

# Quantitative partition of the rhizosphere microbiota assembly processes

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**Abstract:** The soil microbial reservoir and plant recruitment are predominant forces determining microbiota assembly in rhizosphere (i.e. active and passive processes respectively), but to date, no straightforward method to evaluate the respective contribution of forces to the rhizospheric microbiota assembly rules is available. We propose herein a promising way to quantitatively partition the assembling forces of rhizosphere microbiota using ordination metrics. We anticipate that this new method can not only weight the plants individual contributions to microbiota assembly in rhizosphere, but can also indirectly provide a way to quantitatively evaluate soil health by the contribution from plant selection.

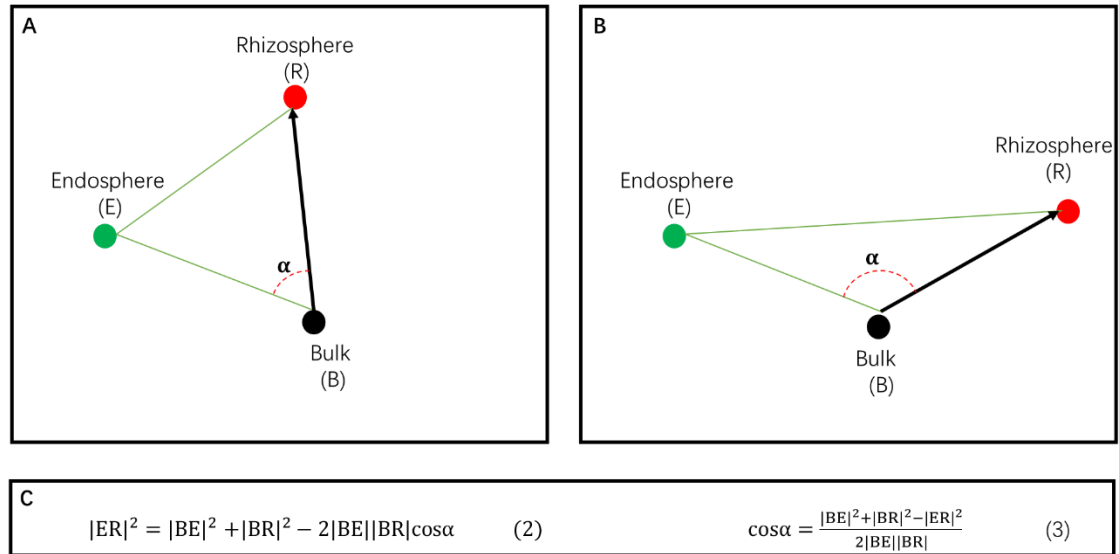
The rhizosphere is of central importance not only for plant nutrition, health and quality but also for microorganism-driven ecosystem functioning and nutrient cycling in agroecosystems [1-3]. It is well established that plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere, indicating that both plant selection and the soil microbial reservoir confer forces that serve to structure the rhizosphere microbial community [4-7]. It is also well known that plants can recruit particular microorganisms to the rhizosphere from the soil reservoir through root exudates and signaling compounds as a deterministic process of rhizosphere assembly [8]. Measurements of the degree of plant influence on the assembly of the rhizosphere microbiome would offer an improved assessment of the co-evolved fraction of rhizosphere microorganisms. It has

34 recently been demonstrated that plants can leave a 'fingerprint' of their endosphere  
35 microbiome on the soil reservoir [9]. In an environment where plant diversity is low, one would  
36 expect there to be a pronounced influence of plants on the soil reservoir. This would be  
37 reflected in a reduction of heterogeneity between microbial communities in the bulk soil,  
38 rhizosphere and the root-endosphere. A similar trend is expected when the soil microbial  
39 reservoir has been eroded, perhaps due to a biotic or abiotic stressor. In both these cases the  
40 apparent weight of the plant contribution to the rhizospheric microbiota is expected to vary  
41 (i.e. bulk soil, rhizosphere and endosphere microbiota more similar). Therefore, the measure  
42 of the weight of the respective plant filtering/recruitment coupled with the influence of the  
43 bulk soil microbial reservoir should allow the interpretation of the level of disturbance to the  
44 soil reservoir.

45 An experimental design where bulk soil, rhizosphere soil and plant roots are sampled  
46 and microbial communities determined for each of the three compartments can easily  
47 produce a contingency matrix [10, 11]. Based on the matrix, beta-diversity are usually  
48 calculated, showing the compositional similarity of microbial community between each other.  
49 Thereby, the distances between samples from bulk soil (B), rhizosphere soil (R) and plant  
50 endosphere (E) based on Bray-Curtis dissimilarity as the equation (1), where  $n_k$  is the  
51 abundance of OTU  $k$  at the  $i$  and  $j$  samples.

$$52 \quad \text{Distance}_{\text{Bray-Curtis}} = \frac{\sum |n_{ik} - n_{jk}|}{\sum (n_{ik} + n_{jk})} \quad (1)$$

53 Based on the distances, a triangle can be constructed in a plane (Fig. 1). Consequently,  
54 the angle ( $\alpha$ ) size between the distance from bulk soil to endosphere (|BE|) and the distance  
55 from bulk soil to rhizosphere (|BR|) can be easily calculated from this ordinations.

