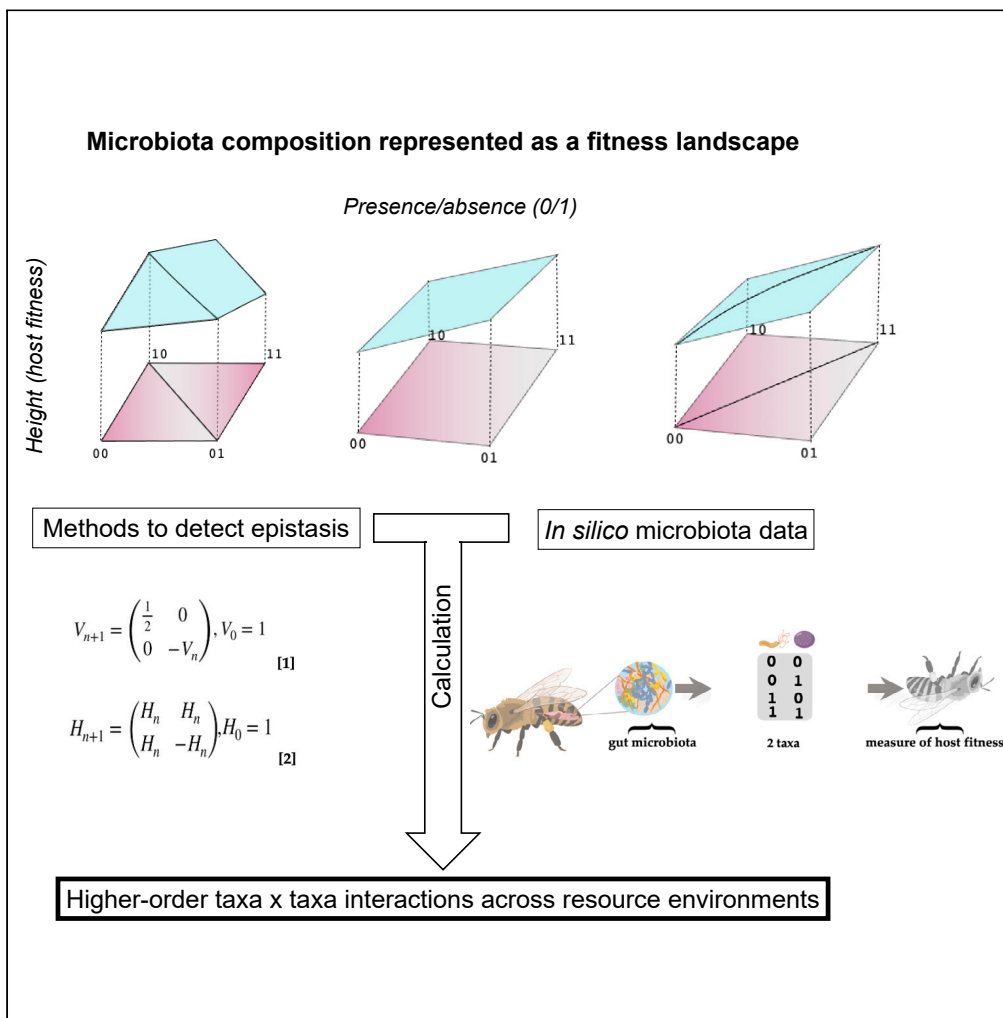


Article

Deconstructing *taxa x taxa x environment* interactions in the microbiota: A theoretical examination



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Highlights

Higher-order microbial interactions can shape important host phenotypes

Epistasis techniques can be used to quantify higher-order microbial interactions

This mathematical framework is applied to an *in silico* insect gut microbiota

This method can be easily integrated into the analysis of experimental datasets



Article

Deconstructing *taxa x taxa x environment* interactions in the microbiota: A theoretical examinationSenay Yitbarek,^{1,10,*} John Guittar,^{2,3} Sarah A. Knutie,^{4,5} and C. Brandon Ogbunugafor^{6,7,8,9}

SUMMARY

A major objective of microbial ecology is to identify how the composition of microbial taxa shapes host phenotypes. However, most studies focus on pairwise interactions and ignore the potentially significant effects of higher-order microbial interactions. Here, we quantify the effects of higher-order interactions among taxa on host infection risk. We apply our approach to an *in silico* dataset that is built to resemble a population of insect hosts with gut-associated microbial communities at risk of infection from an intestinal parasite across a breadth of nutrient environmental contexts. We find that the effect of higher-order interactions is considerable and can change appreciably across environmental contexts. Furthermore, we show that higher-order interactions can stabilize community structure thereby reducing host susceptibility to parasite invasion. Our approach illustrates how incorporating the effects of higher-order interactions among gut microbiota across environments can be essential for understanding their effects on host phenotypes.

