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Vascular optimality dictates plant morphology away from Leonardo's rule

S. B. D. Sopp a,1,2 and R. Valbuena b,a,1,2

^aSchool of natural sciences, Bangor University, Bangor, LL57 2DG, UK; ^bSwedish University of Agricultural Sciences, SLU Skogsmarksgränd 17, SE-901 83 Umeå, Sweden

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Metabolic Scaling Theory (MST) provides an understanding of scaling 1 in organismal morphology. Novel empirical data on the apparently uni-2 versal pattern of tip-to-base conduit widening across vascular plants 3 motivate a set of generalized MST (gMST) relationships allowing for variable rates of conduit coalescence and taper and a transition be-5 tween transport and diffusive domains. Our model, with coalescence 6 limited to the distalmost part of the conductive system, reconciles 7 previous MST-based models and extends their applicability to the 8 entire plant. We derive an inverse relationship between stem volume taper and conduit widening, which implies that plant morphology is 10 dictated by vascular optimality and not the assumption of constant 11 sapwood area across all branching levels, contradicting Leonardo's 12 rule. Thus, energy efficiency controls conduit coalescence rate, low-13 ering the carbon cost needed to sustain the vascular network. Our 14 model shows that as a plant grows taller, it must increase conduit 15 widening and coalescence, which may make it more vulnerable to 16 drought. We calculated how our gMST model implies a lower carbon 17 cost to sustain a similar network compared to previous MST-based 18 models. We also show that gMST predicts the cross sectional area 19 of vessels and their frequency along the relative length better than 20 previous MST models for a range of plant types. We encourage fur-21 ther research obtaining data that would allow testing other gMST 22 predictions that remain unconfirmed empirically, such as conduit 23 coalescence rate in stems. The premise of energy efficiency can 24 potentially become instrumental to our understanding of plant carbon 25 allocation. 26

Metabolic scaling theory | Plant science | Ecology

he exact nature of biological scaling relationships has 1 been argued about for centuries, typically cited as begin-2 ning with Leonardo da Vinci who first proposed that total 3 sapwood area is constant at all levels of branching, such that 4 the summed areas of sapwood in the terminal twigs is the 5 same as the sapwood area at the base. Many biological models 6 have since taken inspiration from Leonardo's rule to model both plant exterior branching networks and their vascular sys-8 tems, despite there being little evidence of the rule occurring 9 consistently (1). Metabolic Scaling Theory (MST) (2-4) is the 10 leading theory of explanation on organism form, utilising prin-11 ciples of area preservation for both the external and vascular 12 branching networks within plants. 13

MST originally modelled the plant vascular system as a 14 Widened Pipe Model (WPM), wherein conduits widen from 15 tip to base (2, 3, 5) (Fig. 1A). A decade later MST adopted a 16 coalescing vascular model (4) which allows conduits to merge 17 along the conduit path from plant tip to base, to maintain con-18 ductive area fraction (Fig. 1B). Recent empirical observations 19 (5-8) suggest a transition between the vascular models of West 20 et al. (2) and Savage et al. (4). Rosell & Olson (8) detail how 21 distinct transport and diffusive domains characterise plant 22

vascular systems with a widened pipe model along the stem 23 and a coalescing, possibly area preserving model, at the end 24 of the network (Fig. 1C). These domains were proposed after 25 the findings of Lechthaler et al. in leaves (7) which is in line 26 with the wider literature (2, 4, 9-11) and further supported 27 within stems by Koçillari *et al.* who modelled conduit shape 28 through the Pareto front of trade-off between carbon cost and 29 hydraulic conductance (6). To date the WPM is the leading 30 theory explaining the shape of the vascular system (6). The 31 empirical observation that conduit widening tip to base and 32 conduit coalescence rates are variable along the network open 33 up an avenue of investigation for the development of MST. 34

In this contribution, we deduce a MST-based model stem-35 ming from an energy efficiency premise alone, with a con-36 sequential reduction in total volume (Fig. 1D). Ours is a 37 generalised MST model because it incorporates variable rates 38 of conduit widening and coalescence, allowing predictions from 39 the leaves in addition to stems. We investigate the implications 40 of a vascular model of transitional functionality, from a trans-41 port (widened pipe model based) to a diffusive (coalescence 42 based model) vascular network, building upon the framework 43 of MST. We show that the premises of constant sapwood area 44 across all branching levels and resistance maintenance are 45 mutually exclusive. Furthermore, we demonstrate that if stem 46 taper is variable then the cumulative volume of branches in 47 sequential branching generations cannot be preserved while 48 maintaining an energy-efficient network. Thus we challenge 49 the notion of constant sapwood area tip-to-base (Fig. 1D) 50 and argue that if resistance is to remain constant along the 51