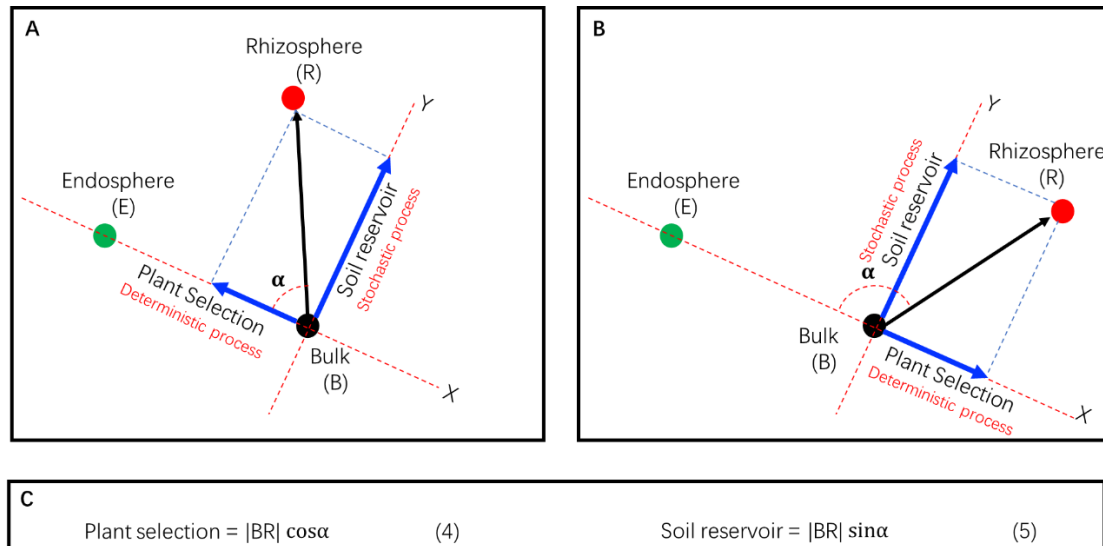


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57 *Fig. 1 The triangle in a plane based on Bray-Curtis distances among the microbial*  
 58 *communities' structures in bulk soil (B), endosphere (E) and rhizosphere (R).  $\alpha$  is the angle*  
 59 *degree between the  $|BE|$  and  $|BR|$ . Panels A and B indicate the two kinds of treatment*  
 60 *arrangement in a plane. The back arrow indicates the assembling vector of rhizosphere*  
 61 *microbiota recruiting from the bulk soil. Panels C shows the equations for calculating angle*  
 62 *( $\alpha$ ) size.*

63

64 Then, we may link the endosphere (E) point with the bulk soil (B) point. Based on this line,  
 65 an X-axis was constructed, then at the point B, a vertical line was drawn to be the Y-axis (Fig.  
 66 2). Thus, the vector representing the contribution of rhizosphere microbial assembly from the  
 67 bulk soil (BR) can be decomposed along the X- and Y-axis. The resulting vector, along the X-  
 68 axis, weights the effect from "plant selection", since 1) the endophyte assemblages  
 69 predominantly depends on the plant genotype, 2) the root endophyte assemblages  
 70 overwhelmingly relies on the soil microbial reservoir since very few species stem from seed-  
 71 borne or other negligible environments.



72

73 *Fig. 2 Decomposition of the rhizosphere microbial assembling forces into plant selection*  
 74 *vector and Soil reservoir vector. X-axis was constructed based on the link line between bulk*  
 75 *soil (B) and endosphere (E), and Y-axis was drawn as a vertical line at the point of B. the blue*  
 76 *vectors indicate the partitioned vector of rhizosphere microbiota assembling. Panels A and B*  
 77 *indicate the two kinds of treatment arrangement. Panels C shows the equations for “plant*  
 78 *selection” and “soil reservoir”.*

79

80 The other resulting vector, along the Y-axis and always pointing to the rhizosphere,  
 81 indicates that the soil reservoir randomly influences microbial assembly in the rhizosphere.  
 82 This stochastic process influenced by the soil microbial reservoir effect can be weighted as  
 83 “Soil reservoir”.

84 As described above, we successfully weighted the major processes impacting the  
 85 assembly of the rhizosphere microbiome due to plant selection (a deterministic process) and  
 86 from the soil reservoir (a stochastic process). These effects were termed as “plant selection”  
 87 and “soil reservoir”, respectively.

88 However, it is interesting to observe that: 1) if  $\alpha < 90^\circ$  (Fig.2A), the value of the plant  
 89 selection effect can be positive, this means the vector points to the direction of the  
 90 endosphere microbiome, indicating the plant selection confers a recruiting power for  
 91 microbial assembly in the rhizosphere; 2) if  $\alpha > 90^\circ$  (Fig.2B), a negative value of plant  
 92 selection is obtained, this means the vector points in the reverse direction in relation to the endosphere.  
 93 This negative value indicates that plant selection is a repulsive effect in assemblage selection,  
 94 perhaps due to incompatibility between plant growth and the indigenous microbiota. We

95 suspect that this incompatibility may be observed in disease-conducive soils, as previous  
96 studies have reported the recruitment of beneficial microbes for fitness maintenance [12, 13].  
97 Thus, if the bulk soil microbial reservoir is characterized by microbial taxa detrimental to plant  
98 fitness, a plant-derived negative selection may take place.

99 Overall, this proposed method provides a platform to partition contributions in  
100 rhizosphere microbiome assembly from plant selection (a deterministic process) and soil  
101 reservoir (a stochastic process). For example, this method can be used to quantify the soil  
102 reservoir effect with one cultivar plant in different soils or between different cultivars in one  
103 soil type. This weighting method may also provide a comparable parameter to evaluate soil  
104 health for plant growth.

105

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109

110 **Author contributions:** NL, PV and QRS designed the study. NL, CX, PV and QRS wrote the  
111 manuscript.

112

113 **R code accessibility:**

114 R code for |ER|, |BR|, |BE|,  $\alpha$ , plant selection, and soil reservoir calculation based on Bray–  
115 Curtis dissimilarity can be provided by correspondence.

116

117 **Compliance with ethical standards**

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119 **Conflict of interest:** The authors declare that they have no conflict of interest.

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