INTRODUCTION

Animal guts contain complex microbial communities whose structure and function depend upon the interactions among microbes and the host. Gut microbiota serve as key actors in host health, impacting development, metabolism, and the immune system.^{1,2} The development of axenic and gnotobiotic model hosts has made it possible to experimentally study how the microbiota influences host traits of interest.³ However, most studies rely on correlations between microbiota taxa and host health, ignoring the potential influence of higher-order interactions among taxa within the community.^{4–7}

The study of microbial communities has undergone a recent flowering of perspectives that have added theoretical and empirical rigor to the study of how communities are constructed and interact.⁸ Researchers have even invoked ideas such as the fitness landscape—a notion with origins in evolutionary genetics—as a framing for the study of microbial communities.⁹ Relatedly, ideas such as higher order epistasis, have also been invoked in the context of microbial communities. A useful non-technical definition of epistasis is “surprise at the phenotype when mutations are combined, given the constituent mutations’ individual effects,”¹⁰ and it has been one of the more cutting-edge and provocative ideas in all of evolutionary genetics. This idea has such reach because it changes how we consider the shape of adaptive evolution, with profound implications for many aspects of evolutionary biology, including predictive evolution¹¹ and synthetic biology.¹² Similarly, interactions among microbes in a community strongly influence our picture of the structure and function of microbial communities.¹³ These studies fortify that—such as genomes, communities or neural circuits—gut microbiota are complex systems defined by the interaction between individual entities or parcels of information (in this case, component taxa in the microbiota). Consequently, the further examination of how interactions between microbes and taxa influence organismal phenotypes constitutes a true frontier of the study of the microbiota.

A long-standing goal of ecology is to capture the vast diversity of multispecies interactions—the unpredictable effects that arise when multiple species are present in an ecosystem.^{14–17} For example, animals harbor diverse microbial communities that are variable in their composition, governed by stochastic processes, which influences the overall behavior of the system.³ This problem has more recently become the object of inquiry in communities of microbes.^{18–21} Many ecological studies involving complex network structures typically focus on

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pairwise interactions.^{16,22} Only very recently has the literature demonstrated that higher-order interactions are at play in these systems, an important area for further inquiry, given how they may potentially complicate (or even undermine) simple models of microbial community function.²¹

Higher-order interactions in the gut microbiota of *Drosophila* spp. impact lifespan, fecundity, development time, and community composition.¹³ With a gut community comprising five core taxa, Gould et al. (2018) found that three-way, four-way, and five-way interactions accounted for 13–44% of all possible cases depending on the host trait. Yet, lower-order interactions (zeroth, first, and pairwise) still accounted for at least half of all the observed phenotypes in the system. Work by Sanchez-Gorostiaga et al. (2018) examined the contributions of multi-species interactions to determining community function (i.e., amylase expression). In the presence of higher-ordered interactions, the predictive power of the additive null model (absence of interactions) in predicting community function decreases. However, by accounting for both behavioral and population dynamics effects in their null model, higher-order interactions did provide good predictions for community function. These studies demonstrate that higher-order interactions can have important implications for the predictive power of bottom-up approaches to designing complex communities and determining their functional traits.²¹ Further, the aforementioned studies provide examples of how higher-order interactions can be measured and suggest that they are relevant for understanding how microbial taxa influence certain phenotypes. While the importance of diversity and host interactions is clear, to our knowledge no studies have attempted to specifically disentangle the effects of higher-order interactions between taxa across environmental contexts.^{23–26}

One major barrier to these studies is the paucity (or non-existence) of the datasets structured such as those in an evolutionary genetics framework, such that existing methods might be used to resolve interactions.^{27,28} For example, the problem of constructing a set of insects that each carry a different combination of constituent taxa of interest grows exponentially with the number of taxa. And unlike some genetic systems, constructing a different insect with a different set of bacterial taxa (corresponding with the possible combinations of taxa) is currently a non-trivial technical challenge. Nonetheless, the use of combinatorial complete datasets—insects containing all combinations of taxa (even few in number)—to explore higher-order interactions (beyond a single taxon or pairwise interactions) could help to inform how taxa interact in framing organismal phenotypes. Higher-order interactions could, in principle, be used to examine how our predictions for taxa-taxa interactions will be contingent on the host context in which a certain distribution of taxa exists.

In this study, we reframe how we consider higher-order interactions in an insect gut using a theoretical approach. We apply a relatively simple mathematical method called the Walsh-Hadamard transform (WHT), which has been used to demonstrate how higher-order interactions between mutations influence fitness or other organismal traits.^{10,29,30} We use this method to explore how higher-order interactions among gut taxa can influence host fitness across micro-environments. In this study, we use WHT to quantify higher-order interactions in an *in silico* dataset resembling the type of data that can be collected in microbiota experimental systems.