Significance Statement

We outline a model that generalises allometric scaling theory to the entire plant by transitioning between distinct vascular domains. We show how the entire network can be optimised based solely on the maintenance of hydraulic resistance, by adjusting the rate of conduit widening and coalescence. This economises the carbon employed in developing an energy efficient vascular system that combines transport and diffusive functions. We deduce an inverse relationship between the widening of conduits tip to base and the tapering of branch volume base to tip. This relationship contradicts the largely accepted Leonardo's rule that the combined area of stems is preserved along the length of the plant. Our postulates may explain the greater vulnerability of large trees to climate change.

The authors declare no competing interests

¹S. B. D. Sopp contributed equally to this work with R. Valbuena.

 $^{^2\}text{To}$ whom correspondence should be addressed. E-mail: s.sopp@bangor.ac.uk, ruben.valbuena@slu.se



Fig. 1. Models of summated branching network volumes, illustrating how total network volume (outer shape in black) and total conductive volume (inner shape in red) change with varying tapering coefficients (t_R , t_r and t_l), and Coalescence rates (m_k and p_k) (see Eqs. 1-7). Four network models are presented, showing the differences between (A) West *et al.* (3), (B) Savage *et al.* (4), (C) Rosell & Olson (8) and (D) our gMST relationships of plant morphology, reaching from the plant base to the tip of the leaves. Values encircled in green are explicitly given as premises by the authors, while other values are inferred.

vascular network while conduit number increases, allowing for 52 a diffusive domain in leaves for the efficient distribution of 53 resources, then there must be a reduction in branch volume. 54 Thus vascular optimality influences the external plant mor-55 phology. We deduce relationships showing that this reduction 56 in volume is necessarily commensurate with the increase in 57 58 coalescence rate in leaves. Lastly, we detail how carbon investment is comparable or lower than previous MST models 59 due to it being offset be reduced volume. In the discussion we 60 consider the implications for the carbon investment needed for 61 plant growth and the potential use of our model to investigate 62 causes of differential tree mortality. 63

64 Theory Development and Results

MST describes a set of scaling relationships that model branch 65 dimensions across branching generations (2, 4). The hydraulic 66 architecture of vascular plants can be characterised through 67 branching ratio (n), conduit radii (r_k) and length (l_k) (2, 3), 68 and conduit coalescence ratio (m_k) (4) at any given branching 69 generation (k = 0, 1, ..., N) (Fig. 2). In the following sections 70 we deduce generalised MST relationships for (a) the area of 71 conduits (r_k) and their total summed area (R_k) , (b) the length 72 of branches (l_k) in association with their volume, and (c) an 73 energy efficient system that maintains hydraulic resistance 74 (ω_k) along the network. We deduce that the premise of energy 75 76 efficiency alone can be used to derive MST relationships, and moreover show how (d) it determines the overall volume of 77 the plant, and elaborate on (e) the relationships that dictate 78 the transition from a transport domain to diffusive domain 79 and (f) the associated overall carbon cost. Finally, using data 80 from Koçillari et al. (6) we show (g) empirical support for 81 aspects of our generalised MST model. 82

a) Conductive area preservation / Conduit radii. Here we describe the relationships pertaining to conduit area and its widening along generations. West *et al.* (3) included a widening term (t_R) in the MST relationships $(r_k^2 = n^{t_R} \cdot r_{k+1}^2)$, for which t_R is the rate of change between r_k^2/r_{k+1}^2 and n on



Fig. 2. Illustration of a vascular network with generations of branching k = 0.2 and conduit coalscence/furcation j = 0.3. Conduits are shown in red, whereas the external branching network is shown in black. The branching locations are denoted with horizontal blue dashed lines, whereas the blue dotted lines denote conduit coalescence/furcation nodes. Example values for m_k and n are given across each generation, whereby m_k increases with coalescence rate at higher generations.

