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Singh, B. K., Liu, H., & Trivedi, P. (2020). Eco-holobiont: a new concept to identify drivers of host-associated microorganisms. *Environmental Microbiology*, 22(2), 564-567, which has been published in final form at <https://doi.org/10.1111/1462-2920.14900>

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Eco-holobiont: a new concept to identify drivers of host-associated microorganisms

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Abstract

Host microbiomes play a critical role in host fitness and health. Whilst the current ‘holobiont’ concept framework has greatly expanded eco-evolutionary and functional understanding of host-microbiome interactions, the important role of biotic interactions and microbial loop (compositional linkage between soil, plant, animal) in shaping host-microbiome are poorly understood. We proposed an ‘eco-holobiont’ concept to fill the knowledge gap.

Keywords: biotic interactions; eco-holobiont; holobiont; microbial loop.

Close association with commensal microbes is known to influence the capacity of a host to cope with adverse environments and have critical biological and ecological implications for plants and animals. Our knowledge of microbiome assembly, host-microbiome interaction and communication, and the microbial role in modulating ecosystem functions is rapidly evolving but remains incomplete^[1-3]. Multiple pathways of host-microbiota interactions exist, both positive and negative, but these interactions are mostly beneficial and symbiotic^[2]. As a result, hosts and their microbiomes are increasingly defined as a unit, known as the holobiont, where the microbiome provides a genomic and functional extension to their hosts^[2,4]. However, only a fraction of variation in host associated microbiome is explained by the host’s genetics and abiotic factors (e.g. soils, climates)^[2,4,5]. Additionally, we have little understanding on the impact of interactions between microbiomes of different ecosystem components (host, habitats, biotic interactions) on the assembly of host microbiomes. We argue that addressing these knowledge gaps are critical to advance the ecological understanding and help translate host microbiome knowledge into health and industrial applications.

Host-microbiome interactions are likely driven by evolutionary and ecological relationship and currently it is assumed that a host microbiome is generally structured by host genetics and nutrients^[2, 5]. However, growing evidence indicates that abiotic (e.g. soil, air) variables and biotic interactions (e.g., a microbial loop across multitrophic levels) can significantly contribute to the structure of host microbiomes^[6]. Predation and competition occur in the rhizosphere and endosphere (inside plant tissues), which can significantly alter microbial composition and structure and affect microbial competition for nutrients. For example, recent findings suggest that soil microbiomes are profoundly influenced by the predators (e.g. protozoa, nematodes) either directly by grazing and/or indirectly by trophic cascade effects when the predators affect the structure and composition of soil microbiomes^[7].

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1462-2920.14900

It is also well known that soil bacteria and fungi interact and influence each other's community structure via extensive cooperation and competition^[8, 9]. Similarly, it is likely that host microbiomes are also heavily regulated by biotic (trophic) interactions including predation and infection (by phages). However, no study has investigated this in the detail. Overall, biotic interactions (e.g. competition, predation) can potentially generate cascading impact on the microbiome of the aboveground plants and animals feeding on them.

Co-evolutionary theories assume that host and microbiomes evolved together and can benefit each other in many circumstances. However, the process of the holobiont assembly is less understood. It can be argued that different species of microbiota of the same host species are subject to different selection pressures. This argument is supported by the presence of host-pathogen or transient species within holobionts. However, most of host-associated microbiota have beneficial or neutral impact on host functions. To explain this, a new mechanism called 'an ecosystem on leash' was proposed for host-microbiota interactions^[10]. It was postulated that plants and animals have developed mechanisms to promote growth and activities of microbes with beneficial traits by providing specialised nutrients and protection against immune system. This concept thus includes both the top-down mechanism of microbiome assembly where the host has selected the beneficial microbes and bottom-up approach by microbial species interaction^[10]. However, in an ecosystem, strong interactions at multiple trophic levels occur which could also influence the overall microbiome assembly and ecosystem health via a circular microbial loop. For example, a soil with healthy microbiome provides suitable habitats for primary productivity, which then provides foods for pollinators and herbivores. These all then return organic matters to soil for microbes- completing a circle that is influenced by each component of the interacting ecosystem (soil, plant, animal).