In our theoretical model of an insect gut microbiota, we have chosen to examine function across environments, framed as resource availability, as it can vary due to spatial and temporal differences, and in terms of the quantity and quality of required resources. Lower resource levels have been found to be costly for resistance to parasites in *Drosophila melanogaster*.³¹ A key component of resource availability is nutrition, which is likely to influence host resistance to natural enemies. In microbial systems, increased resource availability resulted in greater host resistance to parasites.³² The lack of nutritional content (quality and quantity) is a well-known stressor for insect microbes in many settings, including the gut microbiota.^{33–36} However, experimental studies involving model systems rely on high nutritional quality diets to understand factors affecting susceptibility to infectious diseases.³⁷

Using this framework, we can examine underappreciated aspects of the microbiota: questions surrounding the notion that the microenvironment of the insect gut may shape higher-order interactions between taxa, with important consequences for host health and fitness. Our study examines the consequences of higher-order microbial interactions for host susceptibility (i.e., likelihood of host being infected) to disease risk. We demonstrate how higher-order interactions can underlie host microbiota robustness to intestinal parasite invasion, reducing host susceptibility to disease risk, and that these interactions are highly dependent on environmental context. Specifically, our theoretical study will illustrate how interactions may occur within the microbiota, and offers future directions. We introduce this approach with the hope that it, or a related method may eventually be applied to a tractable experimental system for real-world validation and believe that insect systems are among the most promising candidates for these examinations.

RESULTS

Reaction norm

The reaction norm is an instrument used in ecology to demonstrate how traits are influenced by environmental context.⁴⁸ The reaction norm demonstrates that two insect guts, corresponding to 00000 (no taxa) and 11111 (the presence of taxa of every kind) have the largest susceptibility to infection relative to other insect microbiota combinations. The high susceptibility pattern is consistent across the nutrient content that insects consume (Figure 1). In contrast, we find that host susceptibility is drastically reduced for all other insect microbiota combinations (examples include combinations: 001100; 11011; 11101).

Comparison of the orders of interactions among taxa across microenvironments

Figure 2 demonstrates the sum of the absolute values of the interaction coefficients. Here, we can observe the raw magnitude (whether positive or negative in sign) of higher-order interactions as a function of interaction order. Note how the eminence of the higher-order effects changes as a function of nutrient content. At low nutrient contents, fourth-order effects are the most impactful on overall host susceptibility. At approximately 20%, the fifth order dominates the community (corresponding to the five-way interaction of taxa in the *in silico* insect gut

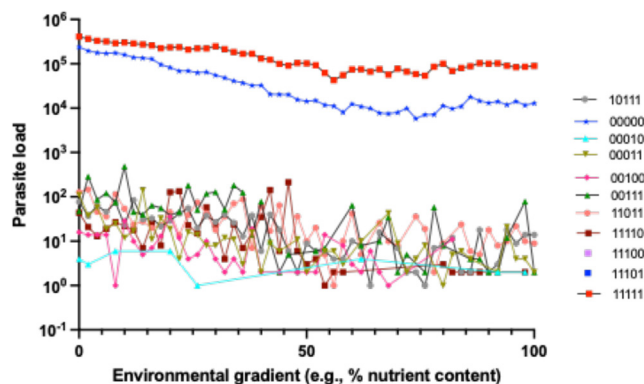


Figure 1. A reaction norm representing host susceptibility of *in silico* insect guts as a function of environment (nutrient microenvironment)

The x axis represents the nutrient content that insects consume, ranging from 0% (deprived) to 100% (a full, standard nutrient content). Individual data points correspond to insect guts containing different combinations of taxa. The y axis represents host susceptibility, a proxy for the susceptibility of a given insect to infection by parasites. Note that only a subset of the 32 taxa are represented in this, as many of the *in silico* insect guts have infection levels that are very low.

represented by 11111). The change in order of eminence also applies to the second-order (pairwise) and third-order interactions. At low nutrient contents, the pairwise interactions exert a more meaningful influence on host susceptibility than the three-way interactions. At approximately 20% nutrient content—not far from that nutrient percentage where a switch between fourth and fifth order effects manifests—the three-way interactions supplant the pairwise effects in their overall influence on host susceptibility.

