

**Title:** Fungal infection alters the selection, dispersal, and drift processes structuring the amphibian skin microbiome

**Running title:** Community assembly of the microbiome

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# **Abstract**

Symbiotic microbial communities are important for host health, but the processes shaping these communities are poorly understood. Understanding how community assembly processes jointly affect microbial community composition is limited because inflexible community models rely on rejecting dispersal and drift before considering selection. We developed a flexible community assembly model based on neutral theory to ask: How do dispersal, drift, and selection concurrently affect the microbiome across environmental gradients? We applied this approach to examine how a fungal pathogen affected the assembly processes structuring the amphibian skin microbiome. We found that the rejection of neutrality for the amphibian microbiome across a fungal gradient was not strictly due to selection processes, but was also a result of species-specific changes in dispersal and drift. Our modeling framework brings the qualitative recognition that niche and neutral processes jointly structure microbiomes into quantitative focus, allowing for improved predictions of microbial community turnover across environmental gradients.

## 13 Introduction

14 The contribution of microbial communities to host functions is a result of the ecological and evolutionary  
15 forces shaping microbial composition (West *et al.*, 2019; Adair & Douglas, 2017; Nemergut *et al.*, 2013).  
16 Community ecology has focused on four general processes affecting community assembly – dispersal, drift,  
17 selection, and speciation (Vellend, 2016, 2010). Dispersal describes the processes by which individuals im-  
18 migrate into a local community. Drift describes the probabilistic nature of births, deaths, and immigration  
19 within a local community that can lead to deviations from expected population patterns that would result  
20 from fitness differences alone. Selection, in contrast, describes any deterministic niche and fitness differences  
21 among species in a community (Vellend, 2010). Finally, speciation describes the processes by which new  
22 species are created. These four assembly processes also shape the composition and function of microbial  
23 communities (Zhou & Ning, 2017; Nemergut *et al.*, 2013; Hanson *et al.*, 2012). An ongoing challenge in com-  
24 munity ecology is to understand how the relative roles of these assembly processes vary across environmental  
25 gradients to better predict changes in communities in space and time (Stegen *et al.*, 2013; Vellend, 2016).

26 Neutral theory provides a framework to understand how altering dispersal, drift, and selection processes  
27 leads to observable changes in community structure (Rosindell *et al.*, 2012). The core assumptions of neutral  
28 theory are that species have equal fitness, occupy the same niche, and that species abundance in a local  
29 community is driven by ecological drift and dispersal of individuals from a metacommunity (Rosindell *et al.*,  
30 2011; Hubbell, 2001). In microbial ecology, neutral theory has provided an important null model with which  
31 studies have identified particular communities or species within a community that do not follow neutral  
32 predictions. These findings point to the importance of selection processes such as environmental filtering or  
33 competition for the assembly of the local community (e.g. Loudon *et al.*, 2016; Lee *et al.*, 2013; Ofiteru *et al.*,  
34 2010). Deviations from neutral patterns in microbial communities have been attributed to community-level  
35 perturbations such as host disease (Venkataraman *et al.*, 2015), time since a disturbance (Ferrenberg *et al.*,  
36 2013), local community age (Burns *et al.*, 2016), the specialization of bacterial species (Burns *et al.*, 2016;  
37 Lindström & Langenheder, 2012; Langenheder & Székely, 2011), the richness of the metacommunity (Ayarza  
38 & Erijman, 2011), changes in community composition across environmental gradients (Dumbrell *et al.*, 2010),  
39 and measurable fitness differences between bacteria species (Cira *et al.*, 2018).

40 While the aforementioned studies recognize the joint role of dispersal, drift, and selection on commu-  
41 nity assembly, their conclusions generally rely on testing whether particular communities or species within  
42 a community fully conform to the expected patterns of neutral theory. A rarely considered hypothesis in  
43 microbial studies, however, is that parts of neutral theory hold, while others are violated. Specifically, devi-  
44 ations from neutrality do not necessarily preclude the importance of dispersal and drift relative to selection

processes, but could instead be a result of differences in dispersal and drift between local communities due to an environmental gradient or differences between species (Janzen *et al.*, 2015; Etienne, 2009). In this study, we synthesize theoretical developments in neutral theory (Haegeman & Etienne, 2017; Harris *et al.*, 2017; Etienne, 2009; Sloan *et al.*, 2006) to build a flexible community assembly model that allows us to explicitly test how the relative importance of community assembly processes change across environmental gradients. Our study addresses an important knowledge gap in microbial community ecology by allowing for a more nuanced test of neutral theory. In particular the method avoids prematurely rejecting the importance of dispersal and drift or overstating the importance of selection based only on the failure of a base neutral model. While our method can be applied to any community given the assumptions are met, we focus on an application in microbial ecology.

Our model builds on extensive research that uses abundance patterns to understand community assembly processes (e.g., Preston, 1948; Hubbell, 2001; Haegeman & Etienne, 2017). However, alternative approaches exist that use community-level phylogenetic patterns to decompose the roles of selection, dispersal, and drift on community assembly based on deviations from phylogenetic null models (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009; Stegen *et al.*, 2012). Abundance-based approaches differ from phylogenetic approaches in a two notable ways. First, while phylogenetic null models are undeniably successful for understanding the assembly processes shaping communities (e.g. Stegen *et al.*, 2013), they require phylogenetic assumptions that are not necessarily met for all ecological communities (Mayfield & Levine, 2010). Abundance-based approaches make no assumptions about phylogeny and can be applied to communities where phylogenetic approaches cannot. Second, abundance-based and phylogenetic-based approaches use different ecological patterns to understand community assembly processes and thus should be viewed as complementary, rather than redundant, approaches for understanding community assembly.

