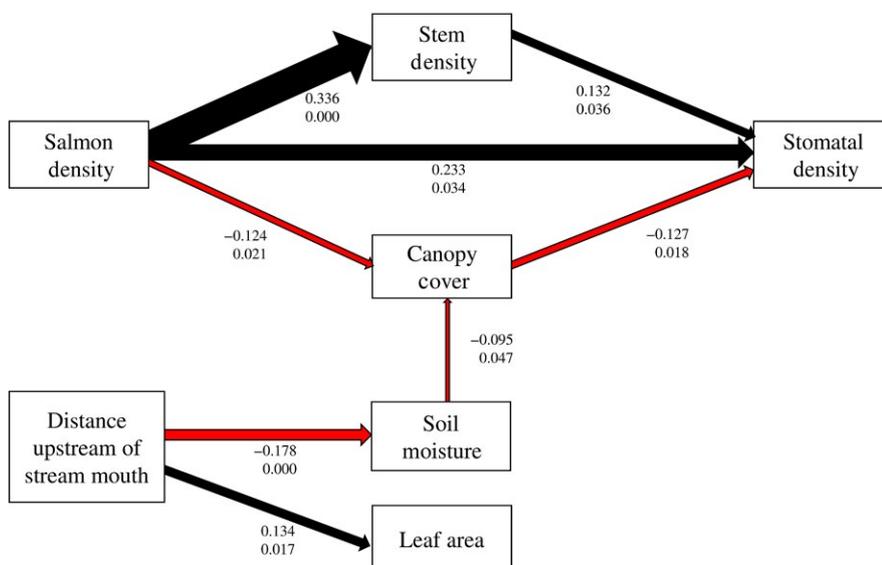
### 3 | RESULTS

Salmon densities varied between 0 and 49 kg salmon/m of spawning reach length (overall mean = 12.9 kg/m, *SD* = 12.7;

see Table 1). Soil volumetric moisture ranged from 9%–93% (mean = 35.1%, *SD* = 17.5), with stem density ranging from 2 to 76 stems (mean = 23.6, *SD* = 16.2). Canopy cover ranged from 26.2%–100% (mean = 63.9%, *SD* = 13.5). Basic correlations between stomatal density and the predicted main effects are summarized in Table 2 and displayed in Figure 1a–d. The final linear mixed model is summarized in Table 3. As predicted, stomatal density varies with salmon density (see Figure 1a), but the effect depends on soil moisture. At low soil moisture (15%), the main effect plus interaction is equal to  $-27.94 + (4.28 \times 15) = +36$ , whereas at high soil moisture content (90%) the combined effects are equal to +357.

The model also shows that stomatal density decreases with canopy cover (Figure 1b), which is as expected given that stomatal density increases as light intensity increases. Stomatal density varies with stem density (Figure 1d), an effect dependent on soil moisture. These results show that salmon density has an overall positive effect on stomatal density, with the strength of the relationship increasing with soil moisture. The final model shows a strong fit to the data ( $r^2 = 0.77$ ), predicting that stomatal density increases from ~200 to 250  $\text{mm}^{-2}$  across the range of salmon densities among streams, after controlling for the other variables. Stomatal density increases by 1.12 stomata/ $\text{mm}^2$  with each additional kilogram of salmon per metre of stream.

The reduced structural model is portrayed in Figure 2. (See Supporting information Table S1 for full details of the structural equation model.) The conditional  $r^2$  for stomatal density (including both direct and indirect effects) is 0.61. This analysis confirms that stomatal density is strongly affected by salmon density (standardized path coefficient (SPC) = 0.233). Further, stomatal density is directly affected by canopy cover (SPC = -0.128) and stem density (SPC = 0.132). The model also indicates indirect effects of salmon on stomatal density via stem density (indirect SPC = 0.044) and via canopy cover (indirect SPC = 0.019). There is a weak indirect effect of the distance from the stream mouth on stomatal density via soil moisture and canopy cover (indirect SPC = 0.002).