a logarithmic scale assuming that $m_k=1$ (a pipe model that widens toward the base (6)). Allowing m_k to vary creates a more generalised relationship:

$$rac{r_k^2}{m_k \cdot r_{k+1}^2} = rac{R_k^2}{R_{k+1}^2} = n^{t_R},$$
 [1] 9

where R_k is the radius of a total volume containing all the conduits at the base of the branch k (red volume in Fig. 1). Thus, t_R is the rate of change between R_k^2/R_{k+1}^2 and n on a logarithmic scale for any value of m_k . There is a relationship between the branching ratio n and conduit coalescence m_k , such that $m_k = n^{p_k}$, where $p_k \ge 0$ (4). A relationship for individual conduits can thus be re-written as:

$$\frac{r_k^2}{r_{k+1}^2} = n^{(t_R + p_k)} = n^{t_r},$$
[2] 99

where t_r is the rate of change between r_k^2/r_{k+1}^2 and n on a 100 logarithmic scale under the assumption that conduits coalesce 101 $(m_k \neq 1)$. Hence, whenever there is conduit coalescence we 102 shall make a difference between t_r and t_R (4), under the 103 equality $t_r = t_R + p_k$ that derives from Eq. 2. Some authors 104 suggest that total conductive fraction may be preserved along 105 branching generations $(r_k^2 = n \cdot r_{k+1}^2$ within a volume filling 106 network) (4, 11), and the model of Savage *et al.* (4) allowed the 107 conditions for which $t_R = 0$ while a given conduit widening rate 108 may still exist (these conditions would be $p_k = 1$ and $m_k = n$). 109 Further work estimated species-specific values for these traits 110 (12, 13). Recent empirical observations suggest that there may 111 be a part of the plant for which those conditions are met, but 112 not along all branching generations (7), and thus we explicitly 113 made m and p variable according to k (Fig. 2). Thus a t_r 114 value between 0 and 1, captures the change in conduit area 115 along branching generations $(r_k^2 = n^{t_r} \cdot r_{k+1}^2)$ by including 116 both widening and coalescence. The recent experimental data 117 (6-8) can be modelled through Eq. 2 whereby their findings 118 suggest a transition from $t_r = 1/6$ at the plant base to $t_r = 1$ 119 at the plant tip (within a volume filling network), coinciding 120 with the two different models suggested by West *et al.* (3) and 121 Savage *et al.* (4), respectively (Fig. 1). 122

b) Volume preservation / Branch length. Here we describe the 123 relationships pertaining to branch volume and its tapering 124 along generations. The lengths of conduits within any given 125 branching generation can be modelled as equivalent to the 126 length of that segment, and their cubic power proportional 127 to its volume. Thus, l_k^3 is described through the term 'ser-128 vice volume' referring to the volume of cells supported by a 129 branch of a given size (2). MST's original formulation gave 130 a relationship between sequential branch lengths that relied 131 upon branch volume preservation, which lead to the deduced 132 allometric scaling (3). Empirical observations however indicate 133 that this premise occurs inconsistently within natural systems 134 (1). To remedy this, a branch segment taper term (t_l) can be 135 included in the original MST formulation (1): 136

37
$$\frac{l_k^3}{l_{k+1}^3} = n^{(1/t_l)},$$
 [3]

1

where branch length decreases t_l ($t_l \leq 1$) gives the rate of 138 change between l_k^3/l_{k+1}^3 and n on a logarithmic scale, whereby 139 a value of $t_l = 1$ results in branch volume preservation $(l_k^3 =$ 140 $n \cdot l_{k+1}^3$ and $t_l < 1$ brings a reduction in branch volume $(l_k^3 =$ 141 $n^{1/t_l} \cdot l_{k+1}^3$). West *et al.* (3) use branch volume preservation 142 $t_l = 1$ as a premise of MST, and thus t_l is a term not included in 143 the original MST relationships. Like the widening of conduits 144 tip to base, the empirical observations on the tapering of 145 volume base to tip shows that it varies with relative length $t_l =$ 146 f(l) (6), consequently decreasing along successive branching 147 generations, and generating the values of $1/6 < t_l < 1$ as 148 limiting boundary conditions. 149

