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Topological scaling and plant root system architecture: developmental and functional hierarchies

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SUMMARY

Topology is an important component of the architecture of whole root systems. Unfortunately, most commonly applied indices used for characterizing topology are poorly correlated with one another and thus reflect different aspects of topology. In order to understand better how different methods of characterizing topology vary, this paper presents an exploration of several different methods for assigning order within branched root systems on the basis of (*a*) developmental (centrifugal) vs. functional (centripetal) ordering sequences and (*b*) whether orders are assigned to individual links or groups of adjacent links (segments). For each ordering system, patterns of scaling in relation to various aspects of link and segment size are explored using regression analyses. Segment-based ordering systems resulted in better fits for simple scaling relationships with size, but these patterns varied between developmental vs. functional ordering as well as the different size metrics examined. The functional (centripetal), link-based ordering system showed complex, non-linear scaling in relation to numbers of links per order. Using a simple simulation model of root growth, it is demonstrated that this method of characterizing root topology in relation to root size might be a more powerful tool for characterizing root system architecture than in the use of simple, single-index characterizations of topology.

Key words: Allometry, architecture, fractal geometry, nutrient uptake, root systems, topology.

INTRODUCTION

One of the most conspicuous, yet poorly characterized and quantified features of whole plant root system form is topology – the pattern of root branching. Together with the overall size of a root system (e.g. root length) and the geometry of orientation and morphology of individual roots, topology determines the *in situ* space-filling properties of a root system within the soil and thus is an important component of nutrient uptake capacity (Fitter, 1982, 1985, 1986, 1987; Fitter *et al.*, 1991; Berntson & Woodward, 1992; Berntson, 1994; Nielsen *et al.*, 1994) and anchorage (Ennos, 1992). A number of different methods have been derived for characterizing the topology of branched structures like plant root systems (MacDonald, 1983). In a systematic review of topological indices (Berntson, 1995), I focused on some of those most commonly applied within the biological literature. Each of these indices is a single value used to describe the pattern of branching corresponding to how topologically 'compact' or 'symmetric' a given structure is (MacDonald, 1983). The different indices were poorly correlated within one another and showed contrasting ontogenetic patterns for several basic models of branching development. However, it was not possible to determine which of the examined indices were *a priori* a better measure of branching than any other.

The purpose of this paper is to examine the assumptions which underly different methods for characterizing topology and assess which approaches provide tools which can be productively used for characterizing branching patterns within plant root systems. In this paper I explore several different ordering systems used for characterizing branching patterns. An ordering system consists of the rules used for assigning orders to different portions within a branching structure and is the necessary first step used in virtually all quantitative topological indices. To provide a common framework for comparing ordering systems, I will derive 'topological indices' using regression techniques to characterize the size

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(e.g. extent of branching) in relation to each ordering system I explore. By exploring different ordering systems my goals are to provide (1) a more solid understanding for why different topological indices vary from one another, and (2) information which will allow researchers to select a method which is most appropriate for their needs when characterizing branching patterns within plant root systems. Although my focus is on root systems, much of the analysis presented here could also be applied to the shoot systems of plants.

COMPARING DIFFERENT METHODS FOR QUANTIFYING BRANCHING PATTERNS

Terminology

Topologically, it is convenient to regard plant root systems as rooted binary trees (Clapham, 1990). For purposes of clarity and consistency with previously published descriptions of plant root systems (Berntson & Woodward, 1992), I will refer to the root, vertices and leaves of a rooted binary tree as the base, links and root tips, respectively.

The direction and units of ordering binary trees

In this paper I compare and contrast four different systems for ordering portions within rooted binary trees. These systems consist of two orthogonal ordering criteria (Figs 1, 2). The two ordering criteria are the direction and unit of ordering. A preliminary report of this approach is available in Berntson (1996). Here I present a detailed analysis. A general review of ordering systems for branching systems is provided by MacDonald (1983).

The direction of ordering can be either centrifugal ('F', developmental) or centripetal ('P', functional). A centrifugal ordering system starts at the base of the system (or root of the binary tree) and orders are assigned in increasing magnitude away from the base. This direction of ordering is 'developmental' in that it reflects the direction of growth within root systems. A centripetal ordering system starts at the root tips and orders of increasing magnitude are assigned toward the base. This direction of ordering is 'functional' in that order reflects topological distance from root tips (*sensu* Fitter, 1987). Distance from root tips is correlated with age and can be an important determinant of the morphology and physiological capacity of a given section of root (Clarkson, 1996). These contrasting directions of ordering result in a distribution of orders which are nearly opposite to one another. Determining how the size of a root system scales with these different directions of ordering might allow us to determine which method of ordering best characterizes topological patterns within root systems. If root architecture scales with developmental order, then it might be possible to use these scaling relationships to describe developmental patterns within plant root systems. Conversely, if root topology scales with functional order, then these scaling relationships might be used to relate branching patterns and processes to physiological activity.

Units of ordering are either individual links ('L', also known as 'nodes') or contiguous, linear groups of links (segments, 'S'; *sensu* Fitter, 1986). When the ordering units are links, demarcation between adjacent orders is unambiguous. Links are the topological equivalent of edges. Morphologically, a link is defined as a linear (unbranched) portion of root bound on either side by either branching (other roots) or a root tip. Demarcation between adjacent segments can be ambiguous, depending on the criteria used. For example, the developmental ordering system (May, Chapman & Aspinall, 1965; May *et al.*, 1967) often used for describing the growth of plant root and shoot systems can be ambiguous when clear patterns of dominance or differential growth are not observed between adjacent developmental orders. This difficulty can arise when the developmental axis stops growing before the laterals (Cannon, 1949), or there is no clear developmental axis (Cannon, 1949), thereby requiring judgment calls on the part of the researcher which can take into account additional aspects of morphology and geometry. An advantage of using a link-based ordering scheme is that a greater number of orders is identified within a root system which might provide a greater level of resolution for examining branching patterns within an individual root system (Crawford & Young, 1990). By contrast, segment-based ordering systems might assign equal order to links of equivalent form and/or function, thereby potentially leading to more simple and meaningful topological classification within a root system.