DISCUSSION

In this study, we explore the importance of higher-order interactions among taxa that compose an insect gut microbiota. Using *in silico* and mathematical approaches, we demonstrate how higher-order interactions can be measured in a complex system of interacting microbial taxa. In our theoretical scenario, higher-order interactions are present and generally increase in relevance with the order of interaction. Notably, the environment (nutrient content in this case) has a meaningful influence on how higher-order interactions among taxa manifests. This result highlights an aspect of higher-order interactions that is so far largely under-appreciated: that the environment and context in which taxa exist can have a meaningful impact on how taxa interact. Consequently, simply noting that non-linear and higher order interactions between taxa may exist is no longer sufficient in how the insect microbiota is discussed: we must consider, and measure, how environments may influence how interactions manifest. Though our results arise from a theoretical examination of *in silico* insect microbiota, they are results nonetheless and highlight the potentially vast scope of the higher-order interaction problem that could define the true dynamics of gut microbiota. Specifically, the outlining of a method that can be used to deconstruct higher-order interactions in biological systems, across environmental contexts, represents a potentially useful contribution to the study of the microbiota.

Though empirical data of the size and scope used in this study are currently challenging to generate, this intractability may be temporary, and future methods may permit the generation of data similar in structure to those explored in our theoretical examination.¹³ Note that the calculations of higher-order interactions, can be considered without knowing the specific mechanism that underlies the nature of these interactions. One additional benefit of these results is that they can identify those settings (combinations between microbiota and a given microenvironment) that should be the focus of mechanistic study. For example, by identifying the taxa involved in large pairwise interactions, one can then examine the mechanistic basis underlying this pairwise interaction through manipulative experiments.

Our results are generally consistent with recent findings, for which diverse communities are more effective at resisting invasions including *E. coli* invasion of soil communities,⁴⁹ plant root bacterial communities,⁵⁰ and experimental invasions in bacterial communities.⁵¹ Collectively these studies show that outcomes of invasions are determined by available resources to the microbiota. Yet, the scale at which these studies examine microbiota interactions does not capture the emergence of higher-order interactions. In contrast, our main result shows that, in a theoretical microcosm, higher-order microbial interactions dictate the ability of parasites to invade, and that these interactions vary across nutrient environments.⁵² It's important to note that the most diverse community in our model had the highest parasite load (e.g., see taxa 11111 in Figure 1). While it is acknowledged that the microbiota is beneficial for host health by resisting invading pathogens, changes across microenvironments might aid pathogen invasion.⁵³ For example, interactions between the microbiota and host immunity can facilitate pathogen infection by exploiting the metabolic and immunological environment.^{54,55} Experimental studies focused on interactions between the native host microbiota and pathogens indicates a range of outcomes encompassing antagonistic, cooperative, and mutualistic interactions.^{56,57} Experimental evolution approaches are needed to determine under which environmental conditions microbes might facilitate invading pathogens or become pathogenic. Evolutionary dynamics can also shape interactions between the host microbiota and pathogens.⁵⁸ Invading pathogens can potentially overcome host microbiota resistance by evolving virulence factors through either direct competition or by triggering host inflammation.^{59,60} Furthermore, invading pathogen might be able to modify their within-host environment to select host-associated microbes that can promote their invasion and long-term survival.⁶¹ Simple gut microbiotas have been engineered to provide