c) Resistance maintenance (energy efficiency). Here we illus-150 trate how resistance maintenance relates to conduit radii and 151 branch volume widening/tapering terms. Eqs. 2-3 are integral 152 to the assessment of hydraulic resistance along a branching 153 system, which can be modelled through the Poiseulle formula 154 that gives the hydraulic resistance of a conduit (ω_k) as $8\mu l_k/r_k^4$, 155 where μ is fluid viscosity (14). Resistance can be maintained 156 along the entire vascular structure, which would minimise en-157 ergy loss in the vascular system. West et al. (3) demonstrated 158 this by comparing the total resistance along a single conduit 159 (from plant base to plant tip) to that of the conduit resis-160 tance in the petiole, showing that a single conduit widening 161 162 value could create an energy efficient vascular architecture in a volume filling network, independent of path length. There-163 fore within an optimal network, the resistance of a conduit 164 in branch k is approximately equivalent to the resistance of 165 a conduit in its daughter branch (k+1) $(\omega_k \approx \omega_{k+1})$. Alter-166 natively, using Eqs. 2-3 which involve the empirical evidence 167 that there is a variable widening rate in the radius t_r and 168 length t_l of conduits, the maintenance of hydraulic resistance 169 along generations can be assessed as: 170

$$\frac{\omega_k}{\omega_{k+1}} = \frac{n^{2t_r}}{n^{(1/3t_l)}} = n^{(2t_r - 1/3t_l)} = 1,$$
[4]

whereby if resistance is maintained $2t_r - \frac{1}{3t_l}$ must equal 0. This allows resistance to be constant along a conduit path, for any values of n and k. As an alternative to West et al. (3), gMST works exclusively under the premise of hydraulic resistance preservation for energetic efficiency. Furthermore it allows the fraction of conductive area to vary along the branching network, depending on both stem tapering base

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d) Vascular optimality dictates plant morphology. Here we 182 describe how resistance maintenance could influence plant 183 morphology. The energy efficiency premise outlined implies 184 that the transition from a transport domain to a diffusive 185 domain can only be yielded by a reduction in branch volume, 186 thus altering plant morphology (Fig. 1D). This transition 187 occurs because the increase in coalescence needed for a diffusive 188 domain must be accompanied by stem tapering if resistance 189 is to remain constant over the vascular network (Fig. 2). If 190 resistance maintenance and the need for an efficient diffusive 191 network are both factors determining natural selection, then 192 this reduction in volume demonstrates how vascular optimality 193 impacts morphology. Eq. 4 leads us to deduce that there is a 194 constant relationship between the widening and coalescence of 195 conduits tip to base and the tapering of branch volumes base 196 to tip, that describes the change in morphology: 197

$$r = \frac{1}{6t_l}.$$
 [5] 198

Hence, as the widening of conduits has been experimentally 199 observed to change along the network (6-8), then volume need 200 not be preserved, meaning resistance preservation could solely 201 regulate the vascular system. In (Fig. 1D) we show that 202 given the relationship deduced in Eq. 5, the model of total 203 conduit volume observed by Rosell & Olson (8) necessarily 204 leads to an ensuing model of plant morphology that modifies 205 the total volume accordingly. In other words, as conduit 206 widening tip to base has been observed to decrease along 207 branching generations, the tapering of the total volume of 208 successive branching generations must increase, impacting the 209 MST scaling. 210

t

e) Transition from transport to diffusive network. Here we de-211 scribe how the network transitions between transport and 212 diffusive domains along the length of conduits, showing where 213 along the plant's length this functional transition occurs. The 214 internal vascular system can coalesce independently from the 215 exterior branching network, and thus the generations of con-216 duits j and branches k operate at different scales (Fig. 2). All 217 equations can be calculated for j as for or k, bringing different 218 values of widening for the internal vascular network which we 219 denote with a tilde \tilde{t}_l (Fig. 1 D). The internal vascular net-220 work can be modelled independently of the external branching 221 nodes using the MST relationships: 222

$$\frac{l_j^3}{l_{j+1}^3} = n^{(1/\tilde{t}_l)}, \qquad [6] \quad 223$$

where l_j represents the length of a conduit generation j between coalescence nodes and \tilde{t}_l gives the rate of change between l_j^3/l_{j+1}^3 on a logarithmic scale (Fig. 2). It is possible to deduce at what distance from the stem tip that the functional transition between a transport and diffusive vascular network occurs, by describing it through the summation of an infinitely scaling geometric series.