Our community assembly model tests four distinct hypotheses regarding how selection, dispersal and drift shape communities and how these processes change across environmental gradients. The first hypothesis is consistent with the base neutral model that is often tested in microbial communities (e.g. Venkataraman *et al.*, 2015; Sloan *et al.*, 2006) and predicts that dispersal, drift and selection are all unaffected by the environmental gradient of interest (Fig. 1A). The second hypothesis predicts that the environmental gradient affects community-level dispersal and drift, but does not affect selection processes (e.g. Lee *et al.*, 2013; Etienne, 2009, Fig. 1B). The third hypothesis predicts that the environmental gradient again only affects dispersal and drift processes, but does so in a species-specific way (Haegeman & Etienne, 2017; Janzen *et al.*, 2015, Fig. 1C). The fourth hypothesis predicts that the environmental gradient affects selection processes, after accounting for the effects of the gradient on dispersal and drift (Fig. 1D). These four hypotheses are useful for understanding community assembly because they sequentially build complexity onto the base

neutral model such that rejection of the base model can be understood in terms of how it jointly fails to describe the dispersal, drift, and selection processes that shape a microbial community of interest.

We apply our approach to understand the relative role of community assembly processes structuring the skin microbiome of amphibians across a gradient of infection intensities of the pathogen *Batrachochytrium dendrobatidis* (Bd). Bd is an aquatic fungus that infects the skin of amphibians and has been the causative agent of amphibian declines and extinctions worldwide (Scheele *et al.*, 2019; Fisher *et al.*, 2012). Upon infection of an amphibian, Bd increases in abundance (i.e. pathogen load) on the skin of the host. The onset of the disease chytridiomycosis and amphibian mortality is highly load-dependent, where amphibians with higher Bd loads have substantially higher death rates (Stockwell *et al.*, 2010; Vredenburg *et al.*, 2010). The amphibian skin microbiome has been proposed as a potential protective factor against chytridiomycosis (Becker & Harris, 2010; Harris *et al.*, 2009). While studies have found a clear association between the composition of the amphibian microbiome and population-level outcomes of Bd invasion (Bates *et al.*, 2018; Jani *et al.*, 2017), the demonstrable effect of the amphibian skin microbiome as a protective mechanism against Bd infection has been variable (Jani & Briggs, 2018; Woodhams *et al.*, 2012; Harris *et al.*, 2009). Field and laboratory results suggest that the magnitude of the Bd effect on microbiome can be greater than the magnitude of the microbiome effect on Bd (if any) (Jani & Briggs, 2018, 2014). However, we do not yet understand which community assembly processes Bd affects to induce the observed changes in the skin microbiome. We ask the question: which community-level processes of the amphibian skin microbiome does Bd affect, if any? By exploiting the flexibility of neutral theory, we find that we can identify the relative roles of the assembly processes structuring the amphibian microbiome and microbial communities more generally, allowing for improved predictions of microbial community changes across environmental gradients

## Material and Methods

### Study system and data collection

We examined how Bd affected the microbiome assembly processes on the Mountain yellow-legged frog complex (*Rana sierrae/muscosa*, henceforth *R. sierrae*). *R. sierrae* are native to the Sierra Nevada mountains of California, USA and have suffered significant Bd-induced populations declines (Vredenburg *et al.*, 2010). We used previously published field data from seven populations of *R. sierrae* Jani & Briggs (2014). The number of frogs from each population are given in Table S1 and range between eight and 30 individuals. Adult and sub-adult frogs were captured in each population, their skin was swabbed using a standardized protocol, and Bd intensity and the bacterial community was quantified for each swab (for details see Jani & Briggs, 2014).

We characterized the bacterial communities using 16S rRNA gene amplicon pyrosequencing. Sequences were quality filtered, aligned, classified, and clustered into operational taxonomic units (OTUs) using the program mothur (Schloss *et al.*, 2009), as previously described (Jani & Briggs, 2014). For simplicity, we use OTU and ‘species’ synonymously. All seven populations were sampled in August or September 2010 with some populations sampled on multiple dates (Table S1). The multiple sampling dates were within two weeks of each other and previous analyses showed that variation in the microbiome composition driven strictly by date over these two weeks was small (Jani & Briggs, 2014). Therefore, we pooled across dates to maximize the gradient of Bd loads for each lake.

We also used data from a complementary laboratory experiment previously published in Jani & Briggs (2018) in which frogs from two different source populations were crossed with water from six different lakes and one treatment of sterile water. Six frogs were assigned to each Frog source  $\times$  Water source treatment and all frogs were housed in separate tanks ( $n = 84$  total frogs). Frogs were monitored for 21 days, after which three frogs from each treatment were challenged with Bd (200,000 zoospores per frog for three consecutive days) and three remained uninfected as controls. All frogs were monitored for 60 days following exposure and Bd and bacterial community swabs were taken approximately every seven days throughout the experiment (see Jani & Briggs, 2018, for a full description of the experiment).

## Hypothesis 1: Base neutral model

We used the Unified Neutral Theory of Biodiversity (UNTB) as the baseline for our community assembly models (Hubbell, 2001). The UNTB assumes that species have equal fitness and completely overlap in their habitat niche, the local community is saturated, local dynamics are linked to regional dynamics via dispersal from a metacommunity, and point mutations maintain species diversity in the metacommunity (Rosindell *et al.*, 2011; Etienne *et al.*, 2007). The probabilities of species  $i$  increasing in abundance by one ( $P(N_i + 1|N_i)$ ), decreasing in abundance by one ( $P(N_i - 1|N_i)$ ), or remaining the same ( $P(N_i|N_i)$ ) given that a death has occurred somewhere in the local community are given by

$$\begin{aligned} P(N_i + 1|N_i) &= \frac{N_T - N_i}{N_T} \left( mp_i + (1 - m) \frac{N_i}{N_T - 1} \right) \\ P(N_i - 1|N_i) &= \frac{N_i}{N_T} \left( m(1 - p_i) + (1 - m) \frac{N_T - N_i - 1}{N_T - 1} \right) \\ P(N_i|N_i) &= 1 - P(N_i + 1|N_i) - P(N_i - 1|N_i) \end{aligned} \tag{1}$$

where  $N_i$  is the abundance of species  $i$ ,  $N_T$  is the total community size (in terms of total abundance) summed across  $S$  species,  $p_i$  is the relative abundance of species  $i$  in the metacommunity, and  $m$  is the migration probability (Hubbell, 2001). We did not model speciation in this formulation of the neutral model and instead

assumed a fixed metacommunity  $p_1, \dots, p_S$  (Sloan *et al.*, 2006). In our study, the bacterial community on the skin of a single frog represented a local community.