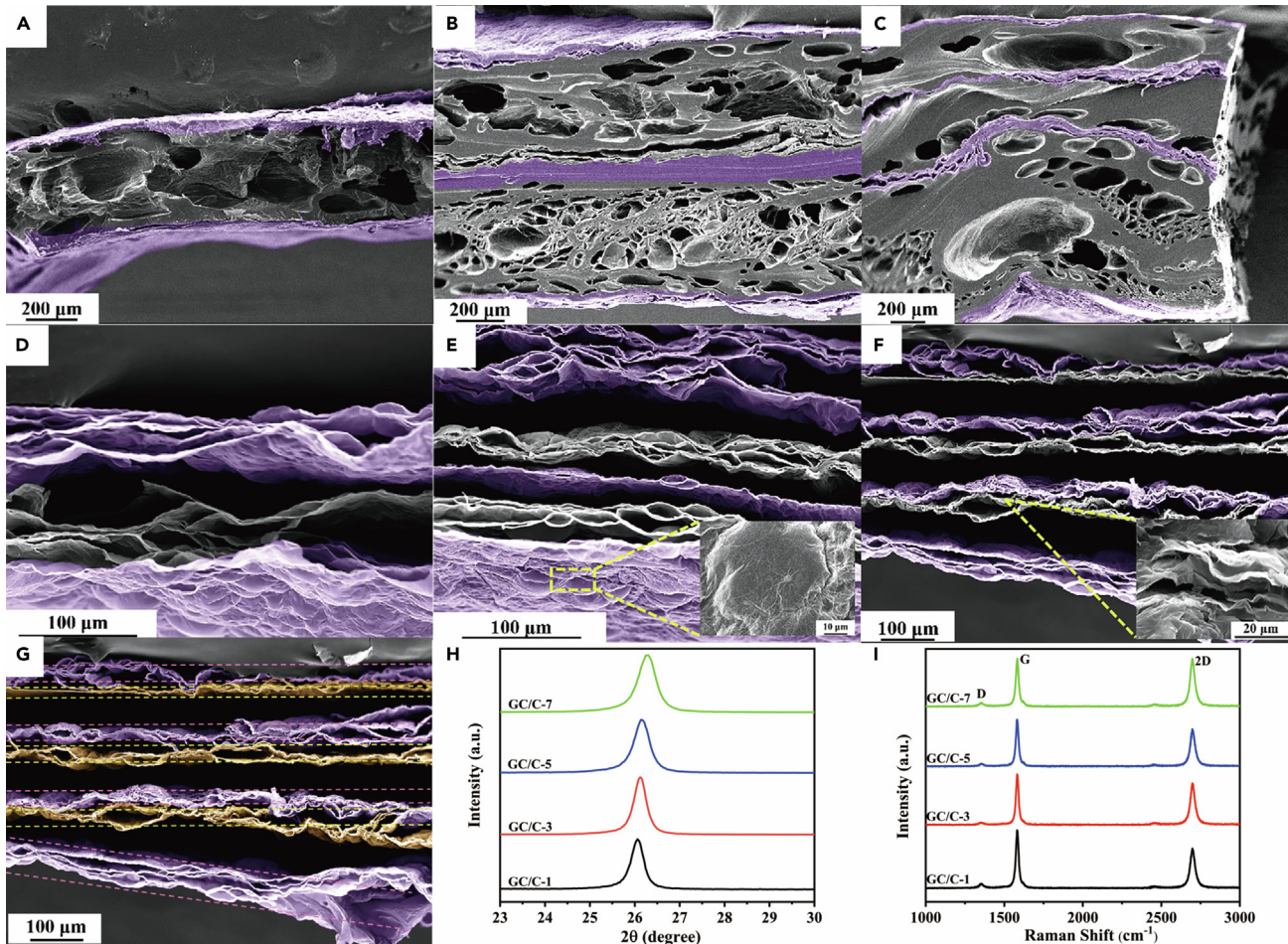


Figure 2. The magnitude of higher-order interaction among taxa as a function of environment (e.g., nutrient content)

Absolute value, averaged magnitude interactions across interaction orders, derived from the data in Figure 1, and computed as described in the STAR methods section. The purpose of this depiction is to illustrate how the magnitude (not sign) of the interactions change with interaction order. Of special note is how the importance of the order of interactions changes as a function of environment (nutrient content; x axis). In this scenario, there is a nutrient content threshold (~20%) where the patterns of the interactions change. In our theoretical insect microbiota system, we define “nutrient content” as a diet comprising a range of nutrients in a standard insect diet. In insect populations, the nutrient content of the host’s food can be controlled by the addition of methyl cellulose (an indigestible bulk agent) in the standard food medium. A diet of 0% would correspond to an extremely low nutrition diet, and 100% to a high-quality diet composed of the standard food amount for insects. Consequently, the nutrient gradient 0–100% represents varying degrees of resource availability.

hosts with important functions, including the uptake of nutrient sources and disease resistance.⁶² To test our model, insect gut microbiota could be engineered to explore how higher-order microbial endosymbiont interactions protect against pathogen infection by enhancing the nutritional status of the host. The long-term effectiveness of microbiota manipulations will need to consider pathogen evolution across environmental contexts.

As mentioned previously, the mathematical approach used in this study—the Walsh-Hadamard Transform—has been previously used by theoretical population geneticists to measure non-linear interactions between mutations.^{10,52} Several empirical datasets in genetics and genomics have demonstrated that the sign of interaction effects can change readily with the identity of the interacting parcels.^{10,30,40} Given these results, we predict that the taxa that compose the gut microbiota might be similarly defined by higher-order interactions, and that these interactions will change appreciably with insect microenvironment. The capacity for measuring the effects of higher-order interactions on host fitness is an important step toward understanding the effects of microbiota on their host.

Limitations of the study

A limitation of our approach is that we do not explicitly incorporate nutrient dynamics in our model. Rather, the nutrient aspects are invoked conceptually in our theoretical model to give it grounding in an environmental context that is meaningful in insect microbiotas. Microbial interactions have traditionally been built on MacArthur’s consumer-resource model that describes competitive interactions among microbes

for a pool of common resources.^{14,63} The MacArthur consumer-resource model assumes that microbes consume nutrients at a fixed rate and that the number of microbes present in each environment cannot exceed the number of growth-limiting nutrients. However, multiple independent mechanisms suggest that microbes don't take up nutrients in an additive manner.⁶⁴ For instance, microbes can physiologically adapt their metabolic strategies by taking up mixtures of nutrients at varying rates depending on environmental conditions.^{65,66} Microbial cross-feeding can also impact nutrient utilization strategies by microbes and shift community dynamics from mutualistic to competitive interactions.⁶⁷ Inhibitory effects by some microbes, via the production of antimicrobials, can also exclude other microbes from the uptake of nutrients. These examples illustrate that microbes can have different resource specialization strategies. While our model does not account for metabolic strategies of microbes, we argue that our model can serve as a useful null model for describing higher-order microbial interactions across a nutrient gradient. Future expansion of the model should incorporate the spectrum of metabolic strategies of microbes and quantify their associated effects on higher-order interactions.