It is likely that holobiont assembly is influenced by both environmental abiotic (e.g. soil, water and air) variables and biotic (e.g. plant-animal) interactions^[11]. The soil microbiome serves as the primary source for plant microbiomes, which is strongly and sequentially filtered by the rhizosphere, rhizoplane (root surface) and endosphere. Such active filtration is putatively mediated by root exudation that creates habitat gradients in terms of physical (pH), nutrient and chemical properties combined with plant immune systems (**Figure 1**). It is known that flower and leaf microbiomes are sourced from soil and/or air. These microbiomes are then exchanged between flowers and pollinators or between leaves and herbivores at a regular basis. Specific habitat-specialised microbes are known to present in pollens, nectar and insect guts and therefore regulate microbiome assembly in both plants and animals^[12, 13, 14]. Ample evidence is available to suggest that plant-insect interactions (both positive- pollination, and negative- herbivory) are significantly influenced by host microbiomes^[12, 13]. Additionally, transfer of pathogens or beneficial microbes from plants to insects or insects to plants can start a cascade of change in host physiology, which then affect the structure and activities of host microbiomes. Although such biological interactions are further modulated by less known abiotic variables, we have little system-based understanding on the multi-trophic host-microbiome interactions and how the dynamic microbial loop influences the host and environment.

We argue that a significant shift in our experimental approach is needed to identify fundamental ecological processes that influence the assembly and functionality of soil-plant-animal microbiomes. We propose a new system-based ‘eco-holobiont’ approach to expand our current understanding. This approach includes microbiomes of interacting organisms within an ecosystem that is regulated by biotic and abiotic interactions. We argue to expand the current ‘holobiont’ approach, which is considered to be shaped by host genetics and local environments, to also explicitly consider the roles/feedbacks from microbiomes associated with inter-kingdom biotic (plants and animals) and abiotic (e.g. soil) components. It is a holistic approach combining holobiont and other ecosystem (eco-holobionts) components (**Figure 2**). Recent studies provide supports for this approach ^[15, 16, 17]. For example, recent findings demonstrated that despite distinct in microbial composition, plant and gut habitats share similar microbial taxa at high taxonomic level (e.g. Firmicutes, Bacteroidetes, Actinobacteria) and host functions ^[15]. It was also reported that each host microbiome was a subset of what existed in the environment and in other organisms present on an island^[16]. By sequencing various components of ecosystems (e.g. soil, water, primary producers, herbivore and carnivore), this study found that soil harbours the highest diversity in the island, followed by plants and algae but microbiome was subset of soil/ water microbiomes. A subset of plant microbiome was harboured by herbivores while carnivore has a least amount of microbial diversity – a subset of herbivore microbiome ^[16]. Pollination processes, where some evidence of microbial loop between soil, plant and pollinator already exist^[18], can provide a good starting point to generate mechanistic understanding of host-associated microbiome assembly. Such approach can estimate relative contributions and identify microbial components that are regulated by host genetics versus components that are acquired by environmental conditions, i.e. what they eat and with whom they interact.

Similar to ‘One Health Concept’, which suggests that environmental and animal microbiomes can affect both human microbiomes and health^[19], our eco-holobiont concept proposes that interacting biotic (plants, animals) and environmental (e.g. soil, water, air) microbiomes forms a circular microbial loop that can impact the assembly of holobionts of all living organisms within an ecosystem. We argue that such a systems-based approach is needed to expand our current knowledge on drivers governing community assembly of host microbiomes and functions, and overall ecosystem health. Without such an approach, it is likely that we will miss key information on seeding/ transmission (host genetics vs interactions with other biotic and abiotic factors) and host colonisation by microbial communities.

