Plant Soil (2017) 418:129–139
DOI 10.1007/s11104-017-3358-y

COMMENTARY

Unwrapping the rhizosheath

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Received: 29 June 2017 / Accepted: 20 July 2017

Keywords Mucilage · Nutrient acquisition · Rhizosheath · Rhizosphere · Root hair · Water uptake

Introduction

In this commentary, we consider the novel results presented by Brown et al. (2017) in their article in this Issue of *Plant and Soil* titled "The rhizosheath – a potential trait for future agricultural sustainability occurs in orders throughout the angiosperms". Brown et al. (2017) report a robust method for screening the presence/absence and strength of rhizosheaths. They then use this method to identify the presence of rhizosheaths in many plant species across many angiosperm orders, leading them to suggest a central role for the rhizosheath in the breeding of crops for future agricultural systems. Given these findings, we present and discuss some issues that require consideration if we are to advance research on the rhizosheath for practical application.

Distinguishing the rhizosheath and rhizosphere: Conceptual confusion?

The rhizosphere is often defined as the volume of soil influenced by the root, a concept first introduced in 1904 by Lorenz Hiltner (Hiltner 1904). The rhizosphere, therefore, consists of the zone of soil surrounding plant roots characterised by root exudation and an abundance of microorganisms, along with the soil that is influenced by the root in other ways (e.g. the zone of nutrient depletion) (Fig. 1). Microbes in the rhizosphere include saprophytic, pathogenic and symbiotic bacteria and fungi (Mathesius 2015). As most land plants are colonised by mycorrhizal fungi (Brundrett 2009), the rhizosphere concept has been extended to include the volume of soil influenced by the hyphae of mycorrhizal fungi external to the root; this soil volume has been named the 'mycorrhizosphere' (Mathesius 2015) (Fig. 1).

The rhizosheath first appeared in the literature in 1887 (Volkens 1887) and was described as present in desert grasses in 1911 (Price 1911). Originally, the rhizosheath was defined as a "peculiar sheath, composed of agglutinated particles of sand" (Volkens 1887), while now it is often defined as the weight of soil that adheres to roots when they are removed from the pot or field (McCully 1999; George et al. 2014). Therefore, the distinction between the terms rhizosheath and rhizosphere is that rhizosheath refers to the soil that

Responsible Editor: Hans Lambers.

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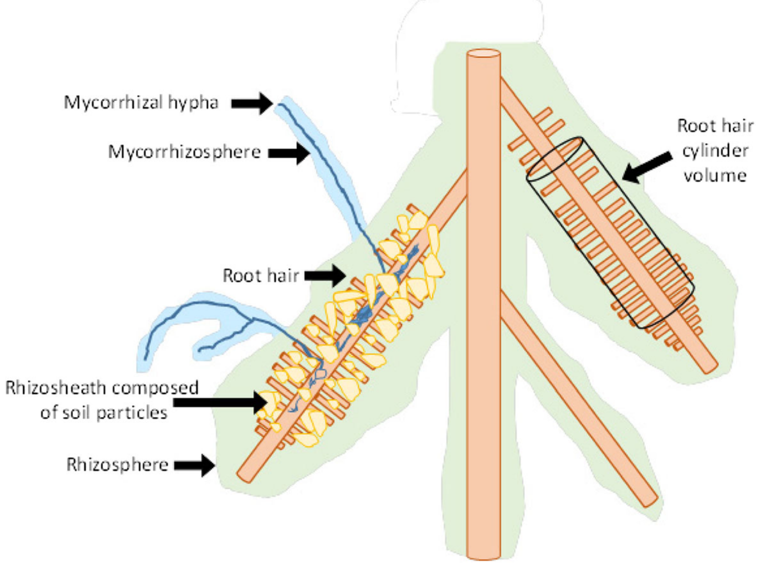

Fig. 1 A diagram showing the zones around a root occupied by the rhizosheath and rhizosphere in the presence and absence of root hairs. The mycorrhizosphere is also shown. The zone defined as the root hair cylinder volume (Yang et al. 2017) is also shown

physically adheres to the root system, while rhizosphere refers to the soil influenced by the root (Mathesius 2015), such that the rhizosphere extends beyond the bounds of the rhizosheath (York et al. 2016) (Fig. 1).