Fitter first introduced the centripetal link based ('PL'; Fitter, 1985, 1986, 1987) and centripetal segment based ('PS'; Fitter, 1982, 1985) ordering systems to plant root systems. Both of these ordering systems were first introduced in the geomorphology literature for describing the topological properties of stream drainage networks. In the PL ordering system, root tips are assigned an order of one (Werner & Smart, 1973). Each proximal link (traversing in the direction of the base) is assigned an order equal to the sum of the orders of the two distal links. The order of the base of the root system is equal to the number of root tips in the system. The PS ordering system was first introduced by Horton (1945) and then revised by Strahler (1952). This ordering system assigns all root tips an order of one (Fig. 2). Each proximal link is assigned an order equal to the larger order of the two distal links or one greater than the order of the distal links if they are

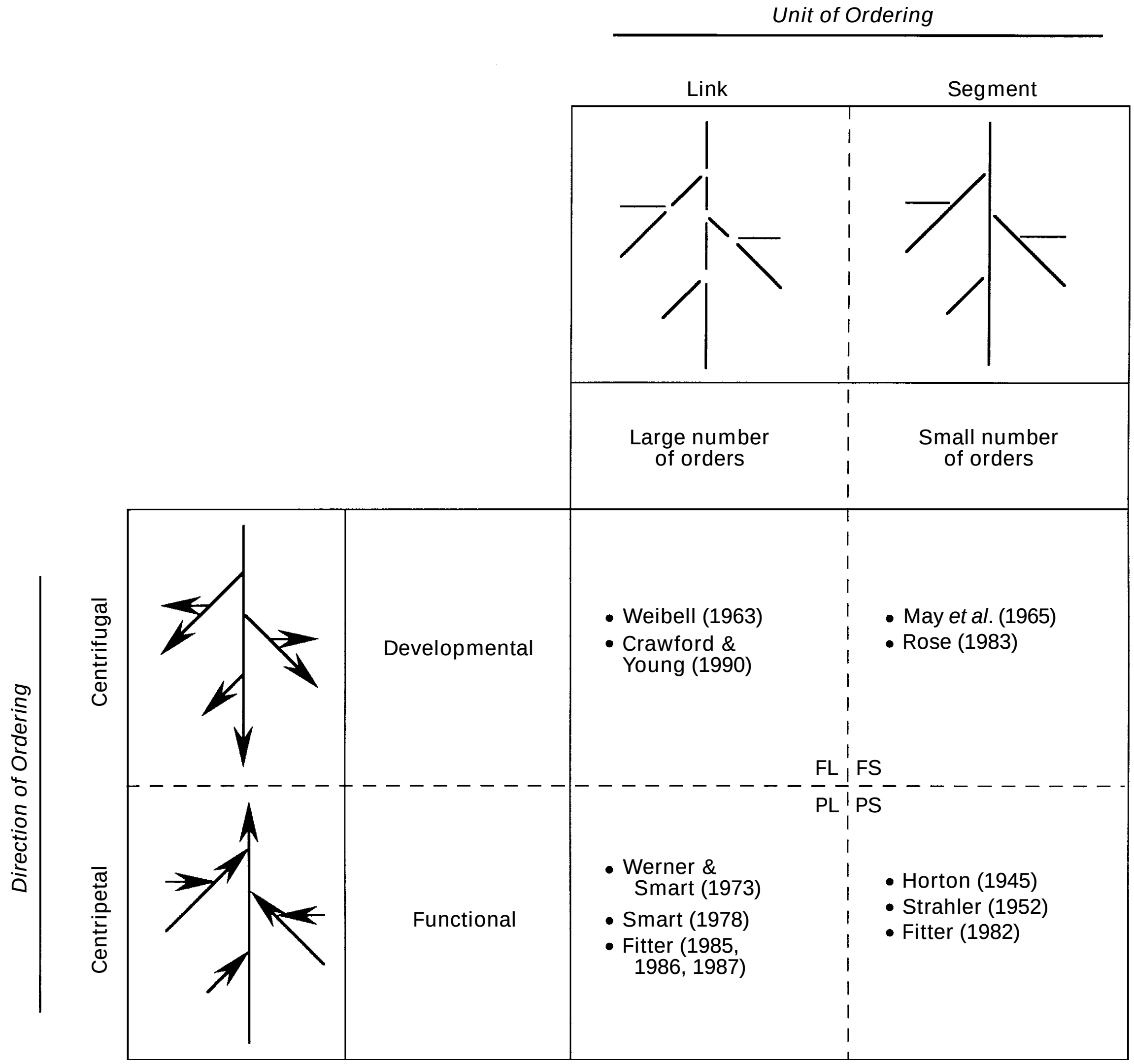

Figure 1. Schematic diagram of the DIRECTION and UNIT based components of the systems used in this paper for assigning different orders (scales) within root systems. The diagram illustrates the basic features of each variation on ordering methodology and gives citations of the introduction and/or application of the particular ordering scheme. See text for additional information and Figure 7 for an application of each of the ordering methods.

equal. Contiguous groups of links of equal order are regarded as a single segment.

The primary centrifugal (developmental) segment based ordering scheme ('FS') which has been applied to plant root systems is the developmental ordering scheme. This scheme has been used extensively in describing patterns of root growth (May *et al.*, 1965, 1967; Hackett, 1968) and in modelling the growth of plant root systems (Lungley, 1973; Rose, 1983; Diggle, 1988; Pagés, Jordan & Picard, 1989; Lynch & Nielsen, 1996). In this scheme, different orders reflect the hierarchical nesting of multiple growth axes in a root system (Fig. 2). In a simple tap-rooted plant with extreme apical dominance, there is a single axis (e.g. the growth of a single root tip) where the length between the base of the root system and the tip of this axis is longer than any other potential axis. This axis is referred to as the developmental axis. Each root tip emerging from this developmental axis constitutes a subordinate developmental axis. Usually the developmental axis is referred to as order zero, subordinate axes to the developmental axis are order one, and so on. In order to express these orders on a log scale I have offset this ordering system by one so that the developmental axis is of order one.