Given the above neutral model, the probability of a relative abundance vector  $\boldsymbol{\pi}$  of  $S$  bacterial species in a local community at equilibrium is approximately (Harris *et al.*, 2017; Sloan *et al.*, 2007)

$$\boldsymbol{\pi} \sim \text{Dirichlet}(Ip_1, \dots, Ip_S) \quad (2)$$

$I$  is the fundamental recruitment number and is interpreted as the number of recruitment events to the local community from the metacommunity given one local birth (Etienne, 2009, 2005).  $I$  combines the parameters  $N_T$  and  $m$  into a single parameter via the relation  $I = \frac{1-m}{m}(N_T - 1)$  (Rosindell *et al.*, 2011).  $I$  captures dispersal and drift and can be conceptually interpreted as the ratio of immigration rate to birth rate (Etienne *et al.*, 2007). As  $I$  increases, species  $i$  in a local community will have less variability about its mean relative abundance  $p_i$  as recruitment from the metacommunity becomes more important relative to drift. In contrast, as  $I$  decreases local community dynamics are increasingly driven by drift (i.e. stochastic births and deaths) such that the relative abundance of species  $i$  has increased variability about its mean  $p_i$ .

## Hypothesis 2: Dispersal and drift vary across local communities

The predictive ability of neutral theory can, in some cases, be substantially improved if  $I$  is allowed to vary across local communities (Jabot *et al.*, 2008; Etienne, 2007). While local community dynamics can still be explained by dispersal, drift and equal fitness, the relative importance of dispersal and drift can differ depending on ecological factors associated with the local community. This suggests that variation in  $I$  between local communities can reflect how biological factors influence dispersal and drift, without breaking the assumption of neutrality within a local community (Etienne, 2009).

In this analysis, we were interested in whether Bd affected dispersal and drift in the amphibian microbiome. A sampled frog is equivalent to a local community and equation 2 can be updated to allow  $I$  to vary across frogs depending on frog-specific covariates such as Bd load (see Appendix S1 in Supporting Information). For example, if Bd and bacteria compete for space on the frog skin and Bd is the dominant competitor, we might predict that increasing Bd load reduces bacterial community abundance, increasing the importance of drift. This mechanism would be reflected in decreasing  $I$  with increasing Bd load.

## Hypothesis 3: Dispersal and drift vary by bacterial species

Hypothesis 2 assumes that frog-specific covariates such as Bd load affect the dispersal and drift of all OTUs equally. However, Bd does not necessarily affect all species in the same way (Woodhams *et al.*, 2015). Under

equation 2 and the neutral model, the marginal relative abundance of bacterial species  $i$  in local community  $j$  is (Sloan *et al.*, 2006)

$$\pi_{i,j} \sim \text{Beta}(I_j p_i, I_j(1 - p_i)) \quad (3)$$

Allowing for species-level differences in how Bd affects recruitment violates the neutrality assumption and equation 3 no longer holds. We can test whether equation 3 fails to describe bacterial species using an alternative model

$$\pi_{i,j} \sim \text{Beta}(I_{i,j} p_i, I_{i,j}(1 - p_i)) \quad (4)$$

where we allow  $I_{i,j}$  to vary with species  $i$ . If a model with varying  $I_{i,j}$  describes the data better than a model with fixed  $I_j$ , then this is evidence that dispersal and drift are not affecting all species in the same way. Because equation 4 is not the exact marginal distribution for equation 1 with varying  $I$ , we cannot consistently interpret  $I_i$  as the fundamental recruitment number for species  $i$  (Appendix S2). Rather, in this context we can consider  $I_i$  as capturing dispersion in abundance for species  $i$ , which we would expect to be the same for all species under a base neutral model.

We allowed  $I$  to vary with both frog-level covariates and between all  $i = 1, \dots, S - 1$  bacterial species (Appendix S1). We only considered  $S - 1$  species as the information contained in the  $S$ th species is redundant based on the sum-to-zero constraint of relative abundances. While not considering the complete multivariate sampling formula as in equation 2 ignores some relevant information in the relative abundance distribution, an assumption of independent species can yield similar inference regarding relative abundance patterns compared to the multivariate sampling formula (Haegeman & Etienne, 2017). We use the independent species assumption for the analyses presented in the main text.

#### 181 Hypothesis 4: Selection processes drive community assembly

We have thus far assumed that recruitment to all local communities (i.e. frogs) came from the same meta-community and that relative OTU abundances on each frog provided an estimate for the relative abundances  $p_1, \dots, p_S$  of the metacommunity (Jabot *et al.*, 2008; Sloan *et al.*, 2006). The first assumption is conceptually valid under metacommunity theory as, by construction, one metacommunity contributes to all local communities within a region (Hubbell, 2001). The second assumption is only valid if all local communities are actually experiencing neutral dynamics. If they are not, then local OTU abundances do not necessarily reflect metacommunity abundances. This could be the case if, for example, local selection processes such as



competitive interactions made it so rare species in the metacommunity were consistently over-represented in the local community.