How to quantify the rhizosheath?

Since 2000, the number of studies focused on processes in the rhizosphere has increased significantly (Tibbett et al. 2012). In many cases, the rhizosphere soil has been defined as the soil that remained attached to the roots after the root systems were carefully excavated and gently shaken (e.g. Veneklaas et al. 2003; Pang et al. 2010, 2015; Ryan et al. 2012; Hilton et al. 2013; Zimmermann et al. 2016). This is technically the rhizosheath and not the rhizosphere, although it does contain a proportion of the rhizosphere soil. This method of capturing the rhizosphere soil is used because it is rapid, easy and does not require complicated equipment or methods. We contend that it is time to insist that soil collected in this way be referred to as rhizosheath soil. A similar plea has been made recently by York et al. (2016) who decry various issues of 'rhizosemantics' and advocate for more accurate descriptions of soil sampled in the vicinity of roots (Table 1).

It should also be noted that terminology differs among studies and with the method used to collect rhizosheath and rhizosphere soil. For example, Delhaize et al. (2012) defined the rhizosheath of wheat (*Triticum aestivum* L.) as the soil adhering to roots after excavation and without shaking or any other treatment. Prendergast-Miller et al. (2014) defined the rhizosphere soil of barley (*Hordeum vulgare* L.) as the soil removed from roots by gentle shaking and the remaining attached soil as the rhizosheath. Swanson et al. (2009) removed plants from field soil and defined the loose soil removed by gentle shaking as bulk soil. Soil removed with more vigorous shaking and by forceps was defined as rhizosphere soil. Swanson et al. (2009) also defined the soil particles attached to the basal regions of nodal roots as an intact sheath as rhizosheath. Brown et al. (2017) defined the

Table 1 The definition of rhizosphere and rhizosheath

Term	Definition	Reference(s)
Rhizosphere	The volume of soil influenced by the root	Hiltner 1904
Mycorrhizosphere	The volume of soil influenced by the hyphae of mycorrhizal fungi external to the root	Mathesius 2015
Rhizosheath	Originally as "a peculiar sheath, composed of agglutinated particles of sand"	Volkens 1887
	The soil that physically adheres to the root system and binding materials such as mucigel	McCully 1999; George et al. 2014; York et al. 2016

rhizosheath as any soil attached to freshly excavated roots after 'hand shaking'. By prescribing soil removal treatments of increasing vigour (hand shaking, sonication in water), they defined four categories of strength of adhesion to roots. 'True rhizosheaths' were regarded as soil that could not be shaken or washed from the root without sonication. Their categorisation of rhizosheath strength was analogous, but different, to that of Watt et al. (1994) who used sonication ('weak'), hot (65 °C) water ('medium') and abrasion ('strong binding') to progressively dislodge rhizosheath soil from roots.

The units used to report rhizosheath size are equally diverse. For example, rhizosheath thickness (Hartnett et al. 2013), volume of sheath per volume of root (Watt et al. 1994), grams of moist soil adhering to roots plus root fresh mass per unit root length (Delhaize et al. 2012), grams of dry soil per metre of root (Haling et al. 2010b) and grams of dry soil per gram of root fresh mass (Fernández Bidondo et al. 2012; Brown et al. 2017). Brown et al. (2017) termed the latter as 'specific rhizosphere weight'. However, for comparisons among diverse species, it might be less informative than expressing rhizosheath weight as grams of dry soil per metre of root, due to the impact of differences in root diameter on the former. The inconsistency of units makes the comparison of rhizosheath size among studies difficult. This confusing range of measurement units also flows onto other rhizosheath-related properties. For instance, the amount of carboxylates measured in the rhizosheath may be reported as per gram of rhizosheath dry weight (Rose et al. 2010), per gram of root dry or fresh weight, or per unit of root length (Kidd et al. 2016); differences among plant species may vary greatly depending on the unit that is used (Kidd et al. 2016).