Adoption of the new ‘eco-holobiont’ approach will provide better understanding of inter-kingdom and environmental microbiome interactions within ecosystems. It can potentially yield new tools for better biodiversity management and conservation policies. At the same time, it can open a new frontier to develop and harness biotechnological products for agri-food, environmental and human health sectors. It is clear the microbiomes of soil, plants and animals within an ecosystem interact regularly and influence the shape and activities of host microbiomes, which in turn impacts multiple host and ecosystem functions. However, future challenges include to identify the magnitude of this inter-kingdom microbial interaction and integrating individual host microbiomes to multi-trophic (e.g. soil, plant and insect)

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interactions at ecosystem scale. This will require sampling across soil gradients, water systems (e.g. lakes, rivers), plants and animal species for microbiome analyses similar to the approach reported previously^[16]. Overall, our eco-holobiont concept adds another layer on an enormously complex system which determines the shape of host-associated microbiomes but also provides a holistic framework to determine the drivers of holobiont assembly and activities. It will be informative and effective if we demonstrate how inter-kingdom biotic interactions, and the associated microbial loop, impacts the holobiont of a species within an ecosystem. New knowledge potentially can provide effective tools for microbiome engineering *in situ* for better industrial, agricultural and health outcomes.

Acknowledgement

Research on host microbiome in BKS laboratory is supported by Australian Research Council ((DP170104634; DP190103714), Horticulture Innovation Australia. BKS also acknowledges support from the Alexander von Humboldt Research Award.

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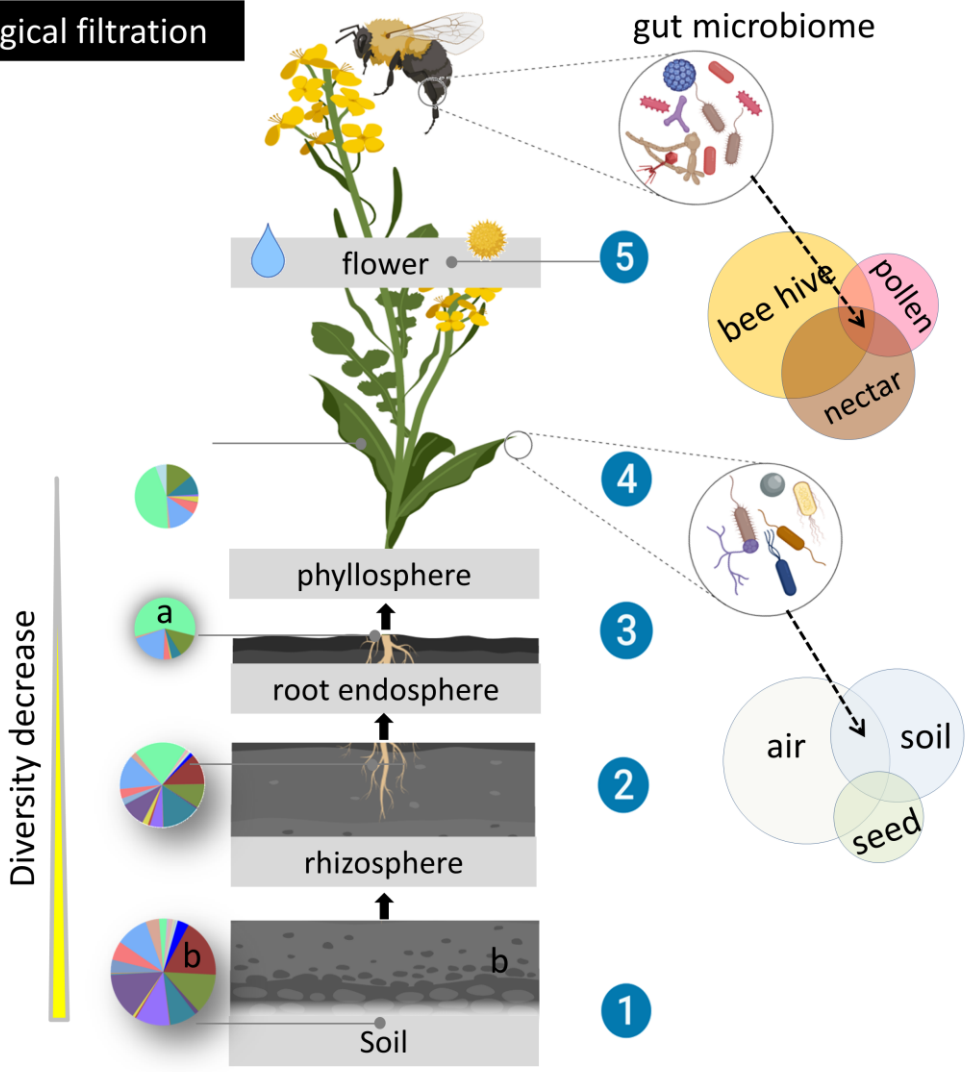
Figure Legends