Conceptually, consider a true, but unknown metacommunity from which local communities recruit (Fig. 1D). If neutral processes are driving assembly, then the mean relative abundance of OTUs across the local communities provides an estimate for the relative abundance of OTUs in the metacommunity (Sloan *et al.*, 2006). This is true even if dispersal varies between communities or between species (Appendix S2). However, if selection processes are operating in the local communities the relative abundances will appear to have been sampled from an “apparent” metacommunity that is different from the true metacommunity (Fig. 1D). The true metacommunity and the apparent metacommunity are linked through an unknown filter that is comprised of any unobserved selection processes (Vellend, 2016). Under the assumption that the true metacommunity is the same but unknown for all local communities at the time of sampling, any changes in the apparent metacommunity would suggest changes in the filter and thus changes in the selection processes operating on local communities. By testing for changes in the apparent metacommunity across locations or environmental gradients, we can implicitly detect changes in selection processes. Importantly, we can compare whether changes in selection processes or changes in dispersal and drift are more important for predicting community-level changes across environmental gradients.

Species-specific dispersal and recruitment rates can also affect a species fitness (Tilman, 1994). Here we distinguish between the effects of dispersal and selection processes on fitness based on the scale at which they are assumed to be acting in the metacommunity framework. Dispersal is assumed to act at an among-local community scale and selection is assumed to act at a within-local community scale. In other words, dispersal ( $I$ ) effects how an individual from a species recruits to a patch, but does not affect the individual’s fitness once in the patch (Vellend, 2016).

The above conceptual framework amounts to fitting dispersion effects (dispersal and drift) and mean effects (selection). The Beta distribution given in equation 4 is defined by two parameters:  $I$  (dispersion) and  $p_i$  (mean). Testing for selection effects corresponds to allowing metacommunity relative abundance  $p_i$  to vary with local community characteristics (Appendix S1).

## Model fitting, comparison, and diagnostics

We first fit the base neutral model (Hypothesis 1) and then fit models of increasing complexity to test our hypotheses on how dispersal, drift and selection affected the *R. sierrae* skin microbiome. While we were primarily interested in the effect of Bd on community assembly processes, we also considered two additional covariates: a lake (for the field)/treatment (water source  $\times$  frog source for the laboratory) factor

and frog body size. Location is a well-known factor driving microbiome composition and we examined how a lake/treatment affected selection, dispersal and drift processes. Frog body size is analogous to local community area and changing area can potentially increase or decrease  $I$ , depending on how total community size  $N_T$  scales with area (Vellend, 2016; Etienne, 2007). Therefore, we included a body size effect on dispersal and drift. We included Bd load in three different ways in the model: as a fixed effect on dispersal and drift, as an OTU-specific random effect on dispersal and drift, and finally as an OTU-specific selection effect (see Table 1, 2). We explored a linear and quadratic effect of Bd on selection. For the laboratory experiment, we also examined a random effect of frog ID on dispersal and drift as some frogs were sampled multiple times.

We fit all models using 19 OTUs with the highest average relative abundance across all samples. The top 19 OTUs made up 80% and 90% of the relative abundance in the field and laboratory samples, respectively and were not identical for the field and laboratory. We examined the sensitivity of our results to different numbers of OTUs in Appendix S3. We compared the fitted models using PSIS-LOO-IC (LOO-IC), a variant of information criteria that compares the within-sample predictive performance between models (Vehtari *et al.*, 2016). We selected the best predictive and most parsimonious model as the model with the lowest LOO-IC. We fit all community assembly models using the probabilistic programming language Stan (PyStan, version 2.18.1.0) (details in Appendix S1; Carpenter *et al.*, 2017).

We assessed the goodness-of-fit of the models using two approaches. First, we computed a Bayesian  $R^2$  that predicted the amount variation in the data described by the selection processes (Gelman *et al.*, 2017). We also calculated a likelihood-based goodness-of-fit by simulating the observed dataset from the fitted models and comparing the OTU-specific simulated likelihoods with the predicted likelihood of the observed data under the fitted model (Etienne, 2007). If the observed likelihood significantly deviated from the predicted likelihood this indicated that the model could not describe the data. This goodness-of-fit test was not meant to compare models, but to test if a fitted model could actually describe the data.

## Results

Bd affected the community assembly of the *R. sierrae* microbiome through a combination of selection, dispersal, and drift processes in the field and the laboratory. In both the field and the laboratory, we strongly rejected “Hypothesis 1: Base neutrality” (Table 1, 2). Goodness-of-fit tests indicated that neither the base neutral model (Model F1, L1) nor the model with within-lake/treatment neutrality (Model F2, L2) could adequately capture the patterns of OTU abundance across frogs, particularly for the most abundant OTUs (Fig. 2A, B). However, the goodness-of-fit test in Fig. 2 was only a within-sample test and did not provide any information about the out-of-sample predictive ability of the neutral model compared to

a model with selection effects. Including selection effects, consistent with “Hypothesis 4”, led to the best predictive model for both the field and the laboratory data (Table 1, Model F8; Table 2). Hypothesis 4 with OTU-specific Bd effects on selection and dispersal and drift was the most parsimonious model for both the lab and field data, suggesting that Bd altered microbial composition across local frog communities by affecting selection, dispersal and drift processes (Table 1, Model F8; Table 2, Model L7). The best predictive model for the field data (Model F8) described 65% of the variation in OTU relative abundance. The best predictive model for the lab data (Model L7) described 41% of the variation in OTU relative abundance.