$$l_t = \frac{l_{j=0}}{1 - n^{\left(-1/3\tilde{t}_l\right)}},$$
[7] 23

where l_t represents total network length, and $l_{i=0}$ denotes 232 the length of the basalmost conduit through the branching 233 system until its first branching node (j = 0) (Fig. 2). The 234 value of \tilde{t}_l varies inversely with stem volumetric taper, ex-235 236 hibited within Fig. 1D by the inverse relationship between 237 the external branching and the internal conductive volume. \tilde{t}_l has a value of 1/6 at the base of the stem, which implies 238 $3/4 \cdot l_t = l_{i=0}$ (see Supplementary Information S1). It is thus 239 plausible that approximately the first three-quarters of the 240 height of a tree from its base has a transport function, with no 241 conduit coalescence, whereas in the topmost quarter the vascu-242 lar network transitions into an increasingly diffusive function 243 with an exponential increase in coalescence. Our estimates 244 would therefore imply about 3-4 coalescence nodes located 245 within twigs, with all other conduit branching occurring within 246 the leaves. Coalescence rates are therefore modelled from the 247 tip of the leaves to the base of the stem at the vascular nodal 248 scale, and thus all observations, whether in the stem or leaves 249 can be modelled within our gMST model. 250

f) Carbon economy. Finally, we quantify the effect on the carbon cost (C_c) of the vascular system, in response to Eqs. 1-5. C_c is often defined as the total volumetric investment in the vessel walls, whereby vessel wall thickness is expected to be proportional to vessel area. C_c can be quantified through the summation of a geometric series:

257
$$C_c \propto \frac{r_{k=0}^2 l_{k=0}}{1 - n^{-t_R} n^{(-1/3t_l)}}.$$
 [8]

Using Eq. 8, the carbon cost of the widened pipe model (3)258 can be calculated using the values $t_l = 1$ and $t_R = 1/6$. For 259 the coalescence model (4) it can be ascertained with the values 260 of $t_l = 1$ and $t_R = 0$. The carbon cost of our gMST model 261 can be predicted with an approximate average value slightly 262 lower than $t_R < 1/6$ (8) as Eq. 5 can be substituted in to Eq. 263 8. For a given length and basal diameter of the first segment, 264 $r_{k=0}$ and $l_{k=0}$, our generalised MST model would result in 265 a total carbon expenditure systematically lower than either 266 West *et al.*'s (3) or Savage *et al.*'s (4) models (all calculations 267 268 can be found as Supplementary Information S2). This carbon efficiency in our model is achieved through the reduction of 269 volume. However this reduction occurs at the expense of height 270 when considering k = 0 dimensions. When comparing the 271 carbon cost of individuals of equal height our model proves to 272 approach that of West *et al.* (3), seen within Fig. 3. Selection 273 must therefore balance the need to transition into a diffusive 274 network with carbon cost, for plants of equivalent height, 275 assuming that resistance remains constant. 276

To investigate the carbon investment that results from fol-277 lowing different strategies in the morphology of the vascular 278 network, we conducted a Monte-Carlo (MC) simulation com-279 bining varying values of t_R and t_l (widening of conduits tip 280 281 to base and tapering of volume base to tip respectively) to illustrate their effect upon total length and carbon cost. The 282 result is given in Fig. 3, with the grey points indicating the 283 MC simulation results, generating a region of plausible plant 284 length and carbon cost values. Our hydraulically optimal 285 equation derived above (Eq. 5) is shown with varying values 286 of t_R and coalescence ratio relative to branching ratio $(p_k,$ 287 denoted as coloured values in Fig.3). The MC results demon-288 strate how coalescence rate and conduit widening influence 289

our gMST model, with increasing values of p_k being associ-290 ated with lower total length and carbon cost for the same 291 hydraulic performance. A lower rate of conduit taper attained 292 by increased conduit coalescence means that plants can grow 293 taller, while maintaining hydraulic optimality with a lower 294 carbon expenditure. Thus Eq. 5 is a highly carbon efficient 295 strategy that allows plants to maintain diffusive functionality 296 while growing taller. 297



Fig. 3. Results of a Monte-Carlo simulation combining values between 0 and 1/6 for t_R and 0 and 1 for t_l , showing the resultant effect upon carbon cost and total plant length. The coloured points indicate the predicted model from Eq. 5 with the colour indicating the plant average values of conduit coalescence ratio relative to branching ratio $\overline{p_k}$ increasing from 0 (blue) to 1 (red).

g) Empirical support and comparison against previous MST 298 models. Our generalised MST model was tested against em-299 pirical data including its implications for vessel frequency and 300 conduit area along the length of the plant. We compared 301 simulated values from our model and previous MST models to 302 data available in Kocillari *et al.* (6). These data included both 303 the stems and sometimes leaves of a wide range of vascular 304 plants, thus allowing us to model the vast majority of the 305 vascular network under our MST presumptions (Eqs. 1-7) 306 against empirical data. 30

