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OPINION PAPER

Back to the roots: standardizing root length density terminology

Tomke S. Wacker · Frederik van der Bom · Benjamin M. Delory · Doris Vetterlein · Johannes A. Postma · Kerstin A. Nagel · Andrea Schnepf · Dorte Bodin Dresbøll

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Abstract The number of studies investigating root length has increased, particularly in the context of root length measurements observed through windows such as minirhizotrons and rhizoboxes. However, there are currently two obstacles constraining their broader utility: (1) the absence of standardized terminology or units for root length data, and (2) the translation from two-dimensional (2D) to three-dimensional (3D) data. Here, we delineate the fundamental disparities between root length measurements obtained from observation windows and via volumetric soil sampling and propose the adoption of more precise terminology to distinguish 2D planar (pRLD) from 3D volumetric (vRLD) root length density measurements. This differentiation should be accompanied with the use of standardized units and should not endeavour to make blanket conversions between dimensions unless this is supported by specific calibration data.

Keywords Root length · Root length density · Minirhizotron · Rhizobox · Terminology · Root imaging

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T. S. Wacker (✉) · F. van der Bom · D. B. Dresbøll
Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark
e-mail: tsw@plen.ku.dk

B. M. Delory
Environmental Sciences Group, Copernicus Institute of Sustainable Development, Utrecht University, Utrecht, The Netherlands

D. Vetterlein
Department Soil Physics, Helmholtz Centre for Environmental Research-UFZ, 06120 Halle (Saale), Germany

J. A. Postma · K. A. Nagel
Plant Sciences (IBG-2), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

A. Schnepf
Agrosphäre (IBG-3), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

Standardizing terminology considering data dimensionality

In recent years, two technological advances have dramatically enhanced the ability to collect root information. Firstly, the establishment of large phenotyping platforms now allows for semi- or fully automated root imaging, often in high-throughput (Nagel et al. 2012; Rasmussen et al. 2020; Svane et al. 2019a; LaRue et al. 2022; Nair et al. 2023). These include diverse setups such as high-throughput rhizobox platforms (Nagel et al. 2012), outdoor rhizoboxes with a large soil volume (Rasmussen et al. 2020), root windows installed vertically in-field (Vetterlein et al. 2021), and in-field minirhizotron facilities (Rajukar et al. 2022, Svane et al. 2019b; Cai et al. 2016).

Secondly, deep learning approaches have enhanced our ability to segment root structures from 2D images, even from highly heterogeneous backgrounds (Wang et al. 2019; Smith et al. 2020; Narisetti et al. 2021; Zhao et al. 2022; Baykalov et al. 2023); and new open-source software tools (e.g. Smith et al. 2022) have made this technology readily available. As a result of these developments, it is now possible to acquire large numbers of high-resolution root images (Svane et al. 2019a; Rajurkar et al. 2022) on which high-throughput image processing pipelines can be applied to segment roots and extract features with minimal intervention by the user (Seethepalli et al. 2021; Bauer et al. 2022; Alonso-Crespo et al. 2023). As these advancements in technology are poised to accelerate the collection of root data, it has become more urgent than ever before to agree and standardize terminology and units. Precise, standardized terminology is critical for researchers to compare ideas and research findings, formulate new theories, pinpoint research inquiries and collaborate across disciplines; and is important in large-scale analyses like systematic reviews and meta-analyses, for which consistent terminologies across studies are a key requirement.

The trait most often extracted from images is *root length*. However, the term *root length* is commonly applied to various measurements from three-dimensional (3D) volumetric soil samples to two-dimensional (2D) windows, despite their differences. To clarify the nature of their measurement, scientists have included various descriptors such as *visible* (Nagel et al. 2012; Bodner et al. 2019), *projected* (Endo et al. 2019), or *captured* (Bourgault et al. 2022), signalling that their root length data was collected from observation windows (Table 1). However, these prefixes tend to describe the process of data acquisition, rather than the fundamental 2D property of the data. Another recurring term in the literature is *root length intensity* (Machado and Oliveira 2003, 2005; Othman and Leskovar 2019; Leskovar and Othman 2021; Table 1), which implies a certain quantity per unit area. Presumably, its use stems from earlier methods in which root length was determined by counting the number of intercepts across a grid (Newman 1966; Tennant 1975). This method was applied to minirhizotron studies (Upchurch 1987) and the count of intersections per gridline length has been reported as root intensity (Thorup-Kristensen 2001). Yet, the term intensity can also be used for the trench profile method (Böhm 1979). Here, *intensity* refers to the number of root segments i.e. counts per unit area (e.g. Bublitz et al. 2022) and the term is thus ambiguous.