There is a clear need for the use of standard terminology that is less open to interpretation when distinguishing rhizosphere and rhizosheath soil, and for definitions of rhizosheath existence, size and strength based on soil adhesion. Improved methodology and consistent terminology would greatly aid the development of a more cohesive literature.

The formation of the rhizosheath in angiosperms

Smith et al. (2011) performed a global survey on the rhizosheath trait in a phylogenetic context in the family Haemodoraceae using field-sampled and herbarium specimens. They found that most of the genera and species possessed 'sand-binding' roots and that only two of the 14 genera in Haemodoraceae contained sister taxa with and without the trait. The survey undertaken by Brown et al. (2017) expanded this work by covering one-third of angiosperm orders and demonstrated that the rhizosheath trait occurs throughout the angiosperms.

The formation of a rhizosheath appears to require two factors—root hairs and mucilage. Root hairs are single-celled tubular extensions to plant roots which greatly increase plant–soil contact and thereby enhance nutrient uptake; they also enmesh soil particles around roots and presumably provide an important physical framework for the extending rhizosheath (Watt et al. 1994; Moreno-Espíndola et al. 2007; Haling et al. 2010a, 2014; Brown et al. 2012; Delhaize et al. 2012; George et al. 2014). Significantly, Brown et al. (2017) found that among the 58 species examined, those that lacked root hairs also had no rhizosheath. McCully (1999) also noted that rhizosheaths only developed where root hairs were present and thus concluded that exudation of mucilage alone is not sufficient for rhizosheath formation. Consistent with this conclusion, the rhizosheath of maize (*Zea mays* L.) mutants with root hair length only 5–10% of the wild type was considerably smaller than the wild type when grown in soil for 60 days (Wen and Schnable 1994). Similarly, rhizosheath weights for mutant phenotypes of barley with either short or long root hairs were an order of magnitude greater than those for mutants lacking root hairs (Haling et al. 2010a; George et al. 2014). Interestingly, it was recently shown by Pausch et al. (2016) that rhizosphere priming, i.e. the decomposition rate of rhizosphere organic matter, differed between wild-type barley and a mutant lacking root hairs in a manner that changed over time. This suggests a complex interaction between root hairs and rhizosphere microbial populations and that the importance of root hairs for rhizosheath formation may be mediated by their influence on rhizosphere microbes.

The gelatinous mucilage that associates with soil particles to form a rhizosheath arises from complex polysaccharides and glycoproteins of microbial and root origin. Mucilage of plant origin has been found at root caps and along the periphery of roots growing through the soil (Vermeer and McCully 1982). Watt et al. (1993) found that mucilage of both plant and bacterial origin contributes to the adhesion and cohesion of maize rhizosheaths, but different mechanisms are involved for root-cap mucilage. Root-cap mucilage is mainly composed of polysaccharide molecules with many complex oligosaccharide branches that have neutral sugars at their terminals: soil adhesion by this mucilage is primarily via hydrogen bonds between the hydroxyl groups of these neutral sugars and the soil particles (Watt et al. 1993). In contrast, bacterial mucilage has a higher protein component and therefore binds soil by different mechanisms; it is also more hydrophilic than root-cap mucilage (Watt et al. 1993). Brown et al. (2017) found that the roots of some species were much more hydrophobic than others, implying a difference in the composition of root exudates, and thus an effect on the ability of roots to bind the soil. Further study of the relative contribution of microbial and root mucilage to rhizosheath formation is required if we are to better understand the factors conducive to rhizosheath formation.

The relationship between rhizosheath formation and root hair length

As the presence of root hairs is critical for rhizosheath formation, it would seem logical that longer root hairs would be associated with larger rhizosheaths. Indeed, in some studies, rhizosheath size has proved a reliable surrogate for root hair length. For instance, Delhaize et al. (2012) demonstrated a strong correlation between rhizosheath specific weight (grams of soil and fresh root per metre of root length) and root hair length among wheat genotypes. The relationship was further substantiated in wheat multiparent advanced generation intercross (MAGIC) populations where the correlation was robust across soil types in the absence of chemical constraints (Delhaize et al. 2015).