The remaining ordering system of the four I consider in this paper, the centrifugal (developmental) link based scheme ('FL'), is not commonly applied to plant root systems. Recently this ordering scheme was applied to the shoot systems of mature deciduous trees (Crawford & Young, 1990), but its primary application has been in describing the branching structure of bronchial trees within lungs (Weibell, 1963). This system assigns an order of one to the base of the root system (Fig. 2). Each adjacent distal link (away from the base) is assigned an order one greater than its adjacent proximal link. Thus, a

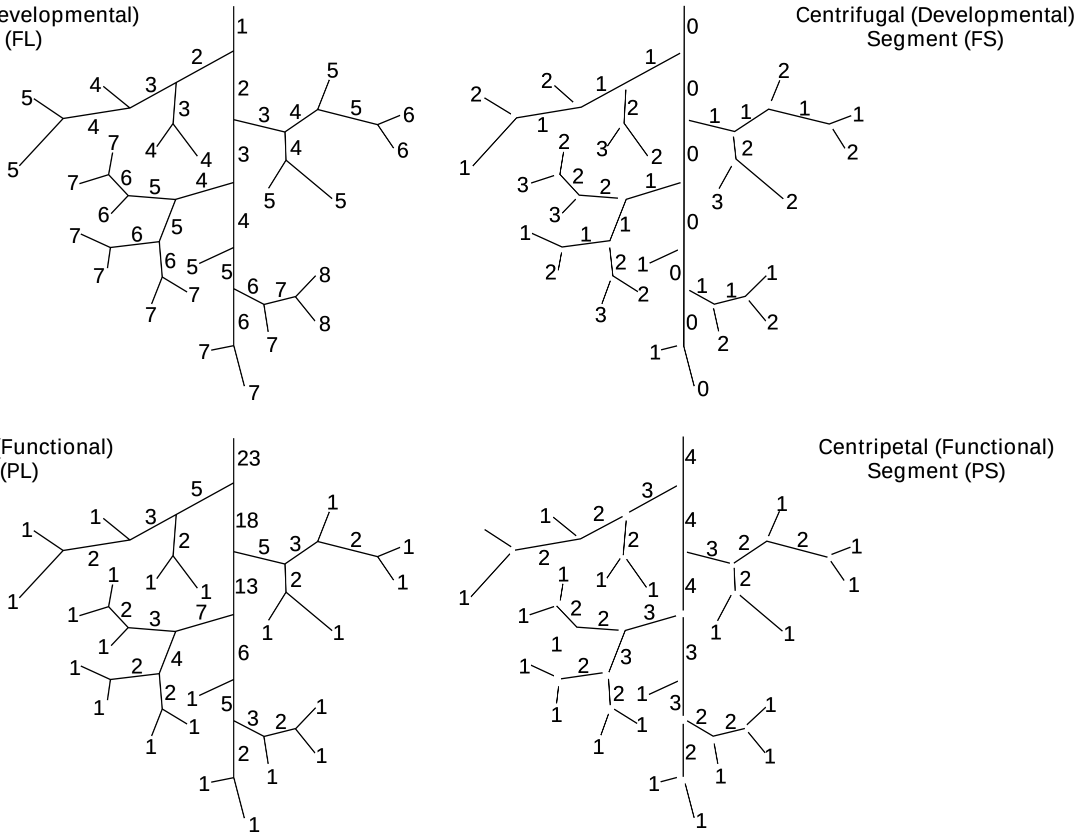

Figure 2. Schematic diagram illustrating the application of each topological ordering scheme used in this chapter. For the two segment based ordering schemes, separate segments are drawn as unconnected lines.

link's order is its topological distance from the base of the system.

Approaches for characterizing the topology of plant root systems

Unlike the measurement of *in situ* spatial distributions of roots within the soil (Rogers & Bottomley, 1987; Brown *et al.*, 1991), reliable and moderately rapid techniques have been developed for the analysis of root system branching patterns. These techniques involve removing an intact root system from the soil and laying it on a flat surface so that no two non-adjacent links touch. The root system is then digitized into a computer and the image is processed using software designed for measuring root architecture (Fitter & Stickland, 1991; Berntson, 1992; Fitter & Stickland, 1992). This method of analysis preserves the topological structure and every link length within the root system, but retains no information about the original *in situ* pattern of deployment of root systems.

Using these computer-automated architectural-analysis techniques, root systems can be stored in computer memory as link data vectors (Smart, 1978; Berntson, 1995) or binary trees (Berntson, 1992). Storing the root systems in computer memory as binary trees greatly facilitates the implementation of simple algorithms for applying all of the ordering systems discussed in this paper. All of the above ordering schemes can be applied from a knowledge of the topological structure of the root system independent of root geometry except for the FS scheme (developmental). In this paper, FS orders were calculated by assuming that different segments (and thus orders) could be distinguished by length of root, where the longer axes represent the lower developmental orders. Initially the longest single axis within the root system (longest distance between any given root tip and the base of the root system) was identified and this was designated the developmental axis (order of one). Each sub-tree (branch) attached to the developmental axis was processed similarly. This process was repeated until all segments within a root system had been assigned an order.

Scaling analysis of topological systems: perspectives from allometry and fractal geometry

To characterize topology and architecture in relation to each of the ordering systems I have described, I examine the scaling relationships between *order* and *size* in plant root systems. The approach I take in deriving these scaling relationships is analogous to the study of allometry (Huxley, 1932; Gould, 1966) and fractal geometry (Mandelbrot, 1983). Allometry is the study of how different components of an organism's size scale with one another through ontogeny (often variation in size within a population is used as a proxy for variation through ontogeny) (Niklas, 1994). The pattern of scaling between different size metrics is referred to as an allometric relationship, and typically takes the form of the following equation

$$y = ax^b \tag{1}$$

where y and x are the size metrics, a is a scaling coefficient which acts as a simple linear modifier, and b is the scaling exponent. When b is not significantly different from 1·0, this relationship is considered to be isometric, indicating that y and x scale linearly with one another. In fractal geometry, an object has a fractal dimension (D) which is a measure of the way in which the object fills space. D is analogous to the allometric scaling exponent. An estimate of D (the box counting dimension, D') can be derived from an equation analogous to eqn (1), where x is the 'scale' of observation (length of the side of a box), y is the number of boxes required to completely cover a two-dimensional object (or fill a three-dimensional object). From this equation, $D = -b$. For standard Euclidean objects, D is an integer value which represents the objects' embedding dimension. For a line, $D = 1$, for a square or a circle, $D = 2$, and so on. For more complex objects D is a non-integer value. For example, the coastline of Britain is a 'line', but its curves and texture mean that it partially 'fills' a plane, and therefore $1 < D < 2$.

Both b and D, can be thought of as scaling exponents which describe how scale (metric x for allometry, spatial scale for fractal geometry) influences the apparent size of an object. In this paper, we will derive topological scaling exponents which are analogous to the allometric scaling exponent and the fractal dimension. The topological scaling exponents will relate topological scale (x, corresponding to the order identified using one of the four ordering systems already discussed) to size (see below for a discussion of the different size metrics examined in this study). It is possible that for a given ordering system and a given measurement of size, the best fit scaling relationship will not account for a significant amount of variation in the size metric. In these cases, we can conclude that the size metric does not scale with the ordering system. By contrast, when we find that the topological scaling relationship accounts for a significant amount of the variation in the size metric, we can conclude that the size metric does scale with the ordering system. This type of comparison will allow us to assess which type of topological hierarchy (e.g. developmental vs. functional) appears to scale with root system size and thus is related to whole root system architecture.