**FIGURE 2** Representation of the reduced structural equation model (SEM). The thickness of each arrow represents the strength of the path coefficient. Red arrows represent negative relationships, black arrows positive. The top value represents the standardized path coefficient and the lower the significance level. Path coefficients are estimated keeping the other variables constant, and taking into account random effects of stream and plant nested in stream

## 4 | DISCUSSION

Plant stomatal density is known to vary in response to the availability of nutrients, light and water. These responses are interpreted as mechanisms whereby a plant adjusts the balance between carbon gain and water loss (required for nutrient transport) to support photosynthetic potential. We predicted that the stomatal density of salmonberry leaves collected along streams in British Columbia's Great Bear Rainforest would vary directly with the number of salmon spawning in the stream, reasoning that the abundant water in these habitats allows stomatal density to be elevated in order to capture the nutrients delivered in salmon carcasses. Our comparison of 16 streams confirmed this prediction. However, the fact that soil moisture alters the relationship between salmon and stomatal density suggests that although abundant, water is not unlimited, and salmonberry seems better able to take advantage of additional nutrients when water is more readily available.

The structural equation model supported the idea that the increase in stomatal density is composed of both a direct as well one or more indirect responses to nutrient enrichment, the latter arising via the effect of nutrient enrichment on canopy cover. Based on comparisons of the total path coefficients, the direct effect of salmon density on stomatal density is 5–100 times stronger than any indirect effect. Effects of soil moisture, leaf area and distances from stream and estuary on salmonberry stomatal density are not detectable using our data. The model also suggests that canopy cover is negatively associated with salmon density. This may be due to associations between canopy cover, salmon density and other variables such as stream width (wider streams have lower canopy cover).

Pacific salmon provide sizeable nutrient subsidies to otherwise nutrient-poor coastal forests (e.g., Moore et al., 2007). We found that the nutrient enrichment effect was substantial, increasing stomatal density by 1.12 mm<sup>-2</sup> per kilogram of salmon per metre of the stream. Over the range of salmon densities in the 16 streams (1.8–49.0 kg per metre of stream), stomatal density was increased by almost 45 mm<sup>-2</sup>, or more than 20%. Experimental studies confirm that stomatal densities are sensitive to nitrogen availability, at least in some species. For well-watered *Solanum tuberosum* L., for example, Yan, Sun, Song, and Liu (2012) reported an increase of 30 stomata/mm<sup>2</sup> when nitrogen in the soil was experimentally increased from 70 mg to 125 mg N (per kg soil), a response as large as we found in our study. There is no indication of an asymptote in our measures, consistent with the notion that these riparian systems are nutrient poor, even in high salmon density streams.

Higher light increases photosynthetic potential (Sáez et al., 2012), and studies show that light intensity and stomatal density are positively related across a broad range (Kong et al., 2016; Mazzanatti et al., 2016; Pazourek, 1970; Petrova, 2012; Rozendaal, Hurtado, & Poorter, 2006). Conversely, the reduced light intensity under a denser canopy should lead to lower stomatal density, as found by this study.

Stem density and salmon density are strongly and positively related (Figure 1d; Pearson's  $r = 0.372$ ), an effect also found by

Hocking and Reynolds (2011). This and other aspects of the performance of salmonberry in response to salmon nutrient subsidies led Hocking and Reynolds (2011) to confirm that salmonberry is a nitrophilic species. In conclusion, we have found that the nutrients derived from salmon carcasses lead to an increase in stomatal density of salmonberry both directly and via indirect pathways. This provides insight into the way plants use nitrogen, including physiological processes that lead to correlations between salmon and riparian plant composition.

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## AUTHORS' CONTRIBUTIONS

R.C.Y., H.H.T.P. and J.D.R. conceived the study. G.G.T. collected the data and processed the samples. J.M. made possible and oversaw the microscopy. G.G.T., J.D.R. and D.J.G. involved in the data analysis and statistics. G.G.T., R.C.Y. and J.D.R. wrote manuscript, with input from all co-authors.

## DATA ACCESSIBILITY

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.63sj6f0> (van den Top et al., 2018).

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## SUPPORTING INFORMATION

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