The impact of higher-order interactions in the gut microbiota on host fitness may result from a range of possible interactions, ranging from competitive to mutualistic.^{68–70} To test the full suite of all possible combinatorial interactions and their associated effects on host traits, it is important to experimentally manipulate microbial communities. For example, the fruit fly (*D. melanogaster*) is an attractive model system for designing combinatorial studies due to relative ease of rearing gnotobiotic flies and modularity of its microbiota.⁷⁰ For example, combinatorial designs of microbial communities in *D. melanogaster* revealed that emerging higher-order effects composed of three, four, and five-way interactions impacted aspects of host fitness such as life span and fecundity.¹³ While the relative simplicity and tractability of fly microbiota facilitates the study of host-microbe interactions, underlying mechanisms can provide insights for more complex mammalian gut microbiotas. In *D. melanogaster*, stable gut colonizers favor specific regions of the foregut, which like mammals, suggest specific niches for gut colonizers.⁷¹ Therefore, strategies that invertebrates and their microbes employ to form stable associations might be informative for mammalian gut microbiotas.^{72–77}

Conclusion

Recent theoretical work suggests that higher-order modeling approaches are able to capture volumes of rich data arising from complex ecological interactions.⁷⁸ We have adapted approaches from evolutionary genetics to the study of host-associated microbiota. In the future, applying these methods to the analysis of experimental data will yield important insight into microbiota dynamics, toward a richer understanding of microbiota assembly, and the many meaningful interactions that it embodies.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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 - Data source
 - Calculating the strength of interactions between taxa
 - The Walsh-Hadamard Transform: A method for measuring higher-order interactions
 - The theoretical model: An insect gut microbiota and varying nutrient concentration

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AUTHOR CONTRIBUTIONS

Conceived idea: SY, SAK, JG, and CBO. Collected data: SY and CBO. Analyzed data: SY and CBO. Integrated and interpreted results: SY, SAK, JG, and CBO. Supervision: SY and CBO. Writing: SY, SAK, JG, and CBO.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Walsh-Hadamard Transform method and dataset	Authors	https://github.com/OgPlexus/MicrobeTaxa1
Software and algorithms		
Code to reproduce the results of this study	Authors	https://github.com/OgPlexus/MicrobeTaxa1

RESOURCE AVAILABILITY

Lead contact

Further information and results for resources should be directed to and will be fulfilled by the lead contact, Senay Yitbarek (senay@unc.edu).

Materials availability

This study did not generate new datasets.

Data and code availability

The *in silico* dataset used in this study and code used to generate them has been publicly deposited on Github and is publicly available as of the date of publication. Links are listed in the [key resources table](#).

METHOD DETAILS

Data source

The data used in this study arise from raw data used to generate theoretical fitness landscapes, composed of five-bit strings that were generated from an *in silico* dataset introduced in a prior study.³⁸ The dataset was originally generated to provide large empirical datasets that could be used to study advanced topics in population genetics, including fitness landscapes, and higher-order epistasis. The datasets are constructed such that they can serve as an exploratory space for any theoretical set of interactors, and therefore, are well-structured for the study of interacting microbial taxa. The data are defined as strings of information (e.g., 01011 or 11001), each with a corresponding “phenotype” value. Therefore, this data is equipped for the analyses as proposed in this study. Here, we generate a theoretical microbiota in an insect gut. For more information on the dataset and its origin, see Meszaros et al. (2019).

For the purpose of this study, it is important that we are transparent with regards to the data source, the notation, and the method for transforming the data into a microcosm for taxa in an *in silico* insect microbiota. In this study, our hypothetical insect guts are encoded as strings of bits. Bits can either be 0 or 1. 1 indicates the presence (+) of a taxon and 0 indicates the absence (–) of a taxon. For example, we can write a string of 0 and 1 corresponding to an insect gut with five interacting taxa (A-E), as demonstrated in below table.