In this paper I characterize the topological scaling exponents by examining how three size components of plant root systems vary as a power of order (exponent b in eqn (1)) in each of the different ordering systems. The size components I examine are: (1) number of links/segments of a given order; (2) total length of root in a given order; and (3) average length of links/segments of a given order. The exploration of size components of plant root systems in relation to topological structure advocated here is not without precedent (Strahler, 1952). Strahler proposed expressing the scaling properties of size components in relation to his ordering system (PS) by as $\mathrm{N}_{\omega}/\mathrm{N}_{\omega+1}$, where N_{ω} is the size at order ω, and $\mathrm{N}_{\omega+1}$ is the size at order $\omega+1$. The two size components Strahler proposed examining that are analogous to those proposed here are (1) number of links/segments of a given order; (2) average length of links/segments of a given order. The statistic determined from the first size component is the Strahler bifurcation ratio (R_b) and the second is the Strahler stream-length ratio (R_l). I have opted not to use Strahler's method of looking at scaling between root size components and branching structure. Rather, I calculate topological scaling exponents that are directly analogous to allometric scaling exponents and fractal dimensions (D). Because the two direction ordering systems I examine here assign order values in opposite directions (centrifugal/developmental and centripetal/functional), the sign of the derived topological scaling exponents (b from eqn (1)) are both positive and negative.

TOPOLOGICAL SCALING AND PLANT ROOT SYSTEMS: MATERIALS AND METHODS

To explore the ordering systems, I examined the root systems of five species of plants: *Senecio vulgaris*, *Arabidopsis thaliana*, *Acer rubrum*, *Betula alleghaniensis* and *Betula populifolia*. *Arabidopsis* and *Senecio* are both short lived annuals. The *Arabidopsis* root systems examined were from 2-wk-old *Arabidopsis* ($n = 5$). Root systems of *Senecio* aged 2, 3 and 4 wk were examined ($n = 20$ for each age; data from Berntson & Woodward, 1992). *Acer* ($n = 8$) and the two *Betula* species (*populifolia*, $n = 7$; *alleghaniensis*, $n = 12$) are trees. For the tree species, root systems from 3-wk-old seedlings were examined. Root systems of each species were analysed as described above (see Berntson 1992). The root systems (stored as binary trees in computer memory) were ordered using each of the four direction and unit ordering combinations (Figs 1, 2) and the number, total length and average length of each link/segment per order were determined using a computer program (Berntson 1992). Topological scaling relationships were derived using linear, type-I regressions where log(order) was the independent variate and log(number, length and average length) were the dependent variates. To avoid type-II errors in comparing multiple P-values when examining regressions, a sequential Bonferroni-correction was employed to adjust P-values (Rice, 1989).

RESULTS

Simple topological scaling relationships in plant root systems

There are several interesting trends that emerge from examining the results of the regressions (Table 1). In general, only segment-based ordering systems showed high adjusted-r^2 and only link-based ordering schemes showed high levels of significance. Topological scaling relationships for the segment ordering schemes consistently had high adjusted-r^2. The low levels of significance of these regressions is the result of few orders being used in the regressions (between three and six). When so few data points are used in a regression, the power of significance testing is low even though the regressions explain a large fraction of the variation observed in the dependent variable. Conversely, the high levels of significance observed in the PL ordering scheme (for number and total length of root per order) with relatively low adjusted-r^2's (0·68 and 0·55) is the result of large numbers of orders included in each regression and thus greater power of significance testing. Crawford & Young (1990) recognized that this discrepancy would result from the utilization of segment vs. link ordering schemes and thus advocated the use of link-based ordering. For the results presented here, I suggest that the segment based ordering schemes are a more valid means of determining simple exponential functions relating topological order and size within plant root systems due to the substantially greater amount of variance they explain (high adjusted-r^2's).

The FS and PS regressions showed different though complementary patterns of r^2's in relation to the different size components. The FS ordering scheme showed a high r^2 with the average length of root segments, but not with the number of segments or total length of root per order. The pattern with PS was the opposite, showing high correlation with the number of segments and total length of root per order. Given that the PS & FS ordering are so different from one another, these results are not surprising.

Table 1. *Summary of type-I linear regressions (eqn 1) relating topological order (different ordering systems listed on left of table,* x*) versus different size metrics (*y*, see text for a discussion of terminology and methods)*

	Number	Total length	Average length
FL			
% Significant	6·7	3·3	6·7
r^2	0·10	0·07	0·08
FS			
% Significant	1·2	0·0	1·2
r^2	0·39	0·17	0·94
PL			
% Significant	95·6	87·8	26·7
r^2	0·68	0·55	0·16
PS			
% Significant	3·5	0·0	0·0
r^2	0·97	0·89	0·59

Presented are the percent of all regressions significant at the $P < 0{\cdot}05$ level (P-values Bonferroni-corrected for $n = 92$, P-value cut-off of $0{\cdot}05/92 = 5{\cdot}26e^{-4}$) and the average r^2.

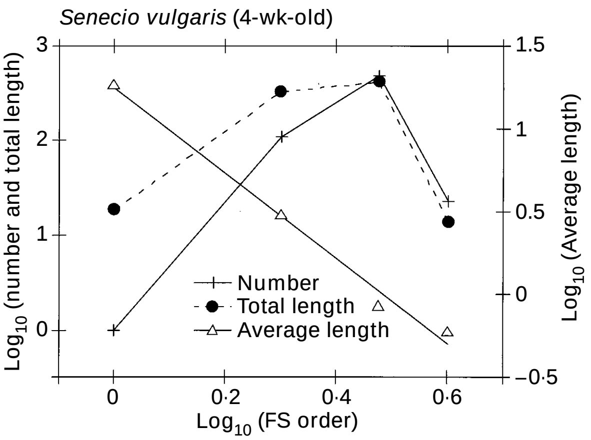

Figure 3. Centrifugal–Segment (FS) ordering scheme in relation to various size components of a 4-wk-old *Senecio vulgaris* root system. Note that the relationship between average lenght and order is linear. By contrast, the number of segments and total length per order show substantial departures from linearity because of low values observed in the highest FS orders.