Nevertheless, there are conflicting findings on the relationship between rhizosheath size and root hair length. Rhizosheath size in barley was poorly correlated with root hair length, suggesting factors other than root hair length contributed significantly to rhizosheath formation (George et al. 2014). Haling et al. (2010b) also examined this relationship using a limited number of barley and wheat lines by regressing rhizosheath size (g/m) against the volume of the root hair cylinder (defined as the annulus around roots delineated by root hair length – Fig. 1). Variation in root hair cylinder volume only explained 52% of the variation in rhizosheath size in wheat and 66% of the variation in barley. Among 100 chickpea (*Cicer arietinum* L.) genotypes with various origins and diverse genetic backgrounds, no correlation was evident between the specific rhizosheath weight (grams of dry soil per gram of root dry weight) and root hair length ($R^2 = 0.001$, $P > 0.05$, Jiayin Pang et al., unpublished data).

Brown et al. (2017) fit a linear model to the relationship between specific rhizosphere weight (grams of dry soil per gram of root fresh weight) and root hair length across a wide range of species. However, the relationship was extremely poor, and the scatter plot indicated that relationships between root hair length and rhizosheath size vary substantially among plant species. Indeed, these relationships may vary considerably within a species. For example, Fig. 2 shows a two-fold difference in specific rhizosphere weight (gram of dry soil per gram of root dry weight) among six chickpea genotypes, all with a mean root hair length of 0.35 mm (Jiayin Pang et al., unpublished data). The reasons for such differences are unknown but could include more subtle variations in root morphology than mean root hair length, such as root hair density (Watt et al. 1994) or variation in rhizosphere properties due to root physiological processes such as exudation of mucilage and other compounds, rhizosphere soil moisture and rhizosphere pH, and the impact of all of these on rhizosphere microbial communities.

Rhizosheaths and tolerance of edaphic stresses

Water uptake in drying soil

Rhizosheaths were first noted on drought-tolerant plants from semi-arid environments. So it has been surmised

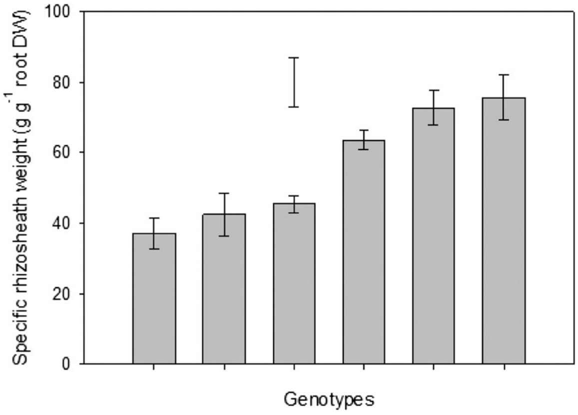

Fig. 2 Large variation in specific rhizosheath weight among chickpea genotypes with a mean root hair length of 0.35 mm when grown in washed river sand for seven weeks with a low soil P level of 10 μg P g^{-1} dry soil (mean ± s.e.m., $n = 4$, LSD at $P = 0.05$)

from very early times that the rhizosheath assisted plants to cope with dry soil conditions (e.g. Volkens 1887; Price 1911; Hartnett et al. 2013). The formation of the rhizosheath mitigates against the development of air gaps around roots of desert species that shrink radially in response to moisture deficit (North and Nobel 1997). The hydraulic resistance of root–soil air gaps is high, and their virtual elimination by the presence of a rhizosheath protects root–soil contact and the potential for water uptake (North and Nobel 1997). Thick rhizosheaths also encase hydrated, dormant roots of perennials that persist through long and severely-droughted summer periods e.g. for the rush (*Lyginia barbata* R.Br.) (Shane et al. 2010) and perennial grasses adapted to desert dunes (Danin 1996). In these contexts, rhizosheaths assist the survival of plants in harsh, dry environments.