Apart from the dimensional properties of the data, the observed root length itself is inevitably influenced by the imaging dimensions. These can vary substantially among approaches, for example, image dimensions of minirhizotron studies in literature vary between 2.43 cm^2 and 400 cm^2 (Cai et al. 2016; Postic et al. 2019; Svane et al. 2019b; Rajurkar et al. 2022). Despite this influence of sample dimensions, studies have often reported *total root length*, being the cumulative length of all imaged roots (e.g. Lemming et al. 2016; Bodner et al. 2019; Bourgault et al. 2022; Chiteri et al. 2022). However, there is no clear differentiation between the total measured root length and the total root length of the entire plant, which can lead to confusion, as the imaged root length will always be smaller because not all roots are visible at the observation window (Kuchenbuch and Ingram 2002; Nagel et al. 2012; Alsalem et al. 2021). Consequently, it would be more precise to explicitly refer to *root length* in context of the sample origin i.e. the *root length* [cm] that is visible within the image [cm²] (Johnson et al. 2001). This implies standardizing root length per unit of image area (cm cm^{-2}), analogous to the way root length data from soil coring is commonly standardized per unit of soil volume as *root length density* (RLD [cm cm^{-3}]).

Following these considerations, we propose using the term *planar* to describe a 2D sample origin, resulting in *planar root length* (pRL [cm]) and *planar root length density* (pRLD [cm cm^{-2}]) depending on the property of interest (Table 2). *Planar* relates to objects lying in a plane and thus fundamentally describes a two-dimensional image environment. Mathematically, a plane can exist in a 3D space, and can be curved - corresponding to the surface of a minirhizotron (which has zero Gaussian curvature). Moreover, *planar* avoids ambiguity where other potential terms or prefixes such as *surface* or *area* are also found in other existing root system architectural traits. We acknowledge the long history and sustained use of *root length density* (cm cm^{-3}) in 3D (volumetric) sampling studies, emphasising that the use of *Root length density* for data obtained from 2D images should not be used. Where required, the prefix *volumetric* may be used for explicit distinction between

Table 1 A selection of recent scientific literature investigating root length from observation windows, the applied terminology for root length, their unit, abbreviation when used, and the method used to observe roots. Criteria for selection were root data shown as length measurement, originating from two-dimensional capturing methods. This selection is not exhaustive but rather aims to show the diversity of terminology and units of root length data from observation windows

Used Terminology		Unit	Abbr.	Method	Reference
Root length		cm	–	Minirhizotron	(Clément et al. 2022; Wacker et al. 2022)
		mm cm−2			(Kulmatiski et al. 2017; Peters et al. 2023)
Total root length		cm	TRL	Rhizobox	(Chiteri et al. 2022)
		–	–	Minirhizotron	(Williams et al. 2022)
		m m−2			(Cai et al. 2018)
Total (visible) root length		cm	–	Rhizobox	(Bodner et al. 2019)
Total root length (captured)		mm	–	Minirhizotrons	(Bourgault et al. 2022)
(Projected) root length		mm cm−2	–	Rhizobox	(Endo et al. 2019)
Root length density		cm cm−2	RLD	Minirhizotron	(Morandage et al. 2019; Bauer et al. 2022)
	*	cm cm−3			(Sullivan and Welker 2005; Brown et al. 2009; Garré et al. 2011; Liao et al. 2015; Xiao et al. 2020; Li et al. 2021; Rajurkar et al. 2022; Geng et al. 2023)
			RLDv		(Haarhoff et al. 2021)
	**		–		(Bieluczyk et al. 2021)
	***		RLD	Rhizobox	(Inostroza et al. 2020)
Areal root length density		cm cm−2	–	Minirhizotron	(Johnson et al. 2001)
			RLDa		(Vamerali et al. 2009)
Normalized root length density	**	cm−1	NRLD		(Cai et al. 2017)
Root intensity		cm cm−2	–	Rhizobox	(Chen et al. 2022)
Root length intensity		mm cm−2	La	Minirhizotron	(Othman and Leskovar 2019; Leskovar and Othman 2021)

Table 1 (continued)

Used Terminology	Unit	Abbr.	Method	Reference
	cm cm−2			(Machado and Oliveira 2003, 2005)
Root length surface density	mm cm−1	RLSD	Minirhizotron	(Noor et al. 2022)
	mm cm−2			(Postic et al. 2019; Louvieaux et al. 2020)

* raw data has been converted with a conversion factor based on the assumption that depth of view equals 1 mm (Garré et al. 2011); 2 mm (Brown et al. 2009; Liao et al. 2015; Rajurkar et al. 2022); 2.5 mm (Xiao et al. 2020; Li et al. 2021; Gengt et al. 2023); or 3 mm (Sullivan and Welker 2005)

**raw data has been converted using a conversion factor

***raw data has been converted by dividing the root length observed on the outside of a rhizotron by the volume inside the rhizotron

planar and 3D sample origins, giving *volumetric root length* (vRL [cm]) and *volumetric root length density* (vRLD [cm cm^{-3}]).