The orders within the FS system represent the hierarchies of developmental axes. If rates of elongation and maximum length of each root segment are inversely related to its FS order then we would expect to see a consistent pattern between average segment size and order. The reason why the FS system shows low r^2's in relation to the absolute size of each order within the root systems (number of segments and total length of root per order) is because of a delay in production of roots between successive developmental orders. For example, the relationship between FS order and number and total length per order rises until the highest order where it drops off substantially while average length shows a strong linear relationship with order (Fig. 3). In models of plant root growth the delay between the initiation of subsequent FS orders has been described using empirical constants (e.g. Rose, 1983). Thus, the total amount of root (in terms of number or size) in a given developmental order does not scale simply for the higher developmental orders. Because the rate of growth of different FS orders is consistently different, the average length of roots of different developmental orders does show simple scaling properties between different FS orders.

The interpretation for why PS ordering shows simple scaling properties with segment number and total root length per order is not as simple as the relationship between FS ordering and average segment length. PS ordering is not developmental and thus does not represent hierarchies of developmental axes as does FS ordering. As a result, PS ordering changes within a root system as it grows. The addition of new root tips leads to a cascade of changes in the ordering of individual links and thus the ordering of segments within the root system. The application of this ordering system for describing branching structure topology (R_b) has resulted in linear relationships in every reported usage in the literature. In biological systems, this ordering system has been successfully applied to plant root systems (Fitter, 1982), the shoot system of trees (Leopold, 1971; Barker, Cumming & Horsefield, 1973; Whitney, 1976), fungal hyphae (Park, 1985), foraging trails of ants (Ganeshaiah & Veena, 1991), and the bronchial trees of lungs (Horsfield, 1980). Thus, it is not surprising that this ordering scheme has shown simple scaling with number of segments per order. Total length of root per order is roughly analogous to total number of segments per order, both being measures of the total amount of a root system per order. It is interesting to note that average segment length does NOT show simple scaling properties with PS order. This lack of a simple scaling relationship indicates that whatever determines average length for the different PS orders (a combination of root elongation rates and location of branching events) is not consistent between PS orders.

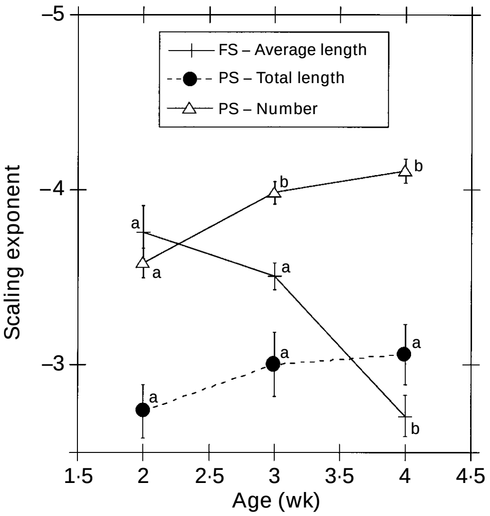

Figure 4. Ontogeny of scaling exponents for *Senecio vulgaris* root systems. If letters differ between different times for any given scaling exponent then the values are significantly different at $P < 0{\cdot}05$ (Bonferoni-corrected least-squared-means comparisons). Bars are single standard errors. Note: scaling exponents are negative for the FS (developmental) ordering system because average segment length decreased with increasing order.

Ontogeny of topological scaling exponents

In order for any derived topological scaling exponent to represent a robust measurement of the processes of development which lead to changes in root architecture through time, the topological scaling exponent needs to show minimal variation through ontogeny. When a topological scaling exponent shows significant variation through ontogeny, it does not reflect a consistent process through ontogeny and thus inference from one time to another is tenuous. Figure 4 presents a summary of the three main ordering schemes – metric component regressions that showed high r^2's for 2-, 3- and 4-wk-old *Senecio vulgaris*. Both the number of segments and total length per PS order showed trends of increasing topological scaling exponents with time – though only for number of segments per PS order was this trend significant (ANOVA, $P = 0{\cdot}4351$ and $P < 0{\cdot}0001$, respectively). For number of segments per PS order, the difference was due to a lower topological scaling exponent at week two. Average segment length by FS order showed a highly significant decrease in topological scaling exponent through time (ANOVA, $P < 0{\cdot}0001$), where the lowest topological scaling exponent was at week four. These observations of the ontogeny of the topological scaling exponents of root system branching patterns suggest that, for *Senecio*, the scaling of length by PS order is relatively constant through ontogeny. However, the scaling of segment numbers by PS order and average segment length by FS order are variable through ontogeny.

Complex (non-linear) topological scaling

In some cases, a poor best fit approximation to eqn (1) is obtained due to complex (non-linear) scaling relationships. This problem has been discussed in studies of allometry (Chappell, 1989; Weiner & Thomas, 1992; Thomas, 1993; Ebert & Russell, 1994) as well as fractal geometry. (Mandelbrot, 1983; Mandelbrot, 1988) and others have pointed out that some structures and processes have non-uniform fractal dimensions. Many of these fractals (typically called multifractals) have values of D that vary depending on the processes or parameters that regulate their formation. In cases where a scaling relationship is not accounted for by a single D or other scaling exponent, the observed complexity might result from fundamentally different processes operating between different scales (Burrough, 1981; Krummel *et al.*, 1987; Sugihara & May, 1990). This observation has been applied in ecology as evidence of differing processes acting on different scales in the formation of landscapes (Krummel *et al.* 1987) and complex spatial structuring of vegetation allowing for diverse assemblages of habitats for invertebrates (Morse *et al.*, 1985; Gunnarsson, 1992).

Fitter (1986, 1987) has suggested that the PL ordering system is valuable for describing plant root

Table 2. *Input parameters to topologically explicit modification of Rose's (1983) numerical model of root growth (Berntson 1995)*

		D	H	CD	MA
DOmax	FS Order	∞	2	5	3
ER	1	1	1	3	1
	2	1	1	2·5	1
	3	1		1·5	0·1
	4	1		0·4	
	5	1		0·2	
	6	1			
	∞	1			
IBD	1	1	1	1	1
	2	1	n.a.	1	1
	3	1		1	n.a.
	4	1		1	
	5	1		n.a.	
	6	1			
	∞	n.a.			
T	1	0	0	0	0
	2	0	0	1	0
	3	0		2	0
	4	0		3	
	5	0		4	
	6	0			
	∞	0			

Refer to text for a more detailed description of model. FS Order, centrifugal segment-based ordering system (developmental ordering); D, dichotomous branching pattern; H, herringbone branching pattern; CD, constrained dichotomous branching pattern; MA, multi-axis branching pattern; DOmax, maximum developmental order; ER, elongation rate (length time−1); IBD, interbranch distance (length); T, time (time). Length and time units arbitrary. The highest developmental order values for IBD are not applicable (n.a.) because there is no branching on the highest developmental order.

systems because it results in categorizing root links of equivalent function (proximity to a root tip) similarly. If complex scaling properties are observed between PL order and a size component, this suggests that different functional components of plant root systems show different topological scaling properties. Such an observation would not be entirely surprising, as root sections of different age and different root members can show pronounced physiological differentiation (Waisel & Eshel, 1991; Clarkson, 1996).