Figs. 4 and 5 show the simulated value for our generalised 308 MST model (in green) against empirical data, showing as well 309 the MST models of West *et al.* (3) (in blue) and Savage *et* 310 al. (4) (in red). The results illustrate that the generalised 311 MST model accurately models vessel frequency (Fig. 4) and 312 cross-sectional area (Fig. 5) stretching from the stem to the 313 leaves, compared to other MST models. It should however be 314 noted that previously proposed MST models were intended 315 to model only the stems, i.e. excluding the leaves, but still 316 predicted rampant furcation. 317

Our generalised MST model outperformed other models in predicting empirical observations on vessel frequency (Fig. 4). West *et al.*'s (3) model proved to perform well for the majority of the vascular network, although its lack of conduit coalescence results in poor performance in the latter stages



Fig. 4. Plot of relative plant length vs vessel frequency. Three models are plotted, with our generalised MST model given in green, West *et al.*'s (3) model given in blue, and Savage *et al.*'s (4) model given in red. The black lines represent the 75th, 50th and 25th quantiles for the dataset. Relative length is given by distance from tip divided by total length of plant.

of the network (i.e. toward the leaves). Savage et al.'s (4) 323 model yielded a rapid increase in vessel frequency, similar 324 to our model, although at an earlier stage of the network. 325 Kolmogorov-Smirnov tests showed that our generalised MST 326 model predictions of vessel frequencies were the ones that fit 327 best (D = 0.571; p-value = 0.141), since the predictions of 328 West *et al.* (3) (D = 0.777; p-value = 0.009) and Savage 329 330 et al. (4) (D = 0.714; p-value = 0.021) could be proven to significantly differ from the empirical observations. Our 331 generalised internal MST vascular model therefore combines 332 aspects of both models to successfully predict vessel frequency 333 with relative length. 334

Secondly we modelled how our model predicts vessel cross 335 sectional area, by simulating vessel cross sectional area against 336 distance from tip (Fig. 5). In this comparison, only the 337 predictions from Savage et al.'s (4) model significantly differed 338 from the distribution of the empirical data (D = 0.7; *p*-value 339 = 0.0123). Strikingly, the results showed that our modelled 340 rate of widening alongside conduit coalescence yields a similar 341 cross sectional area as West *et al.*'s (3) assumptions of fixed 342 conduit widening with no coalescence. We therefore agree with 343 past suggestions that lumen resistance scales with a number 344 of factors such as pit membrane resistance and vessel/tracheid 345 length (6). Our vascular equations (Eqs. 6-7) are applicable 346 within both leaves and stems, and therefore they attain an 347 gMST model applicable for the entire plant vascular system, 348 i.e. including the leaves which were not included in previous 349 MST formulation (3). 350

351 Discussion

Rosell & Olson (8) describe the hydraulic architecture of trees as having a "transport" and "diffuse" domain within their vascular networks. The branching system can therefore both transport resources to the farthest parts of the organism, and



Fig. 5. Plot of normalised plant vessel cross sectional area vs relative length. Three models are plotted, with our generalised MST model given in green, West *et al.*'s (3) model given in blue, Savage *et al.*'s (4) model given in red. The black lines represent the 75th, 50th and 25th quantiles for the dataset. Relative length is given by distance from tip divided by total length of plant.

then transition to the distribution of these resources to the 356 the tissues where they are needed. Our set of generalised 357 MST relationships (Eqs. 1-5) give the relationship between 358 t_r and t_l which allows this transition between the models of 359 West *et al.* (3) and Savage *et al.* (4). The premise that 360 'service volume' would be constant $(t_l = 1)$ yields a constant 361 value for the widening rate of individual conduits $t_r = 1/6$ 362 (Eq. 5), which minimises resistance for the transportation of 363 resources without losing volume and is thus congruent with 364 West *et al.*'s (3) models and empirical observations at the 365 beginning of the network (6-8). However, when approaching 366 the leaves and within them, t_r must approach one for conduit 367 area preservation while resistance remains constant and branch 368 volume decreases. Maintaining constant resistance with a t_r 369 value of one is facilitated by increasing m_k , which maximises 370 conductance and resource distribution (4, 9). As a consequence, 371 t_l must become substantially lower than one, meaning volume 372 is increasingly lost in the terminal generations. This gives 373 a new model of plant morphology (Fig. 1D) deduced from 374 the resistance maintenance premise alone (Eq. 4), given the 375 variable widening and coalescence rates observed in recent 376 research (6-8). The implications may in fact reach beyond 377 just plants, with possible insights into the morphology of other 378 taxonomical groups (e.g. if animals have larger shares of 379 diffusive functionality in their vascular network, a reduction in 380 volume that modifies their allometric scaling would be implied). 381 Further empirical research in a wide range of fields could be 382 stimulated by our MST modelling based on energy efficiency, 383 such as our predictions of conduit coalescence locations (and 384 lack there of), or the predicted rates of conduit coalescence 385 and volume reduction within branching generations. 386