There were three notable differences in the role of dispersal and drift processes between the lab and field. First, the estimated values of  $I$  were generally lower for the laboratory data, indicating that drift was playing a larger role relative to dispersal in the laboratory compared to the field (Fig. 3, Fig. 4A, C). The one exception was lake YOSE 3, which had comparable levels of  $I$  to the lab, particularly as Bd load increased. Second, while the main effect of Bd on  $I$  was null for the field data, the effect of Bd load on  $I$  trended positively in the laboratory data (Fig. 3). Moreover, while there was strong evidence for OTU-specific drift and dispersal in the field and the lab (Fig. 4A, C), OTUs did not necessarily show the same effect of Bd on  $I$  in the field and the lab (Fig. 4B, D). For example, in the lab *Rubrivivax* showed a significant negative effect of Bd on  $I$  and in the field *Rubrivivax* did not show a significant Bd effect on  $I$  (Fig. 4B, D). Third, frog body size had no detectable effect on  $I$  in the laboratory compared to the negative effect of body size on  $I$  in the field data. The body sizes of laboratory frogs were significantly smaller than the body sizes of frogs from the field and they covered a smaller range of body sizes (laboratory mean: 34 mm, field mean: 43 mm; two-sample  $t$ -test with unequal variance:  $t_{df=154.23} = -7.45$ ,  $p < 0.001$ ; Fig. 3B).

Bd effects on selection processes varied by OTU in the field (Fig. S1, S2). OTU relative abundance showed both positive and negative responses to Bd (Fig. S1, S2). Including a quadratic effect of Bd on OTU relative abundance significantly improved the fit of the model, reducing LOO-IC by 184 units compared to a model with only a linear effect of Bd on OTU relative abundance. Similarly, there was strong evidence that Bd effects on selection processes varied by OTU in the laboratory data (Fig. S3, S4). The best fit laboratory model also included a quadratic effect of Bd on relative abundance and removing this quadratic effect increased LOO-IC by 88 units. Species that were represented in both the laboratory and field analyses showed similar responses to Bd. For example, *Psuedomonas* and *Stenotrophomonas* generally decreased in relative abundance with increasing Bd in both the lab and field, while *Rubrivivax* and *Undibacterium* increased with increasing Bd (Fig. S1, S3).

## Discussion

Bd infection jointly affected the selection, dispersal, and drift processes structuring the skin microbiome of *R. sierrae*. Our results showed that a base neutral model with no selection and constant dispersal and drift across communities and between OTU species could not adequately capture OTU abundance patterns across varying Bd loads. Rather, we found that the magnitude of drift and dispersal processes and how they changed with Bd load varied between OTUs in the field and the lab. However, only allowing for changes in dispersal and drift processes across local communities and among OTUs failed to capture significant variation in the observed microbial communities and invoking implicit effects of Bd on selection processes significantly improved the predictive ability of our community assembly models.

Variation in the fundamental recruitment number  $I$  can highlight the importance of biological mechanisms influencing community assembly across local communities (Lee *et al.*, 2013; Etienne, 2009; Jabot *et al.*, 2008). For example, previous studies have found that communities or guilds of trees with a high proportion of dispersal by biotic mechanisms (e.g. mammals or birds) had higher estimated values of  $I$  compared to communities or guilds of trees that were primarily dispersed by abiotic mechanisms (e.g. wind or water; Janzen *et al.*, 2015; Jabot *et al.*, 2008). In our study, we predicted that local communities (i.e. frogs) with varying Bd loads would have different skin microbiomes at least in part due to Bd-induced changes in dispersal and drift. Particularly, we expected increasing Bd load to decrease  $N_T$  in the local community and increase the importance of drift relative to dispersal, decreasing  $I$  across OTUs in the community. We did not observe this overall effect of Bd on  $I$  in either the field or the lab. We did, however, observe a significant OTU-specific effect of Bd on  $I$  in both the field and the lab, with OTUs showing no change in  $I$ , increasing  $I$ , and decreasing  $I$  with increasing Bd load.

These OTU-specific effects of Bd on  $I$  can be understood in terms of the two parameters upon which  $I$  depends:  $m$  and  $N_T$  (Rosindell *et al.*, 2012). The total community size  $N_T$  can vary across environmental gradients and was the primary parameter that we expected Bd to affect. However, the immigration probability  $m$  can also vary across gradients and can vary between species (Janzen *et al.*, 2015). Therefore, even if increasing Bd load did reduce  $N_T$  by, for example, reducing the overall space on frog skin that could be colonized by bacteria, OTUs could show different changes in immigration probability  $m$  as Bd load increased (e.g. disturbance specialists could increase  $m$ ). In this case, any overall effect of Bd on  $I$  through  $N_T$  could be swamped out by OTU-specific changes in  $m$  with changing Bd load.

Interestingly, however, we did not see consistent OTU-specific effects of Bd on  $I$  in the lab and field. In the laboratory experiment, more OTUs showed a strong positive effect of Bd load on  $I$ , indicating a reduced importance of drift relative to immigration with increasing Bd load. This was again counter

to our prediction that  $I$  would decrease with increasing Bd load. The laboratory experiment attempted to standardize the amphibian microbial communities at the start of the experiment by washing frogs in hydrogen peroxide and housing frogs together in a common garden tank (Jani & Briggs, 2018). Moreover, the laboratory metacommunity was likely dynamic as water was replaced every seven days and frogs shed and excreted into the environment in a confined tank. Considering the uninfected frog skin at the start of the experiment as an approximation to a benign environment relative to an infected/colonized frog, a combination of priority effects, a variable metacommunity, and transient dynamics all could have contributed to uninfected frogs having increased variability in community composition (Vellend *et al.*, 2014; Chase, 2007). Increasing disturbance can in some cases act as a synchronizing force, eliminating initial priority effects and selecting for similar species among communities (Chase, 2007). If Bd acted as a synchronizing force in the lab and offset the variability between uninfected frogs induced by priority effects or variability in the metacommunity between tanks, we would expect to see a trend of increasing  $I$  with increasing Bd load as strong selection processes decreased the importance of drift in community assembly.

In summary, our study highlights two unique ways the fundamental recruitment number  $I$  can change in response to an environmental gradient, both of which are important to consider when linking changes in  $I$  to biological processes influencing dispersal and drift. As discussed above, any changes in  $N_T$  and  $m$  across an environmental gradient can change  $I$ . If an environmental gradient simultaneously alters  $N_T$  and  $m$  in a species-specific way, it can be difficult to uniquely identify whether changes in  $I$  are driven by changes in drift or dispersal. However, if  $N_T$  is known or can be approximated across community samples then including data on  $N_T$  could help further parse apart how drift and dispersal affect  $I$ .