In species less known for their extreme drought tolerance, larger rhizosheaths are also formed under dry soil conditions. *Zea mays* produces more root hairs in dry soil and this, combined with a suspected increase in the adhesiveness of mucilage, results in rhizosheaths that are more coherent and bind more strongly to the root than rhizosheaths formed in wet soil (Watt et al. 1994). It is the contention of Brown et al. (2017) that the rhizosheath on species used in agriculture may also improve the resilience of plant production systems under drought stress.

The exact mechanism by which the rhizosheath assists water acquisition in drying soil remains open to discussion. A large body of evidence indicates that the release of mucilage into the rhizosphere from both the plant and rhizosphere microorganisms helps to enmesh soil particles within the root hair cylinder and substantially modifies the hydraulic properties of the rhizosheath (Kroener et al. 2014; Ahmed et al. 2016). Soil mixed with mucilage maintains a higher water content for any given negative matric potential, has lower saturated hydraulic conductivity, and has different drying and wetting rates than soil alone (Kroener et al. 2014). The mucilage-enriched rhizosheath becomes hydrophobic under drought (Ahmed et al. 2016). Some of these properties may, at least in part, account for the often observed higher moisture content of rhizosheaths relative to the bulk soil in a drying soil (Young 1995; North and Nobel 1997; Benard et al. 2016). North and Nobel (1997) observed that the water content of the rhizosheath was more like that of the root than that of the drier surrounding soil. However, given the matric potential gradient between a root and drying soil, the retention of moisture by the rhizosheath is difficult to explain unless it varies diurnally and the nocturnal water efflux from roots is involved (e.g. McCully 1995). It has been concluded that by keeping the rhizosheath 'wet' and 'conductive', mucilage decreases the matric potential gradient between the bulk soil and the root rhizosheath–soil interface enabling plants to maintain an 'open' path for water uptake and to access more water by drying the soil to a lower water content than would otherwise be possible (Ahmed et al. 2016; Carminati et al. 2016). However, is this entirely the case? If mucilage in the rhizosheath is the conduit for water uptake, then there should be little to stop the rhizosheath from drying as a result of transpiration to moisture contents that approach that of the surrounding drier soil. The extent to which the rhizosheath dries presumably depends on the matric potential of the rhizosheath soil–mucilage mixture.

A recent evaluation of the role of root hairs in water uptake by barley using the cultivar Pallas (with root hairs) and its *brb* mutant Baldy (without root hairs) identified a significantly reduced capacity for water uptake by the hair-less mutant in drying soil (Carminati et al. 2017). The authors argued that root hairs facilitate water uptake by reducing the matric potential gradient (as argued previously for mucilage in the rhizosheath) because they greatly expand the interface between the root and the soil (i.e. effectively the rhizosheath surface – Fig. 1). As such, root hairs are envisaged to act as a conduit of least resistance for water flow from the rhizosheath–soil interface to the root (i.e. water will flow more easily through root hairs than through soil and, perhaps, the mucilage-embedded rhizosheath depending on its state of 'dryness'). Perhaps, root hairs and the mucilage-filled rhizosheath both facilitate water uptake but to different degrees throughout the day as mucilage becomes drier with transpiration. Indeed, Carminati et al. (2017) suggest that drying of mucilage in the rhizosheath may be a possible explanation for hysteresis in measurements of suction in the xylem when the transpiration rate increases and then falls (Carminati et al. 2017).

Irrespective of these speculations, a diurnal cycle in the wetting and drying of mucilage is potentially necessary for the formation of the rhizosheath. McCully (1995) demonstrated nocturnal water efflux from root epidermal and root hair cells and argued that this would encourage mucilage expansion into the rhizosheath and that subsequent drying during transpiration would convert the mucilage into an adhesive that binds rhizosheath soil particles together and to the root.