To interpret these non-linear scaling properties I first wanted to determine the scaling properties of root systems with known growth rules. To this end I performed a series of simulations of root growth where the rules regulating growth varied from the topological extremes of dichotomous branching to herringbone branching (*sensu* Fitter, 1985). The intermediate growth rules I refer to as multi-axis (MA) and constrained dichotomous (CD) branching patterns. These simulations were performed using a topologically explicit modification of Rose's (1983) numerical model of root growth (Berntson, 1995). This model requires input for time of first appearance, elongation and distance between adjacent daughter roots for each developmental order and a maximum developmental order. A summary of the input parameters used in the simulations is given in Table 2. Unlike Rose's (1983) original application of this model, the topologically explicit model creates a binary tree representation of the growing root system.

The results of these simulations are presented in Figure 5. The dichotomous simulation results in perfect linear relationships between PL order and number, with a topological scaling exponent of -1 ($r^2 = 1$). The herringbone simulation results in relationships with a topological scaling exponent of zero for PL order > 1 because there is only one link of each higher order. The topological scaling exponent of the tail end of these simulations was log(number links of order 1)/log(2). The larger the root system, the steeper the slope. The constrained dichotomous simulation resulted in number-order relationships consisting of two linear segments. The PL order which marked the boundary between these segments was equal to order 2 for small root systems (indicating that they were initially herringbone) but increased through ontogeny. The topological scaling exponent between order and number remained constant for small orders through ontogeny. The magnitude of this topological scaling exponent was decreased with increasing maximum developmental order and increasing relative elongation rates at higher developmental orders (results not shown). The multi-axis simulation showed the most complex, non-linear scaling properties between PL order and number of links per order. For the larger root systems (older simulated root systems) there were three distinct regions in the relationship between order and number. Similar to the herringbone pattern, there was a small region (from order 1 to 2) which showed a steeper slope with increasing root system size. Similar to both the constrained dichotomous and the herringbone system, the topological scaling exponent for the largest orders was zero. For intermediate orders, there was convex hump. At the low order end of this hump, the slope was nearly zero. The number of links to which this region corresponds is the number of axes within the root system. The lower orders within this system behave analogously to the herringbone pattern. The difference is that in the multi-axis system there are several herringbones being ordered simultaneously. The right hand side of the convex hump results from the multiple axes being of different sizes and thus once a certain order is reached, the constant offset from a herringbone pattern decays. To characterize these patterns in actual root systems I applied one and three change point linear models (Chappell, 1989) using a least-squares maximum-likelihood

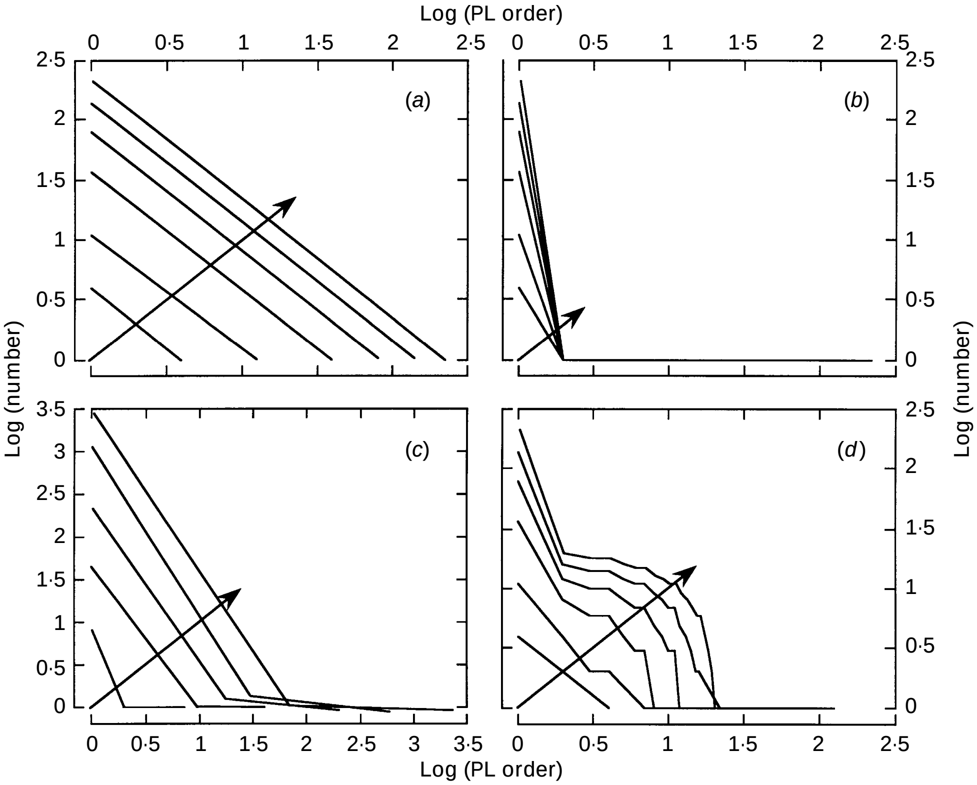

Figure 5. Summary log–log plots of number of links vs. PL order for simulated root growth. The arrow in each plot indicates the ontogenetic tracjectories of each simulation, with the oldest simulated root systems near arrow-head. (*a*) Dichotomous. (*b*) Herringbone. (*c*) Constrained dichotomous. (*d*) Multi-axis.