A second way that  $I$  can “change” over an environmental gradient is by failing to account for relevant selection processes such that the variance induced by these selection processes is then statistically captured by  $I$ . However, this can generally be avoided by jointly allowing an environmental covariate of interest to affect selection, dispersal and drift processes, such that any observed effects of the covariate on  $I$  are conditional on the covariate’s affect on selection processes. If cryptic/unmeasured selection processes are operating on community assembly (e.g., unobserved spatial processes, Stegen *et al.*, 2013), then allowing these processes to be captured by  $I$  is not necessarily problematic if the goal is to understand, for example, whether or not a base neutral model is sufficient to explain community abundance patterns across sites (Etienne, 2007). That being said, the approach we develop here could be used to account for these unobserved selection processes by including random effects when modeling mean relative abundance (e.g., spatial random effects). While beyond the scope of this paper, we adopt the view that variation in abundance is not infinitely reducible with respect to species identity, such that drift processes are an important component of community assembly (Vellend *et al.*, 2014; Clark, 2009).

While community-level and OTU-specific differences in dispersal and drift processes influenced the *R. sierrae* skin microbiome, selection processes also played an important role. In the framework of (Vellend, 2010), selection processes are broad, encompassing any mechanisms that can lead to deterministic differences in fitness between species (Vellend, 2016). Therefore the deterministic response of a species to a given covariate may be due to changes in, but not limited to, constant selection processes such as species sorting (Leibold *et al.*, 2004), changes in negative density-dependent processes such as intraspecific to interspecific competition ratios (Adler *et al.*, 2007), or changes in positive density-dependent processes such as priority effects (Chase, 2007). The approach we used here did not attempt to distinguish between these different selection processes, but instead implicitly modeled selection through changes in mean relative OTU abundance, while accounting for dispersal and drift processes.

We found that the relative abundance of OTUs in the *R. sierrae* microbiome could not be described by a single metacommunity across lakes in the field or treatments in the lab. This is consistent with the importance of environmental filtering in microbiome composition in amphibian systems (Jani & Briggs, 2014; Kueneman *et al.*, 2014) and other microbial studies more broadly (Adair & Douglas, 2017). Moreover, after accounting for environmental filtering by lake (in the field) and treatment (in the lab), our model showed that OTU-specific, Bd effects on selection processes improved our model's predictive ability, even after accounting for OTU-specific Bd effects on dispersal and drift. Consistent with previous results, we found that Bd had positive, negative, and null selection effects on OTU relative abundance (Jani & Briggs, 2018). The key conceptual difference between this work and the results presented in Jani & Briggs (2014, 2018) was that previous work examined effects of Bd load on relative abundance and the current work examined the different community-level processes that Bd altered to cause these effects. An important next step is to test candidate mechanisms leading to the observed selection patterns using time series data of amphibian microbial communities perturbed by Bd infection (e.g., Faust *et al.*, 2018).

While it is increasingly recognized that both selection (i.e. niche) and dispersal and drift (i.e. neutral) processes affect the assembly of microbial communities (Zhou & Ning, 2017; Ofiteru *et al.*, 2010), untangling the relative contributions of these processes is challenging (Stegen *et al.*, 2013; Vellend *et al.*, 2014). Neutrality in microbial communities is commonly tested using a neutral model with a single parameter describing the influence of dispersal to all local communities (Burns *et al.*, 2016; Loudon *et al.*, 2016; Venkataraman *et al.*, 2015; Sloan *et al.*, 2006). However, models that recognize that there are degrees of neutrality provide a more complete view of how dispersal and drift affect community assembly. The framework we developed here is broadly applicable to other microbial communities and represents a synthesis of theoretical developments in neutral theory in both microbial and macroecological communities (Haegeman & Etienne, 2017; Harris *et al.*, 2017; Etienne, 2009; Sloan *et al.*, 2006). By understanding the role of community assembly processes

in the microbiome we can improve our ability to predict changes in microbial composition and function across environmental gradients.

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Table 1: Model selection for community assembly models describing amphibian microbiomes in the field. Lake was a factor with seven levels. Body size was the body size of a frog. Bd was the Bd load on a frog. The “ $\times$ ” symbol represents an interaction between two variables.  $\Delta$ LOO-IC is the PSIS-LOO difference between all models and the Model F8. “Constant” for either drift and dispersal or selection means that the model assumed that the given processes did not vary across local communities (i.e. frogs) or for OTUs within a local community. The “Hypothesis” column indicates the hypothesis with which the fitted model is most consistent. Hypothesis 1: Base neutral model; Hypothesis 2: Dispersal and drift vary across local communities; Hypothesis 3: Dispersal and drift vary by bacterial species; Hypothesis 4: Selection processes drive community assembly.