Nutrient acquisition

North and Nobel (1997) commented that the ability of rhizosheaths to delay the appearance of root–soil air gaps in drying soil should also have benefits in terms of nutrient uptake. The association of the rhizosheath with the maintenance of water uptake in dry conditions should be advantageous, in particular for nutrients acquired primarily via mass flow (e.g. N). They have also been shown, on plants in nutrient-impoverished soils, to host microorganisms capable of associative N-fixation (e.g. Wullstein 1980). There is also clear evidence that the rhizosheath can assist roots in very dry soil to maintain appreciable rates of nutrient uptake provided the plant has at least partial access to water and remains hydrated. For example, roots of oats (*Avena sativa* L.) in a dry soil layer developed a substantial rhizosheath and were able to absorb Zn from the dry soil (<−1.5 MPa suction) at a rate equivalent to ~40% of that achieved in moist soil (Nambiar 1976). However, it is less clear whether this benefit of a rhizosheath can substantially improve tolerance of nutrient stress when nutrient supply is determined predominantly by diffusion (e.g. P deficiency). In a drying soil, tortuosity in the diffusion pathway from the bulk soil to the rhizosheath–soil interface will inevitably constrain the acquisition of P (e.g. Barber 1984).

There are many examples of how root hairs improve P acquisition in P-deficient soils (e.g. Gahoonia et al. 2001; Brown et al. 2012) and of the analogous benefits for P nutrition of plants with longer root hairs irrespective of whether the differences are determined genetically (e.g. Gahoonia and Nielsen 2004; Miguel et al. 2015; Haling et al. 2016) or by environmental influences on root hair length and consequently rhizosheath size, e.g. adverse impacts of soil acidity of rhizosheath size and root hair length (Delhaize et al. 2009; Haling et al. 2010a). Long root hairs allow the development of a large root-hair-cylinder surface area and facilitate the interception of P diffusing towards the root (e.g. Gahoonia and Nielsen 1997; Haling et al. 2016). The root hair cylinder will, in many cases, be roughly analogous to the rhizosheath (Haling et al. 2010b) (Fig. 1). The unanswered question is the extent to which the rather special edaphic environment of the rhizosheath per se assists or protects nutrient uptake by the root and root hairs under these circumstances.

Given that some plant species do not appear to have a rhizosheath and many others only support small rhizosheaths irrespective of their root hair lengths (Brown et al. 2017), the role of the rhizosheath in stress tolerance remains an interesting conundrum.

The importance of mycorrhizal fungi to rhizosheath formation

Many crop plants, including those in the Poales and Fabales where Brown et al. (2017) show rhizosheaths are particularly common, are colonised by arbuscular mycorrhizal (AM) fungi (Thompson and Wildermuth 1989). As AM fungi have long been considered to play an important role in soil structure, it is necessary to assess if they perhaps make a significant contribution to rhizosheath formation.

Hyphae of AM fungi are thought to both physically entangle soils and exude the glycoprotein glomalin which, when released into the soil, has widely been assumed to form glomalin-related soil protein (GRSP) (Zou et al. 2016). The level of root colonisation by AM fungi, the length or density of their external hyphae, and the concentration of GRSP have all been reported to correlate positively with soil aggregate stability or aggregate mean weight diameter (Moreno-Espíndola et al. 2007; Ryan and Kirkegaard 2012; Zou et al. 2016). However, strong evidence has emerged that AM fungi do not play a major role in the formation of GRSP (Walley et al. 2013). Rillig et al. (2015) recently acknowledged that aggregation is a complex, poorly understood process with contributions potentially from many root and mycorrhizal traits. The role of AM fungi in rhizosheath formation or stability has been little researched, but the examples presented below suggest that they are not a significant contributor.

Moreno-Espíndola et al. (2007) used microscopy to measure the proportion of sand particles adhered to by each of root hairs and hyphae in three crops grown in the field. They found that enmeshment of soil particles by root hairs to be far greater than enmeshment by hyphae (e.g. a ratio of 40:1 under maize). On a broader scale, Hartnett et al. (2013) studied 18 grass species in a semi-arid savanna of the Kalahari region of Botswana. They found that drought-tolerant and shorter-lived grasses had greater rhizosheath thickness and fine root development than later successional climax grasses and those characteristic of wetter sites. However, the drought-tolerant and shorter-lived grasses had lower colonisation by AM fungi, and across all grasses, there was no correlation between rhizosheath thickness and colonisation level.