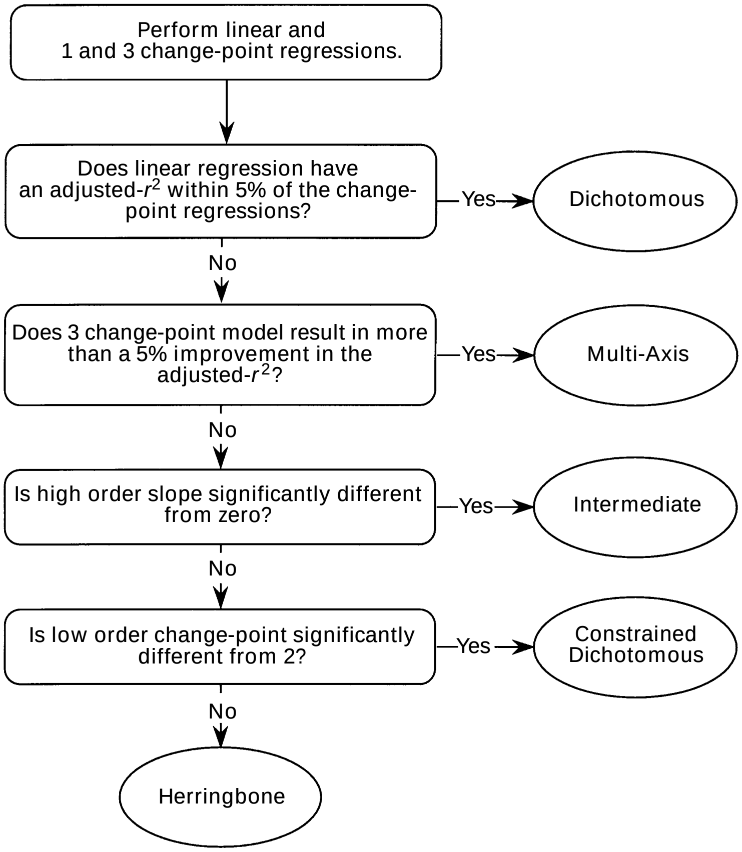

Figure 6. Schematic diagram illustrating the use of change point regressions for characterization of root systems. See Chappell (1989) for details on change-point regression techniques.

Table 3. *Summary of multiple change point regressions* (*see text and Fig.* 6 *for a discussion of terminology and methods*)

		Branching pattern (%)			
	Age (wk)	H	CD	I	MA
Senecio	2	22 (1·00)	22 (0·97)	17 (0·94)	39 (0·99)
	3	0 –	20 (0·97)	30 (0·92)	50 (0·99)
	4	0 –	15 (0·97)	10 (0·88)	75 (0·96)
Non-*Senecio*	●	3 (1·00)	72 (0·97)	16 (0·93)	9 (0·97)

Presented is the percent of all regressions that are representative of H (herringbone), CD (constrained dichotomous), I (intermediate) or MA (multi-axis) branching patterns for each age class for *Senecio* and all the other species pooled together. The average adjusted-r^2 of each set of regression is noted in parenthesis. Refer to the text for a description of criteria used to categorize change point regressions.

estimation (Press *et al.*, 1989). These two models fit multiple linear segments (two in the single change point model and four in the three change point model) to the data. The models allowed me to characterize a root system in relation to the above simulations (H, CD, & MA) on the basis of which change-point model best fit the data. A schematic diagram illustrating the characterization of root systems as one of the above simulated types through the application of the change point regressions is provided in Figure 6. An additional branching pattern is introduced in Figure 6, the 'intermediate' branching pattern. This pattern results from root systems that show no statistically discernible zero topological scaling exponent region at high orders. Instead, these systems show two non-zero scaling regions. These root systems are similar to multi-axis branching patterns, but have low curvature of the 'convex' region and a reduced zero topological scaling exponent region. These regions are pooled together in the 'intermediate' branching pattern. This approach is similar to Steingraeber & Waller's (1986) method for estimating non-stationarity in branching patterns using two-point estimates of R_b, but because this method uses a link-based ordering system it has much greater resolution and statistical power.

A summary of the change point regression method diagrammed in Figure 6 to characterize root system branching patterns is given in Table 3. Sample plots for each of the four branching patterns is given in Figure 7. In no case did any root system exhibit dichotomous branching. *Senecio* root systems exhibited each of the observed branching patterns. The young *Senecio* roots showed a large proportion of herringbone and constrained dichotomous branching patterns. By contrast, the older root systems primarily exhibited multi-axis branching patterns. The other species' root systems primarily exhibited multi-axis branching patterns.

From these data, it is clear that the PL ordering system can be used to characterize different types of branching patterns of root branching structures. Using a simple, topologically explicit model of root development I have shown that different sets of growth rules lead to distinct, easily classified variations in complex scaling relationships between PL order and the number of links. This type of analysis is more informative than simple indices of 'branching' (e.g. how 'compact' or 'elongate' a given tree is: *sensu* MacDonald, 1983), as it is possibly to quantitatively distinguish between different models of development for any binary tree. This can be a particularly difficult problem in simple indices of branching as they tend to show large variations through ontogeny, even for simple models of development (Berntson, 1995).

In addition to being able to distinguish between different models of root development, the PL ordering scheme in conjunction with the change-point curve fitting procedure has the potential for identifying discrete topological scales over which branching processes appear to be constant. An interesting question which arises from having identified these topologically distinct regions is whether they also map out onto distinct morphological or physiological differences within a root system. Root sections of different age and different root members can show pronounced physiological differentiation (Waisel & Eshel, 1991; Clarkson, 1996), and studies of whole-plant nutrient uptake have demonstrated that only a small fraction of an entire root system needs to be physiologically active in order to acquire resources from the soil (Robinson, Linehan & Caul, 1991). Do the distinct topological regions we can identify using the functional link-based ordering system correspond to regions of distinct physiological activity? If they do, then patterns of whole-root system architectural development (and thus spatial distribution of roots) and physiology are coordinated and models of root system development and function could be improved by incorporating these relationships.

