

Commentary

Vertical root segregation theory put to the test

Plant roots are an important yet challenging study subject. We are all aware that roots represent a significant fraction of a plant's vegetative body. Furthermore, though not all plant species have roots, every plant biologist also knows that roots are essential for the survival of the majority of plants. Indeed, for most plants, roots are the first entry point of water in to the plant's vascular system; roots are responsible for gathering > 15 mineral nutrients essential for metabolism; roots mediate microbial mutualisms that are key to gathering some of the most limiting resources; roots interact with myriad pathogens and herbivores in the soil that can reduce survival and reproduction; and competition below ground can dramatically reduce plant growth and reproduction (De Deyn & Van Der Putten, 2005). The problem with studying roots is that they are often buried under opaque soil, and the fine roots of terrestrial plants can be visually indistinguishable, especially within closely related plant functional types. These logistical constraints mean that, even if one chooses to study species-specific characteristics of roots, those studies will likely be: confined to containers, where the researcher can more easily sample roots at the end of an experiment; and confined to individual plants growing alone, where the researcher can easily assign species identity to the roots at the end of the experiment. The advent of molecular tools in the late 1990s provided the ability to identify the roots of plants in a soil sample, meaning that the logistical barrier to studying species-specific characteristics of root ecology in the field was eliminated. These molecular methods rely on either DNA sequencing, different forms of fragment length polymorphisms, or species-specific primers. Increasingly, quantitative polymerase chain reaction (qPCR) analysis can also be used to estimate abundance as well as identify species in a soil sample (Mommer *et al.*, 2011). While these tools have only become cheaper and more accessible since the 1990s, insight into basic questions about species-specific plastic root responses has been sporadic, such studies are still largely confined to containers, and the enormous potential of these molecular tools has remained unearthed. In this issue of *New Phytologist*, Herben *et al.* (2022; pp. 2223–2236) used qPCR in the field to identify the 12 most abundant plant species in a mountain grassland community to estimate root system depth and shape. Their work examines long-held hypotheses about how roots of competing species might distribute themselves in soil layers, hypotheses that have been almost impossible to test without molecular tools for root identification.

This article is a Commentary on Herben *et al.* (2022), 235: 2223–2236.

Vertical root segregation: an old hypothesis that was hard to test

The idea that the roots of different species might segregate by depth is one of the oldest hypotheses for a mechanism that might explain plant species coexistence in diverse communities like grasslands (Parrish & Bazzaz, 1976). The logic is simple: if plant species distribute their root biomass differently with depth, this could minimize interspecific- relative to intraspecific-competition and promote coexistence via negative frequency dependence (Chesson, 2000). Unfortunately, before the advent of molecular tools for the identification of roots, this hypothesis was practically untestable in the field except by excavation, or in ecosystems where plants were rare and widely spaced, such as in deserts (Schenk *et al.*, 1999). Presence–absence data from molecular root identification has shown that most species are present at most depths (Frank *et al.*, 2010), but the segregation hypothesis is implicitly about biomass distributions, and thus a stronger test requires estimates of root biomass. Furthermore, in the decades since the root segregation hypothesis was posed, an active question in the literature has emerged: is vertical segregation a fixed trait, or does it emerge from plasticity in root growth placement?

'The molecular tools for species-level root identification are fully developed, and it is time that we take studies of species-specific differences in root plasticity into the field and test them where they are most biologically relevant.'

Nutrient foraging: a role for plasticity in vertical segregation?

A well-known concept in root biology is the idea that plants proliferate roots into nutrient-rich patches (Cahill Jr & McNickle, 2011). Again, the logic is simple and intuitive: if you were a plant, and if nutrients are concentrated in a small patch of soil, where would you put your roots? You would preferentially put them into the nutrient-rich soil. Hundreds of plant species have been assayed for this ability, and nearly all have some ability to preferentially place roots into nutrient-rich soil (Cahill Jr & McNickle, 2011). At the same time, it is well known that there is usually a declining gradient of nutrients through the soil profile, which provides the basis for the idea that vertical root segregation might also have a plastic component. Indeed, studies in containers have shown nutrient foraging precision and soil nutrient heterogeneity may have a role in the vertical distribution of roots, and that

competitors plastically alter root placement by depth (Mommer *et al.*, 2010; Padilla *et al.*, 2013). However, in these containers, diversity and stem density are generally much lower than what is observed in the field.

A slight detour into eco-evolutionary theory

It is often useful to link hypotheses to formalized theory. Indeed, theory for how evolution shapes niche partitioning under competition is well characterized for both fixed and plastic traits. By plastic trait, I simply mean that plants somehow assess and respond to an external cue by adjusting some aspect of their growth, physiology, allocation, gene expression, biochemistry or any other biological trait. However, models give slightly different answers depending on whether the trait is fixed or plastic, and whether the taxa are animals or plants. First, for fixed traits (e.g. a fixed blueprint for rooting depth) there is substantial theoretical evidence that natural selection can produce diverse communities where a component of niche space (e.g. soil depth) is partitioned among species (Cressman *et al.*, 2017). Thus, if one envisions rooting depth as a fixed blueprint, then eco-evolutionary theory can both explain and predict the evolution of this trait under negative frequency-dependent selection. Second, for plastic traits (e.g. confront a competitor or avoid a competitor) the answer depends on whether the organisms are mobile or not. For example, there is substantial evidence that animals will sometimes confront competitors and sometimes avoid competition (Maynard Smith & Price, 1973). But animals have a ‘superpower’ that plants lack when it comes to avoiding conflict: most animals can choose to just walk away. Since plants are sessile, except in low density containers, it is essentially impossible to avoid competition in nature. Thus, eco-evolutionary models that envision root placement as a plastic trait universally find that confronting competitors is predicted to be more evolutionarily stable, and that avoiding competition through plastic root growth is rarely the best strategy (O’Brien *et al.*, 2007; Herben & Novoplansky, 2010; Smyeka & Herben, 2017; Cabal *et al.*, 2020). If one envisions rooting depth as a plastic growth response, eco-evolutionary theory has trouble explaining how such a behaviour could evolve. Of course, the true utility of hypotheses connected to formal theory emerges not from predictions but from experimental tests that seek to falsify these predictions.

Vertical segregation theory put to the test

The article by Herben *et al.* (2022) published in this issue of *New Phytologist* explicitly examines the question of fixed compared with plastic vertical root segregation in natural field conditions. They took multiple cores from the field from within a plot, divided them by depth, and used qPCR to estimate the abundance of species by depth. They then compared the belowground species distribution with the aboveground stem distribution to arrive at hypothesized root system shapes. Their two key findings were that the rooting depth in the glasshouse containers predicted the rooting depth in the field, but that the highly plastic proliferation of roots into nutrient-rich patches in the glasshouse containers did not predict proliferation of roots into nutrient-rich zones in the field.


They concluded that root placement in the field was more like a species-specific fixed architectural blueprint, and that there was a limited role for plasticity in a multi-species community to shape vertical segregation. Though their results are from just 12 species in one community, they represent the first test of the fixed compared with plastic root segregation hypotheses in the field. More data from diverse species and communities will be needed to know if this is a general pattern.

The difference between container results and field results highlights the need to move beyond the status quo of container-based studies of roots, and to begin to tap molecular tools for root identification as a new standard in belowground plant ecology. The study by Herben *et al.* (2022) sets a new standard for belowground plant ecology and the study of both root segregation and foraging precision. The molecular tools for species-level root identification are fully developed, and it is time that we take studies of species-specific differences in root plasticity into the field and test them where they are most biologically relevant.

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