The biologically optimal resource distribution network is un-

derpinned by Eq. 5. Natural selection should favour vascular 388 structures that maintain hydraulic resistance while increas-389 ing conduit number at the network's termination. Increasing 390 conduit coalescence rate must therefore be compensated for 391 392 by the reduction in external volume. Thus, our theory agrees 393 with previous postulates challenging Leonardo's rule of area preservation (1), giving a unified gMST framework that can ac-394 commodate transport and diffusive domains within the plant. 395

The transition from a transport to a diffusive domain can 396 occur while maintaining a constant level of resistance. This 397 is however achieved through different mechanisms, with resis-398 tance maintenance being generated by widening (t_r) in the 399 transport domain and conduit coalescence (m_k) in the diffusive 400 domain. The implication of this difference is that if selection 401 favours resistance maintenance and some degree of diffusion 402 in the network, volume cannot be maintained with respect to 403 conduit area (Eq. 5). Trees offer an ideal demonstration of this 404 trade off between volume and vascular diffusion, with conduits 405 in the trunk and the first few branching generations coming 406 close to volume preservation, as long-distance transportation 407 is favored. A transition to a diffuse domain occurs within 408 the later branching generations (which we calculated to occur 409 within the last quarter of the total length of the network), 410 allowing resources to be distributed efficiently within leaves, 411 and thus tree volume is reduced leading to the termination 412 of the branching network. Such a transition is in line with 413 currently available data (6-8), and our results demonstrated 414 that incorporating a transition between a transport and diffu-415 sive domain into MST modelling through variable widening 416 and conduit coalescence achieved a better empirical fit to the 417 entire vascular network including both leaves and stems (Figs. 418 4-5). Our prediction is however based on a constant conduit 419 widening rate that may not always be the case. It is possible 420 that the first conduit branching location occurs closer to the 421 tip than estimated, in which case plant morphology may be 422 more acutely impacted by changes in widening rate (Eq. 2 423 and Eq. 5). Future research is therefore needed to obtain em-424 pirical data to evaluate the rate of conduit coalescence within 425 branches. 426

The carbon cost of our gMST model proved to be highly 427 comparable to that of West *et al.* (3) for equivalent plant 428 lengths. However, the height and carbon cost of the plant 429 changes with respect to t_l , and thus with respect to conduit 430 coalescence and widening rates. Consequently the carbon cost 431 432 is lower than all previous MST models if stem volumetric 433 tapering occurs. This implies that the resistance maintenance premise may result in scaling exponents lower than predicted 434 by area/volume preservation (15). Moreover, the coalescence 435 model (4) predicts an enormous increase in the number of 436 conduits, diverging from empirical observations and leading 437 to much higher carbon costs than the widened pipe model 438 (3). Our model therefore predicts that natural selection will 439 440 favour individuals that invest less carbon in the transport and diffusive domains for the same performance, giving more 441 carbon surplus that can be invested in other plant functions 442 like reproduction or growth. Our generalised MST model 443 introduces the same conduit coalescence concepts as Savage 444 et al. (4), but in such a way that does not imply a massive 445 increase in carbon cost, effectively merging the coalescence and 446 the widened pipe model, and better predicting the number of 447 conduits per unit leaf area along the length of a plant (Fig. 4). 448

Fig. 3 demonstrates the impact of coalescence upon carbon 449 cost, where the relationship of Eq. 5 is highlighted in colour, 450 illustrating that it provides a path of carbon cost reduction for 451 any given total length of the vascular network. Selection will 452 favour networks that minimise carbon expenditure while also 453 maintaining resistance across the vascular network as plants 454 grow taller. Selection should thus favour the scenario pre-455 sented here, with a gradual transition to a diffusive domain at 456 the termination of the network, thus minimising excess carbon 457 expenditure while keeping constant hydraulic resistance con-458 stant, through the reduction of plant volume. The carbon cost 459 for otherwise equivalent performance proves to be marginally 460 more than of West *et al.* (3), but more accurately depicts the 461 plant vascular system. 462