Another potential application of identifying the

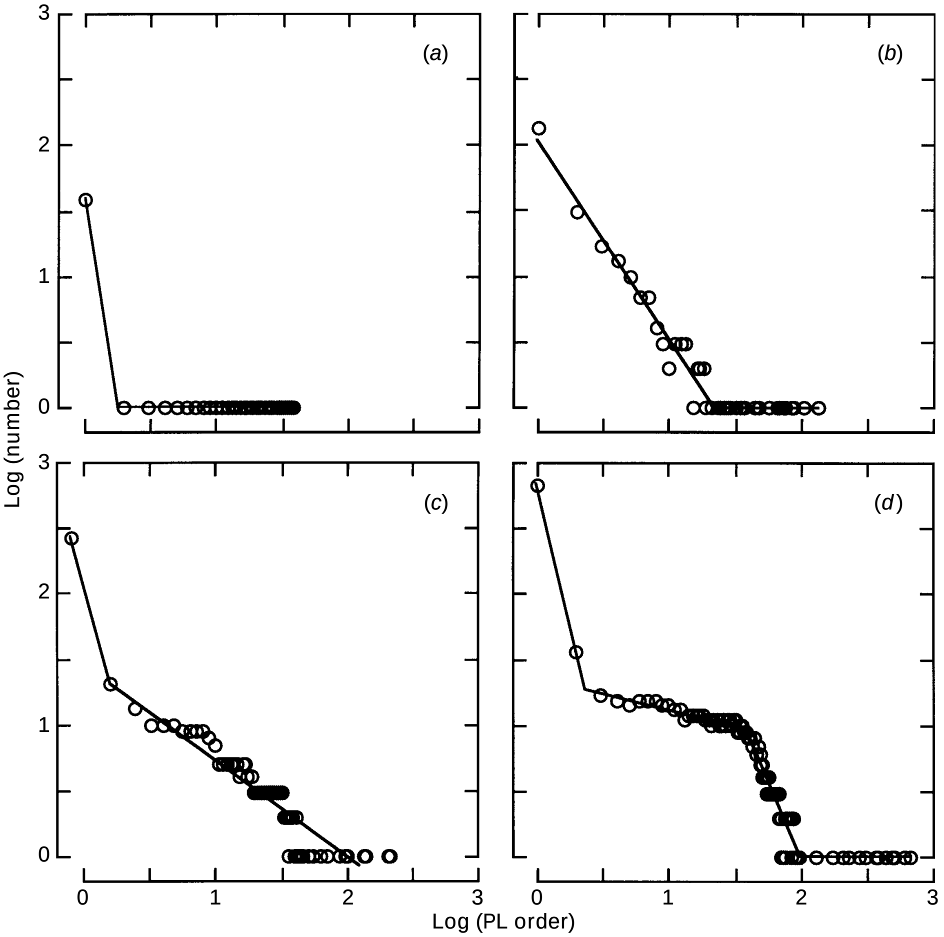

Figure 7. Sample scatterplots of Pl order vs. number of links per order for root systems characterized using the criteria diagrammed in Figure 5. (*a*) Herringbone (2-wk-old *Senecio*). (*b*) Constrained dichotomous (*Betula alleghaniensis*). (*c*) Intermediate (3-wk-old *Senecio*). (*d*) Multi-axis (4-wk-old *Senecio*).

scales of branching processes in relation to the PL ordering scheme is to increase our understanding of the spatial scales that separate root production in very large plants such as trees. The root systems of trees typically contain multiple classes of roots that differ functionally and morphologically. Large woody roots, which might contain the majority of a large tree's standing belowground mass, are long-lived and primarily responsible for structural stability and transport between fine roots and the stem (Lyford & Wilson, 1964; Lyford, 1980). By contrast, the fine roots of trees can be relatively short lived, are the primary resource uptake organs (Hendrick & Pregitzer, 1992, 1993; Fahey & Hughes, 1994; Bloomfield, Vogt & Wargo, 1996) and exhibit different branching patterns than woody roots, foraging more intensively within small volumes of soil (Lyford, 1975). For these reasons, applications of these techniques to the root systems of trees (and possibly other large, long lived perennials) might allow us to quantify the scale over which different growth processes are taking place. This information might allow us to characterize the spatial and temporal scales over which different components of these large, long lived root systems deploy their roots.

DISCUSSION

In this paper I have explored the topological scaling patterns of three different size metrics in relation to four different topological ordering systems, which represent orthogonal combinations of functional vs. developmental and link vs. segment-based ordering. The following four points summarize the results of this study.

(1) Simple, one-power-exponent scaling relationships (eqn (1)) between topological order and the size of root systems for a given order explained a greater proportion of the variation in size metrics for segment based ordering systems than for link-based ordering systems. However, because of the high number of orders in link-based ordering systems, and thus higher degrees freedom, link-based

ordering systems tended to have higher levels of significance.

(2) Different size metrics scale differently from one another, both within and between developmental vs. functional ordering systems. For the centrifugal/developmental ordering system (F), I observed good fits (eqn (1)) only for average length vs. order, and then only for the segment-based ordering system. For the centripetal/functional ordering system (P), I found good fits only for the number and total length. This was observed in both the link- and segment-based ordering systems.

(3) The segment-based functional ordering system (PS) showed the least variation in scaling with size metrics through ontogeny. Of the two size metrics which scaled with the PS ordering system, total length of segments showed less variation through ontogeny than did number of segments.

(4) The lack of a good fit for simple, one-power-exponent scaling relationships (eqn (1), as judged by r^2) between size metrics and the link-based functional ordering system (PL) was due to non-linear scaling relationships. Using change-point regressions and a simple model of root development, I demonstrated that the PL ordering system can be used to identify contrasting sets of rules regulating root development. Additionally, the change-point regression technique made it possible to identify distinct topological regions where different patterns of branching were taking place in the different developmental models.

The selection of any particular ordering system for characterizing topology and architecture within plant root systems has the potential of directly affecting 'apparent' patterns of branching. If a simple, single index of 'branching' is desired, it is important to first determine what size metric is used as the dependent scaling factor in relation to the applied ordering system. In general however, segment based ordering systems show better scaling with the various size metrics I examined, and the functional ordering system tended to show less variation through ontogeny.

The single most informative method for characterizing branching patterns I explored was the link-based functional ordering system. This ordering system showed non-linear scaling in relation to the number of links per order. Because this topological scaling method yields results which are more complicated than simple single-index characterizations of branching patterns, this method allows a more informative understanding of the processes that led to a given branching pattern. Future applications of this technique might be especially important in three different arenas of study. First, by applying a greater variety of models of development, it will be possible to further refine the ability of discriminating between different developmental histories for given root systems and possibly other branching structures. Second, the distinct topological scales derived by applying the change-point regression analysis might correspond to different distinct morphological and/or physiological regions within root systems. Such a concordance would allow for simple parameterizations of more sophisticated models of root development and function. Third, the distinct topological scales might allow us to focus our studies of plants with large root systems if these topological scales correspond to discrete space filling attributes and/or physiological activity.

A simple caveat: what root system topology can and cannot tell us about root system function

The focus of this paper is the topology of plant root systems. When we focus on root system branching patterns, we are examining only one of many aspects of root system architecture. In the absence of simultaneous measures of the geometry and size of plant root systems and the environment in which they are deployed, we cannot infer the functional significance of any particular pattern of branching. The interplay between different aspects of the form of plant root systems as they relate to whole root system architecture and function has been studied and reviewed extensively elsewhere (Fitter *et al.*, 1991; Berntson & Woodward, 1992; Berntson, 1994; Lynch & Nielsen, 1996). Studies of plant root system branching patterns, aside from contributing to an integrated view of architecture, can provide valuable insights into the developmental processes which produce root systems. This study has demonstrated a detailed examination of the relationship between the centrifugal-link-based ordering system and number of links can be used to infer developmental processes from static descriptions of root system topology.

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