Data structure of a hypothetical insect gut

Taxa	A	B	C	D	E
Presence (+) or absence (–)	+	-	+	+	-
Binary representation	1	0	1	1	0

For example, the “10110” string corresponds to an insect gut where taxa A is present, B is absent, C is present, D is present, and E is absent (see table above). The dataset that we are mining, originally derived for studying combinatorial datasets that are common in the study of fitness landscapes, offers tens of thousands of combinatorial sets that correspond to a hypothetical insect gut with interacting taxa.³⁸ We have randomly chosen one such set, containing five individual bits, to explore the central biological concepts of interest in this study: the measurement of higher-order interactions between taxa, and how these interactions might be influenced by the environmental context.

The dataset that we will use is a five-bit string (i.e., a string of five numbers), combinations of presence and absence (+ or –) of five taxa (A-E). The combinatorial possibility corresponds to $2^5 = 32$ theoretical combinations of taxa across four different insect environments.

Calculating the strength of interactions between taxa

As mentioned in the introduction, there are myriad methods for resolving higher-order interactions, and many such methods have been explored in genomic studies.^{29,39–41} A full treatment of the strengths and weaknesses of every method would require a review that is beyond the scope of our study, but some existing work has interrogated multiple methods in the study of epistasis.²⁹ In describing the methods as applied in this study, we have erred on the side of redundancy in our explanations. We believe that this is appropriate, given that our method of choice – the Walsh Hadamard Transform – has never been applied to the study of the microbiota and so could benefit from further explanations.

The Walsh-Hadamard Transform: A method for measuring higher-order interactions

The Walsh-Hadamard Transform allows one to quantify the eminence of interaction effects among potentially interacting objects or parcels. Its main output is a Walsh coefficient, which communicates the magnitude (how large the interaction is) and sign (positive interaction or negative direction) for a given interaction. The method implements phenotypic (host infection risk in our study) values in the form of a vector, before reformatting it into a Hadamard matrix (and is then scaled by a diagonal matrix). The output is a collection of coefficients which correspond to the strength of interaction between taxa.

For example, we can define the Walsh-Hadamard coefficient for the following:

*B*DE

The asterisks (*) correspond to taxa that could either be present or absent. This can be reencoded in binary as: 01011

This Walsh-Hadamard coefficient for this string corresponds to the magnitude of the interaction among the B, D, and E taxa. Importantly, we label the interaction between B, D, and E as a “third order” interaction, as the calculation provides the average strength of interaction between three different taxa: B, D, and E. Understanding the different orders of interaction is the key to gaining a perspective on “higher-order” interactions. In a gut containing five taxa that we are interested in understanding the interaction among, there are five different “orders” of potential interaction.

For example:

0th (zeroth) order interaction would be the insect containing none of the taxa of interest (A-E) present. First-order interactions correspond to the influence of individual taxa on the infection risk. There are five such first-order terms in this theoretical insect microbiota:

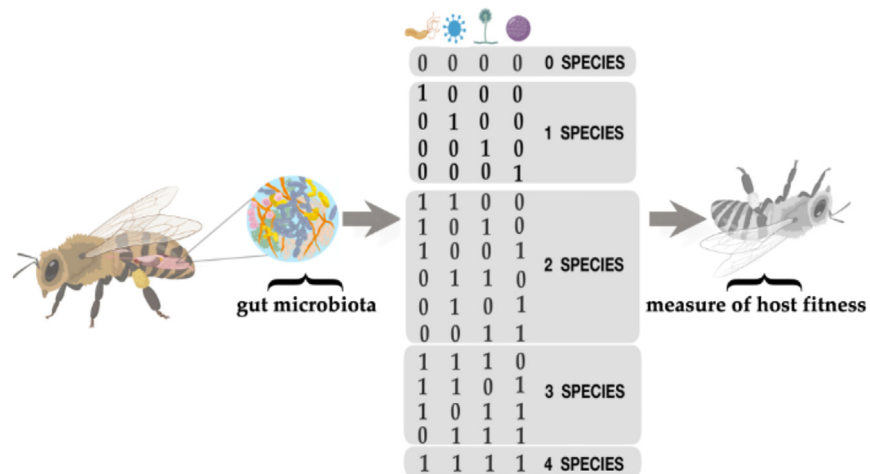
A****
*B***
C
***D*
****E.

Similarly, there are 10 second order coefficients, ten third order, five-fourth order, and one-fifth order (corresponding to the interaction between all five taxa; ABCD or 11111). These Walsh-Hadamard coefficients can be summed within an order. Consequently, a whole theoretical “insect gut” can be described in terms of the overall magnitude of its 0–5th order interactions. For example, we can examine the strength of third-order interactions (in sum) and compare them to the strength of fourth-order interactions.

The Walsh-Hadamard coefficient describes the magnitude to which an interaction map is linear, or second order, third, and so forth. We refer interested readers to two published articles—Poelwijk et al. (2016) and Weinreich et al. (2013)—that outline and apply the method in detail.