The co-variation of MST exponents has been examined em-463 pirically by previous authors (16, 17), but no rigorous theory 464 based reasoning has been put forward to describe the observed 465 co-variation. In this contribution we offer a mechanistic theory 466 relating widening/tapering coefficients in Eqs. 4-5, which may 467 offer a novel explanation for this co-variation. If hydraulic 468 resistance maintenance and carbon cost are key factors of 469 selection, then the co-variation of MST exponents can be 470 derived from the rate of conduit coalescence and widening. 471 Furthermore, branching of the internal vascular network can 472 be independent of branching of the exterior branching nodes. 473 Savage *et al.* (4) partially segregated the internal and external 474 networks by modelling widening and coalescence within the 475 plant vascular system, but maintained that the proportion 476 of conduit area was a fixed proportion of the stem area (Fig. 477 1B). We complete this network segregation and illustrate how 478 energy efficiency dictates variation in coalescence rate through 479 the plant (Eqs. 6-7). This way the plant can show phenotypi-480 cal plasticity to environmental circumstances, such as water 481 availability, thus influencing the height and morphology of the 482 plant in connection with its hydraulic architecture. 483

We reconsider MST in the light of recent empirical observa-484 tions, outlining an MST based model that accommodates for 485 distinct transport and diffusive domains within a single plant 486 vascular system and including the entire organism with both 487 stems and leaves. Our model shows that natural selection 488 should favour plants whose conduits coalesce and widen tip to 489 base, compensated by an overall reduction in plant volume, 490 maintaining hydraulic efficiency (Fig. 1D). We model how con-491 duit coalescence could function within such a system, with the 492 coalescence rate increasing dramatically in the final stages of 493 the branching network, affecting the morphology of the plant 494 and thus its efficient use of carbon. We therefore encourage 495 authors to test both the premises and empirical strength of 496 the proposed model, and compare to other current models 497 that explore the trade-offs between the vascular system and 498 carbon cost, like that of Koçillari et al.'s WPM (6). 490

Materials and Methods

In section f) on carbon economy, Monte-Carlo simulations were 501 carried out with average stem and conduit taper and widening 502 coefficient $(\overline{t_l} \text{ and } \overline{t_R})$ such that all coefficients could be applied in 503 equations for total carbon cost and length. To model gMST within 504 the Monte-Carlo simulation results, only plant average values of $\overline{t_R}$ 505 and $\overline{p_k}$ were required and utilised to output a set of plant average 506 values for $\overline{t_r}$ and $\overline{t_l}$. Thus total length and carbon cost could be 507 calculated under our premise of energy efficiency (Eq. 5) 508

In section g) we compared different MST-based model predictions 505

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against empirical data kindly made available by Kocillari et al. (6). 510 511 We used a Kolmogorov–Smirnov test to compare the predictions of each modelled conduit generation sequence against the observed 512 sample frequency distributions. All calculations are available in the 513 514 R scripts included as supplementary materials.

In order to simulate vessel frequency from our model, Eq. 6 was 515 used to find the lengths of the first 100 j generations, using $l_{j=0} =$ 516 0.75 (an approximation deduced in supplementary information S1). 517 t_l was modelled to exponentially increase to a value of 1 towards 518 519 the 100th iteration. For both our model and Savage $et \ al.$'s (4), we assumed that branching of conduits occurred at each node, and thus 520 calculated the number of conduits in each generation starting from 521 a single conduit, whereas for West et al. (2) it was assumed that 522 no conduit coalescence occurs. The mean basal vessel frequency for 523 524 all plants was then multiplied by the simulated vessel number for each generation to simulate vessel frequency along the distance of 525 the stem. 526

We analysed our model's predictions of vessel cross-sectional area. 527 Using the same assumptions as MST we simulated the relative cross 528 529 sectional area for a conduit with a widening coefficient (t_R) that was fixed for West *et al.* (2) and Savage *et al.* (4), in accordance 530 with Fig. 1, whereas for our generalised MST model this value was 531 allowed to vary. The widening coefficient was combined with the 532 simulated internal node locations (calculated through Eqs. 6-7). 533

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