Model name	$\Delta$ LOO-IC	Drift and dispersal	Selection	Hypothesis
F1	1663	Constant	Constant	Hypothesis 1
F2	973	Constant	Lake	Hypothesis 1 (within a lake)
F3	960	Body size, Bd	Lake	Hypothesis 2 (within a lake)
F4	961	Body size, Bd, Lake	Lake	Hypothesis 2 (within a lake)
F5	446	Body size, Bd, Lake, OTU-specific $I$	Lake	Hypothesis 3 (within a lake)
F6	448	Body size, Lake, OTU-specific $I$	Lake	Hypothesis 3 (within a lake)
F7	416	Body size, Bd, Lake, OTU-specific Bd	Lake	Hypothesis 3 (within a lake)
F8	0	Body size, Bd, Lake, OTU-specific Bd	Lake $\times$ Bd <sup>2</sup>	Hypothesis 4
F9	31	Body size, Bd, Lake, OTU-specific $I$	Lake $\times$ Bd <sup>2</sup>	Hypothesis 4
F10	30	Body size, Lake, OTU-specific $I$	Lake $\times$ Bd <sup>2</sup>	Hypothesis 4
F11	561	Constant	Lake $\times$ Bd <sup>2</sup>	Hypothesis 4
F12	1050	Body size, Bd $\times$ Lake, OTU-specific Bd	Constant	Hypothesis 3

Table 2: Model selection for community assembly models describing amphibian microbiomes in the lab. Treatment was a factor with 14 levels representing different water source by frog source combinations. Body size was the body size of a frog. Bd was the Bd load on a frog.  $\Delta\text{LOO-IC}$  is the PSIS-LOO difference between all models and Model L7. “Constant” for either drift and dispersal or selection means that the model assumed that the given processes did not vary across local communities (i.e. frogs) or for OTUs within a local community. The “Hypothesis” column indicates the hypothesis with which the fitted model is most consistent. Hypothesis 1: Base neutral model; Hypothesis 2: Dispersal and drift vary across local communities; Hypothesis 3: Dispersal and drift vary by bacterial species; Hypothesis 4: Selection processes drive community assembly. Including a random effect of frog on dispersal and drift increased LOO-IC 7 units compared to Model L7.

Model name	$\Delta\text{LOO-IC}$	Drift and dispersal	Selection	Hypothesis
L1	1573	Constant	Constant	Hypothesis 1
L2	1374	Constant	Treatment	Hypothesis 1 (within a Treatment)
L3	1350	Body size, Bd	Treatment	Hypothesis 2 (within a Treatment)
L4	453	Body size, Bd, OTU-specific $I$	Treatment	Hypothesis 3 (within a Treatment)
L5	544	Body size, OTU-specific $I$	Treatment	Hypothesis 3 (within a Treatment)
L6	348	Body size, Bd, OTU-specific Bd	Treatment	Hypothesis 3 (within a Treatment)
L7	0	Body size, Bd, OTU-specific Bd	Treatment, Bd <sup>2</sup>	Hypothesis 4
L8	139	Body size, Bd, OTU-specific $I$	Treatment, Bd <sup>2</sup>	Hypothesis 4
L9	134	Body size, OTU-specific $I$	Treatment, Bd <sup>2</sup>	Hypothesis 4
L10	1025	Constant	Treatment, Bd <sup>2</sup>	Hypothesis 4
L11	593	Body size, Bd, OTU-specific Bd	Constant	Hypothesis 3