Given the above, it may simply be the case that rhizosphere microbes other than AM fungi play a more important role in rhizosheath formation due to greater abundance or exudate production. For instance, when Fernández Bidondo et al. (2012) compared the impact of inoculation of maize with *Bacillus megaterium* with inoculation with two strains of AM fungi, only *B. megaterium* enhanced the specific rhizosphere weight (grams of dry soil per gram of root fresh mass) (up to six-fold). If AM fungi do impact rhizosheath formation, it likely occurs through quite complex processes including potentially negative ones such as reductions in root exudation or root hair length when the fungi are present (Ryan et al. 2012, 2016).

Methodologies for screening rhizosheath weight within and among plant species

Although some studies have shown consistent results for the relationship of root hair length and rhizosheath weight across soil types (Delhaize et al. 2015), many others have found inconsistencies across environments. In Brown et al. (2017), rhizosheaths were observed in a glasshouse screening of species in the Rosales, Malvales, Brassicales and Geraniales which had not previously been reported. Moreover, the frequency of occurrence of the rhizosheath trait among the Fabales and Caryophyllales was greater in the glasshouse screening than that reported in the literature.

Many factors that may differ among environments can affect rhizosheath formation including plant growth stage, growth conditions, soil moisture (Watt et al. 1994; Ghezzehei and Albalasmeh 2015), soil texture (Haling et al. 2014) and soil pH (Haling et al. 2010b). If we consider soil type, Brown et al. (2017) found that while the ‘rhizosheath score’ for each of seven species differed little between two soil types, the difference in specific rhizosphere weight between the two soils ranged from negligible to greater than four-fold, suggesting an interaction between soil properties and host plant species. However, even when screening among lines of a single species, soil type effects may be considerable. For instance, we found that two cultivars of subterranean clover (*Trifolium subterraneum* L.) had heavier rhizosheaths in a sand than in a red sandy loam, but that this effect was more marked for cultivar Woogenellup such that its rhizosheath was significantly heavier than that of Riverina in the sand but significantly lighter in the red sandy loam (Fig. 3). This raises questions about the merit of screening among cultivars in a single soil type when the aim is to identify genetic variation for specific rhizosheath weight or to eventually apply results (i.e. cultivars with greater rhizosheaths) to a broad range of soils and environments.

Plant species with negligible rhizosheaths

Brown et al. (2017) showed that the rhizosheath is present in many species from many angiosperm orders, and is especially common and strong in species from the Poales and Fabales. As these two orders contribute many of our major crop species, such as the cereals and grain legumes, it suggests a focus on the rhizosheath in crop breeding may prove beneficial. However, there remain many significant agricultural species with no or little rhizosheath. For instance, Brown et al. (2017) found that *Brassica rapa* had little rhizosheath despite relatively long root hairs. Moreover, all species of *Allium* and *Asparagus* that they examined had negligible rhizosheaths. We also found that sweet potato (*Ipomoea batatas* L. Lam.) (Order Solanales), a staple crop in many developing countries, had a

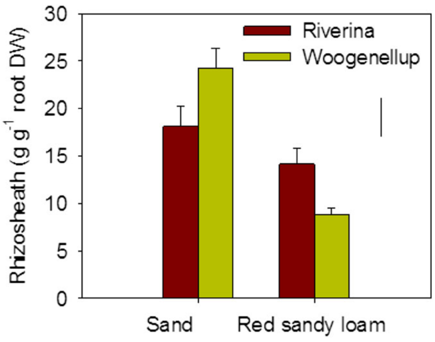

Fig. 3 The significant interaction of cultivar and soil type ($P < 0.001$) on the specific rhizosheath weight of two cultivars (Riverina and Woogenellup) of the annual pasture legume *Trifolium subterraneum* colonised by arbuscular mycorrhizal fungi and grown in a sand (pH $CaCl_2$ 5.6, % sand:silt:clay 95:4:1) and a red sandy loam (pH $CaCl_2$ 4.7, % sand:silt:clay 82:11:7) (unpublished data from Experiment 3 in Ryan et al. 2016) (mean + s.e.m., $n = 10$, LSD at $P = 0.05$)