Linking two-dimensional and three-dimensional data

A major challenge of measuring root traits is how to scale from the observed ‘subsamples’ to the entire root system in order to understand the biological function and plasticity of root systems. This challenge may be particularly relevant for planar root traits due to the different dimensions between the 2D observation and the 3D environment.

Some researchers have used conversion factors to go from planar to volumetric root data, based on geometrical considerations such as the depth of view into the soil matrix (Taylor et al. 1970; Sanders and Brown 1978; Brown et al. 2009) or the position of the viewing plane with respect to the anisotropy of 3-dimensional root growth (van Noordwijk 1985). However, we have not found any scientific validation for this approach, and we question whether this straightforward conversion between two distinct properties with different dimensions makes sense. A large array of physiological processes and interactions determine root growth and development in any given soil environment. Further, the physical barrier of an observation window can alter the root growth. Consequently, conversion factors, would need to be more complex than a simple, single factor per plant or experiment to take the resulting variation into account. Indeed, parameter calibrations have been proposed for soil texture, plant species, root diameter, environmental conditions and soil depth (Upchurch 1987; Box Jr and Ramseur 1993; Samson and Sinclair 1994; De Ruijter et al. 1996; Brown et al. 2009; Taylor et al. 2014; Machado and Oliveira 2003; Bublitz et al. 2022), although these rarely seem to be applied.

Two dimensional methods such as minirhizotrons or rhizoboxes will also introduce biases. Firstly, the observation plane creates a physical obstacle for roots, which can trigger plastic responses and change rooting patterns (Böhm et al. 1977; Downie et al. 2015; Wahlström et al. 2015; Pandey et al. 2021). Further, the experimental setup (e.g. angle of rhizobox (Nagel et al. 2012), pot size (Poorter et al. 2012) and genotype

Table 2 Two varying root length density terms

Scientific term:	(volumetric) Root length density*	planar Root length density
Abbreviation:	(v)RLD	pRLD
Sample origin:	Soil/substrate volume	Planar surface (image, observation window, etc.)
Dimensionality:	three-dimensional	two-dimensional
Unit:	cm cm^{-3}	cm cm^{-2}

*Since root length density (RLD) is a standardized term used for volumetric root length data (Atkinson 2001), we suggest the use of RLD in purely volumetric sampling studies. The term volumetric root length density (vRLD) offers the possibility to explicitly distinguish volumetric and planar observations in the same study

(Correa et al. 2022) can influence what proportion of the root system is visible against the transparent window, and can influence gravitropicity (Liao et al. 2004), anisotropy (Chopart and Siband 1999) and the distribution of roots along different diameter classes (Pierret et al. 2005). As a result, root length along an observation plane may not necessarily be representative of roots growing in the bulk soil. Note that our criticism relates primarily to the attempts to convert planar data as volumetric ones and not to the use of planar systems for root observations.

Volumetric soil sampling poses challenges and significantly underestimates root length, due to loss of fine roots during washing procedures (Noordwijk et al. 1985). Further, as with planar sampling volumetric sampling is affected by the sensitivity to spatial variation (Burridge et al. 2020), and limitations in image analysis techniques for scanned roots (Delory et al. 2017). Similarly, 3D imaging techniques such as X-ray CT, MRI, and neutron tomography have resolution and quality limitations that can limit the detection of fine roots (Vetterlein et al. 2021; Hou et al. 2022). Thus, 2D sampling is likely to overestimate fine root length, while 3D sampling is likely to underestimate fine root length. These diverging biases make conversion between 2D and 3D measurements unreliable, as it is inherently difficult to account for unobserved data.

Considering the above, any approach that uses a simple conversion factor to transfer 2D data to 3D information cannot be justified based on the current knowledge and understanding of root system development. Rather, we encourage scientists to collect 2D data and 3D data *together* to develop greater understanding about how planar 2D observations relate to plant root growth and function in a 3D soil environment. Along these lines a few previous studies have combined the minirhizotron method and the core method (Gregory 1979; Upchurch and Ritchie 1983; Heeraman and Juma 1993; Wahlström et al. 2015) or the profile wall method with volumetric sampling (Bublitz et al. 2022; Vansteenkiste et al. 2014). Even if such data may not lead to new procedures or factors to directly convert between data dimensions, they should deliver valuable insights into root system growth and function, and/or may assist with development of future process-based models. Our suggested improved terminology for root length measurements may support this endeavor by providing a clear framework for the distinction between methods.

Conclusions

We propose adoption of the terms 'planar root length density (pRLD)' and 'volumetric root length density' (vRLD), depending on the measurement context. This terminology provides a direct link to the sample dimensionality (2D vs. 3D), which should prevent data misinterpretation. At the same time, data normalization over the sampling frame gives standardized units. Further, we discussed challenges regarding translation between different measures, dimensions, and scales. Translation between pRLD and vRLD data is complex and system-dependent and misinterpretation of data will arise from using simplistic conversion factors to translate 2D measurement data into 3D space.

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