Fig.1 Biological filtration processes through different compartments of plants. Plants intimately interact with a large number of microorganisms in the rhizosphere and phyllosphere with consequences on the plant growth and fitness. It is estimated that about 10^6 - 10^9 microbial cells exist in one gram of soils (**process 1**). These microbes provide a microbial seed bank for plants for recruiting beneficial microbes at germination stage. The distinct physio-chemical conditions between the rhizosphere (the narrow soil zone surrounding plant roots) and bulk soil, such as pH, O₂ level and nutrients, form a biological gradient which results in gradient increases of particular bacterial taxa (e.g., Gammaproteobacteria, shown as the green in the pie chart, also marked with **a**), and decreases of particular microbial groups (such as Acidobacteria, Archaea and Gemmatomonadetes, shown as the red in the pie charts, also marked with **b**) (**process 2**). Furthermore, the rhizoplane and endosphere continue to apply selection process for the assembly of endophytic communities (**process 3**). In these two compartments, those microbes that elicit strong plant defence responses or lacking the traits attaching to or penetrate root tissues are excluded from entering the endosphere. The biological processes 1-3 consequently result in gradual decrease in microbial diversity from bulk soil to the plant endosphere. The aboveground plants, namely the phyllosphere containing flowers, leaves and stems, have intimate microbial interactions with soil and roots of the belowground plants. Plant leaves, flowers also have a complex microbiota which might have been sourced from seeds, air, soil dispersal and insect visitors. The distinct biotic and abiotic conditions of plant leaf, nectar and pollen cast the strong microbial selection on their environments (**process 4 and 5**). For example, the high osmotic conditions associated with nectar result in the predominance by yeasts of the *Metschnikowia* and bacteria of *Acinetobacter* in nectar. Similar to plants, insect pollinators/herbivores that forage/feeding on plants have a gut microbiome that is key for their health and fitness. Their microbiomes are putatively selected from the hive environment (e.g., hive frames, for bees), air as well as from plants that they visit.