The Walsh-Hadamard Transform generally relies on the existence of combinatorial datasets, where the objects for which we are interested in understanding the interactions between (taxa in this study) are constructed in all possible combinations. Another limitation of the Walsh-Hadamard Transform is that it can typically only accommodate two variants per site, that is, two states per actor. (Note, however, that recent approaches have introduced mathematical methods that transcend some of these requirements⁴²).

In the case of taxa, we can think of this in terms of the presence/absence of a certain taxon, and we can encode this in terms of 0 (absence) or 1 (presence). For each theoretical insect with a different presence/absence combination, we have a corresponding phenotypic measurement (e.g., host infection risk). For example, if we wanted to measure the higher-order interactions among 4 taxa within an insect with regards to their role in host susceptibility (as a model phenotype), we would need $2^L = 16$ individual measurements (insects in this case), with L corresponding to the number of different taxa whose effects we were interested in disentangling. We can encode this combination of 4 taxa in bit string notation (see figure below).



Schematic representation of higher-order interactions in the insect gut microbiota

We represent the presence of microbial species in the gut similarly to the presence of a genetic locus. Species composition of gut microbiota is represented in binary strings. In this configuration, the combination 0011 represents both the presence and absence of two species. For each string combination, we associated a phenotypic measurement, such as infection risk. We quantify “epistatic” interactions between microbes in n dimensional space, where n represents the number of species interacting.

As described above (STAR methods section), each site (0 or 1) in the string corresponds to the presence or absence of a given taxa in a given insect. This notation allows us to keep a mental picture of which taxa are in which insect for which we have a phenotypic measurement and can be used to construct a vector of values. Again, the string 01011 corresponds to an insect with the pattern of absent (0), present (1), absent (0), present (1), present (1). The full dataset includes a vector of phenotypic values for all possible combinations of taxa (see table in data source). Note, again, that these can be divided into different classes based on the “order” of the interaction. This vector of phenotypic values for the 32 will be multiplied by a (32×32) square matrix, which is the product of a diagonal matrix V and a Hadamard matrix H . These matrices are defined recursively by:

$$V_{n+1} = \begin{pmatrix} 1 & 0 \\ 2 & -V_n \end{pmatrix}, V_0 = 1 \quad (\text{Equation 1})$$

$$H_{n+1} = \begin{pmatrix} H_n & H_n \\ H_n & -H_n \end{pmatrix}, H_0 = 1 \quad (\text{Equation 2})$$

n is the number of loci ($n = 5$ in this hypothetical example).

This matrix multiplication gives an output:

$$\gamma = VIIx$$

Where V and H are the matrices described in (Equation 1) and (Equation 2) above, and γ is the Walsh coefficient, the measure of the interaction between parcels of information in a string. Using this, we compute γ values for every possible interaction between bits in a given string. These methods measure every one of these interactions (e.g., all ten 2^{nd} order interactions) between taxa. While our use of a five-bit string structure (as opposed to a three or fifteen bit string) is arbitrary, it communicates the nature of the higher-order interaction problem: Even if we suspect that only five taxa in an insect microbiota are meaningfully influencing a phenotype of interest,^{43–45} the possible ways that these species are interacting, and the number of measurable coefficients between them can be meaningful.

Having outlined the method used to quantify higher-order interactions above, it is important to directly explain the presumptive biological interpretation of the values. The Walsh-Hadamard Transform returns a Walsh coefficient for each interaction of a certain order (e.g., first order, pairwise, third order, and so forth). This corresponds to the relative strength or importance of that interaction in the phenotype being measured. Therefore, the Walsh-Hadamard Transform can help to interpret the overall presence and eminence of higher-order interactions between taxa in a microbiota.

The theoretical model: An insect gut microbiota and varying nutrient concentration

Here, we seek to explore how different gut microbiota environments influence interactions between taxa. As a stand-in example, we will focus on how varying nutrient diets influence host susceptibility to parasites in the gut microbiota. While one could have chosen any number of

environments, we chose to focus on the nutrient diet content in our study design because the resource environment is highly relevant to the insect gut microbiota. In insects, nutrient diet content of the host's food can be controlled by the addition of methyl cellulose (an indigestible bulk agent) in the standard food medium.⁴⁶ Resource levels varying from high-quality diets (containing no methyl cellulose in the food medium) to lower-quality diets (replacing 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% of the food medium with methyl cellulose) have been used to study the role of nutrition to parasite resistance in lepidopteran pest species.⁴⁷ In our theoretical study, we define "nutrient content" as a diet compromising a range of nutrients in a standard insect diet. A diet of 0% would correspond to an extremely low nutrition diet, and 100% to a high-quality diet composed of the standard food amount for insects. Consequently, the nutrient gradient 0–100% represents varying degrees of resource availability.