negligible rhizosheath (Fig. 4). Consistent with the single species of Solanales (*Solanum lycopersicum* L.) included in Brown et al. (2017), the cultivar shown in Fig. 4 had very short root hairs. However, while *Solanum lycopersicum* had a significant rhizosheath, this cultivar did not.

For plants with no or little rhizosheath, it may be beneficial to investigate the rhizosphere as the root will still affect surrounding soil through exudation and other processes. While the absence of the rhizosheath presents technical difficulties, as defining the rhizosphere becomes challenging, there are promising techniques. These techniques are also relevant to the study of the entire rhizosphere for rhizosheath-forming plants.

Techniques to characterise the rhizosphere beyond the rhizosheath

In the laboratory, agar gel containing a pH indicator has been used to study rhizosphere processes as it enables visual assessment of the acidification or alkalisation of the rhizosphere according to the change in colour along the rhizosphere (e.g. Li et al. 2007). However, this method is unrealistic due to the absence of soil microbial communities, and many results could significantly vary from what would occur in soil. A more robust technique was used by Denton et al. (2006) which consisted of a two-compartment pot system, made up of two PVC cylinders stacked vertically with a polyester mesh of 24-μm pore diameter preventing penetration of roots from the upper into the lower compartment. Once a root mat was established above the mesh, the adjacent soil to 8 mm depth below the polyester mesh was defined as rhizosphere soil. A custom-made device was used to sample this rhizosphere soil in 1-mm sections for the analysis of acid phosphatases. However, the properties of the rhizosphere soil defined by Denton et al. (2006) differ from the plant's true rhizosphere as the root mat presumably has higher root density than 'normally' distributed roots.

Fig. 4 Roots of sweet potato (*Ipomoea batatas*) cv. Beauregard showing negligible rhizosheath formation after being grown in sandy loam for 12 weeks. (Photograph courtesy of David Minemba, The University of Western Australia)

Emerging technologies may enhance the ability to study the rhizosphere by enabling investigation in situ without the disturbance caused by removing surrounding bulk soil. Imaging technologies such as X-ray tomography, neutron tomography, magnetic resonance imaging, confocal laser scanning microscopy and optical projection tomography have also been used in the study of root hairs, root–soil contact, and rhizosheath development (reviewed by Downie et al. 2015). Recently, Vincent et al. (2017) developed a high-throughput method to quantify root hair area in digital images taken in situ by using ImageJ and R open source software. Significant further development of these in situ technologies is required before they can provide the same detailed overview of rhizosphere properties that can be obtained by analysing a subset of the rhizosphere that is easily captured as the rhizosheath on roots with bulk soil removed. Thus, for species with a rhizosheath, both technologies may be usefully applied together in the future. For species with no rhizosheath, studying the rhizosphere remains challenging.

Conclusions

To aid the development of a more cohesive literature, standardised terminology for the definition of rhizosheath and rhizosphere, and rhizosheath size and strength is urgently needed. There are many aspects of issues associated with rhizosheath formation that require further exploration, e.g. the interaction between genotype, environment and rhizosheath development and function, the development of protocols for quick and reliable screening of rhizosheath formation, the relationship between the rhizosheath and root hair length and mucilage, and, most critically, the relationship between yield and simple rhizosheath traits (presence, specific weight, strength) in crop species under a range of soil types and environmental conditions. For those plants without a rhizosheath, the development of a simple methodology to assess rhizosphere processes would be useful. There is no doubt that generating a detailed understanding of root, rhizosheath and rhizosphere processes will be essential to manipulate root–soil interactions to ensure sustainable crop production in the future.

Acknowledgements Megan Ryan is funded by an ARC Future Fellowship (FT140100103).

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