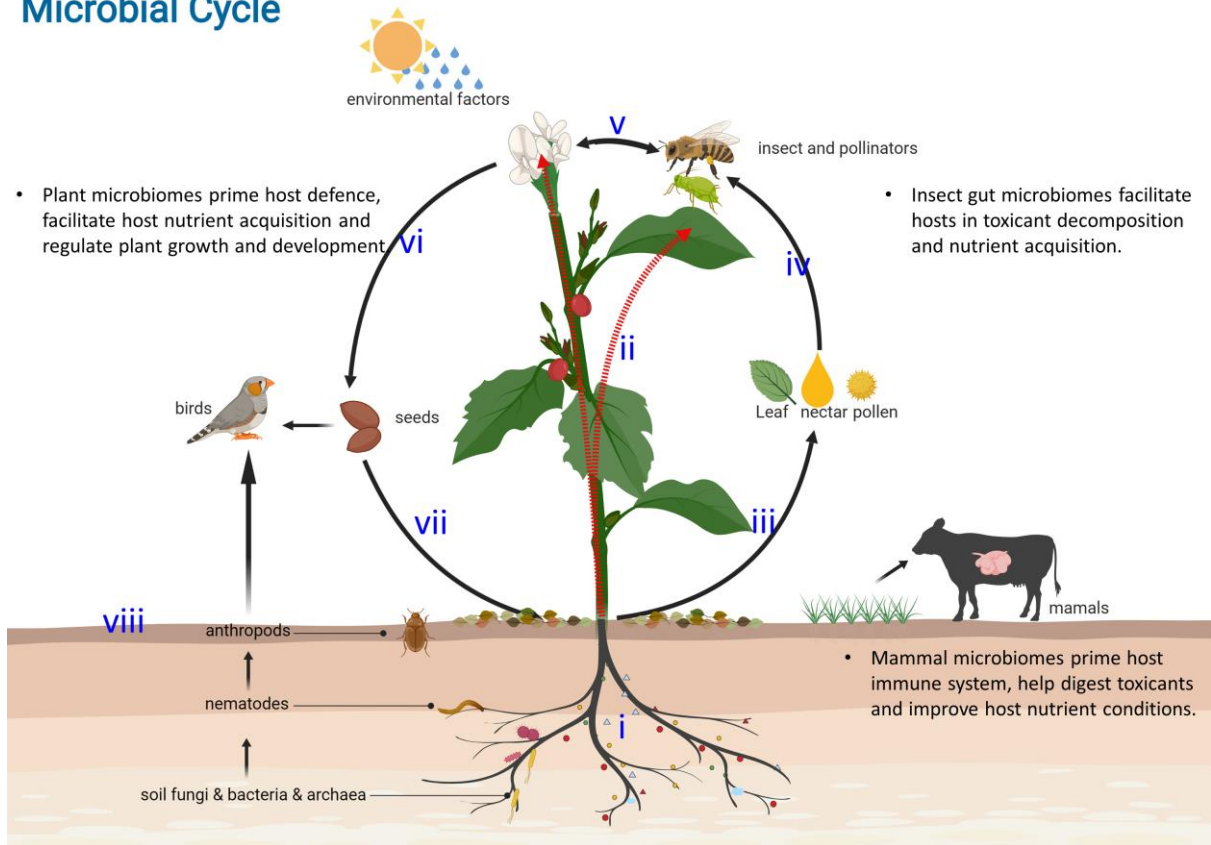
Fig.2 The ‘eco-holobiont’ conceptual framework. We propose that the biotic interactions across multi-trophic levels, and the plant and animal-associated abiotic environments should be considered together to enable a holistic view of the terrestrial ecosystem. Plants invest a large amount of the photosynthesised organic materials into soils in the form of root exudates or plant litters. The organic matters nurture the soil microbial community and soil animals (**process i**). There is evidence that soil microbial diversity positively correlates to the plant diversity. A part of the rhizosphere microorganisms can enter the root tissues via the emerging root sites, and cracks generated by soil herbivores or pathogens, a small proportion of which could further transfer to the endosphere of the aboveground plant flowers and leaves via the xylem and phloem system (**process ii**). Part of the soil microbes can directly reach to leaves and flowers via soil and air dispersal (**process iii**). In the aboveground, there are pollinators and herbivores that can microbially interact with plants (**process iv**). This can be reflected by the fact that pollinators and herbivores will take in plant-associated microbes when they feed/forage on the plants. Moreover, these insects can facilitate the spread of both plant stimulators and pathogens between different plants (**process v**). Thereafter, flower microbiome can be evidently inherited by seeds that will start a new round of microbial recruitment from soils

(**process vi and vii**) completing the microbial loop. Besides the aboveground, the complex interactions composed of prey and predators in the belowground food web is also key part of our ‘eco-holobiont’ concept. A microbial cascade can exist in the process (**viii**). Therefore, microbiome of single host species is not only influenced by host genetics, nutrients and abiotic factors but also by biotic inter-kingdom interactions (e.g. plant and animals) via microbial loop within an ecosystem. We therefore, propose that holobiont study should explicitly include biotic interactions and microbial loop to account for variation in holobiont assembly of a species within an ecosystem.

Biological filtration



Microbial Cycle



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