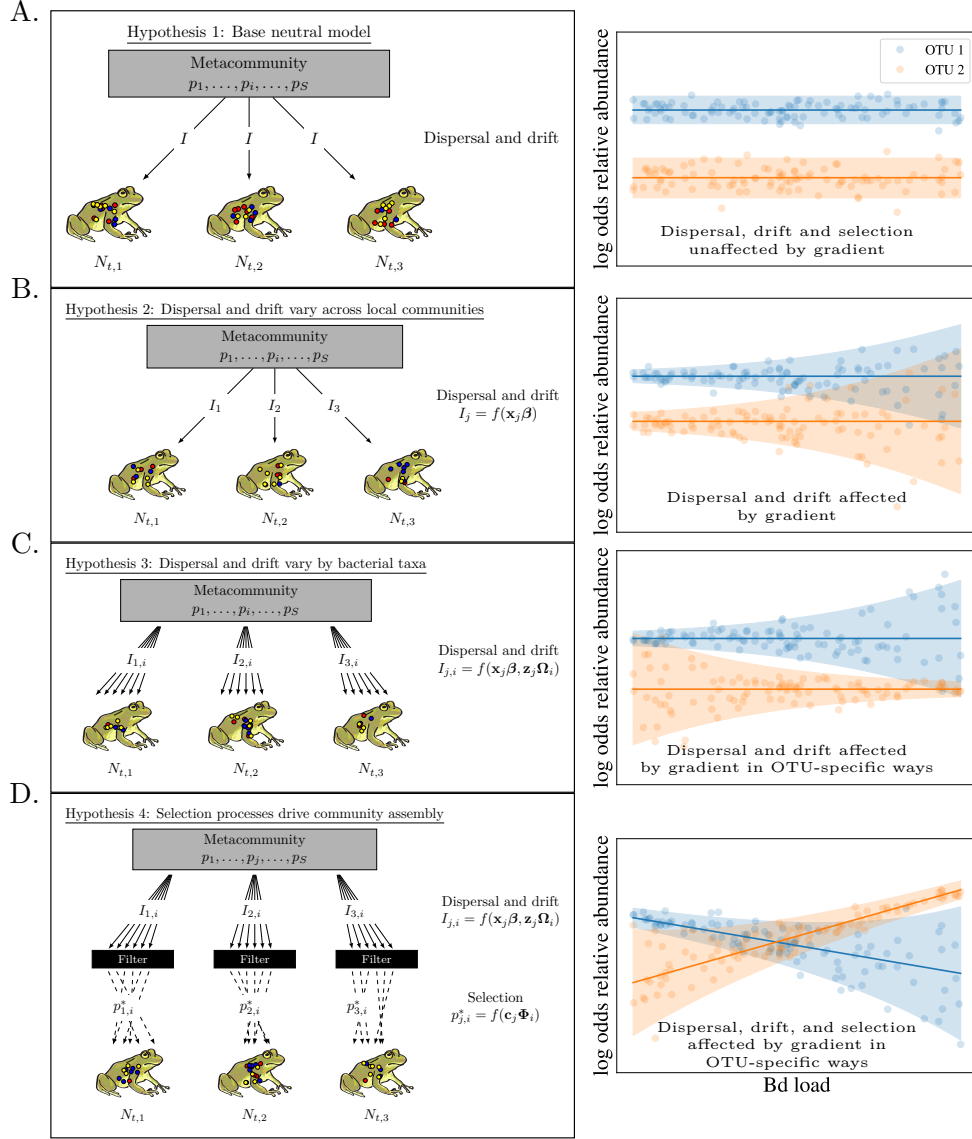


Figure 1: Four hypotheses regarding how selection, dispersal, and drift processes can affect microbial community assembly. The figure describes the community assembly models using local frog communities and their skin microbiomes. ‘OTU’ and ‘species’ are used synonymously. For each row in the figure, the left panel gives a visual description of the hypothesis and the right panel gives an example of how the relative abundance patterns of two OTUs in a community might look under the hypothesis. Each point in the right panel is the log odds relative abundance of an OTU in a local community. Fungal infection load (Bd) on frog skin is used as an example environmental gradient. **A.** Hypothesis 1 was a base neutral model in which dispersal was constant across all local communities and selection processes were absent.  $p_i$  is the relative abundance of OTU  $i$  in the metacommunity.  $N_{t,j}$  is the sampled community size and  $N_{t,j} \ll N_T$  where  $N_T$  is the total community size. Note that dispersal and drift processes captured by the fundamental recruitment number  $I$  are constant across OTUs in the base neutral model, but variance in relative abundance in a local community is a function of mean OTU relative abundance and is given by  $\frac{p_i(1-p_i)}{I+1}$  for the  $i$ th OTU in equation 2. **B.** Hypothesis 2 allowed dispersal and drift to vary across local communities.  $f(\mathbf{x}_j \beta)$  indicates that  $I$  can vary with covariates in the  $j$ th local community  $\mathbf{x}_j$  and  $\beta$  are the effects of the covariates on  $I$ . **C.** Hypothesis 3 allowed dispersal and drift to vary across local communities and between OTUs.  $f(\mathbf{x}_j \beta, \mathbf{z}_j \Omega_i)$  indicates that  $I$  can vary with covariates in the  $j$ th local community and these effects can be specific to the  $i$ th OTU ( $\mathbf{z}_j \Omega_i$ ). **D.** Hypothesis 4 allowed selection processes to affect community assembly through an unobserved selection “filter” that altered the relative abundance of OTUs in the local communities ( $p_{j,i}^*$ ) relative to the metacommunity. The crossing arrows for Hypothesis 4 indicate that the filter may lead to a different rank order of species relative abundances in the local community relative to the metacommunity.  $p_{j,i}^* = f(\mathbf{c}_j \Phi_i)$  indicates that there are OTU-specific effects ( $\Phi_i$ ) of local community covariates  $\mathbf{c}_j$  on observed relative abundance  $p_{j,i}^*$ .

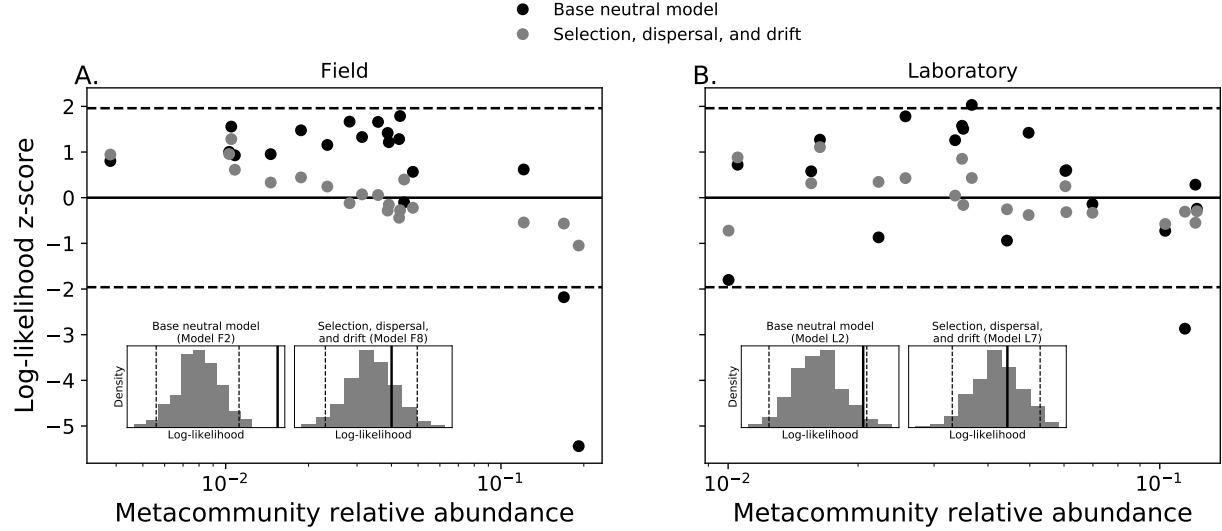


Figure 2: Likelihood-based goodness-of-fit tests for the field (**A.**) and laboratory (**B.**). For both the field and the laboratory, the figures show that the community assembly model that included selection, dispersal, and drift could adequately describe the observed OTU relative abundance data and the base neutral model could not. The base neutral model in both **A.** and **B.** allowed for a variable metacommunity between lakes and treatments, respectively (i.e. Model F2, L2 in Table 1, 2). Each point gives the log-likelihood z-score for one of the 19 most abundant OTUs used when fitting the model. Metacommunity relative abundance on the x-axis was calculated across all lakes/treatments. To generate the z-score, we used the fitted models to simulate 500 datasets with the same structure as the observed dataset. For each OTU in each simulated dataset, we computed the log-likelihood of the OTUs relative abundance vector under the fitted model. This provided a distribution of OTU-specific log-likelihoods given the model. We then compared the observed log-likelihood to the mean predicted log-likelihood, standardizing by the standard deviation of the predicted log-likelihoods. The dashed lines show the 2.5% and 97.5% quantiles of a standard normal distribution and points lying outside this range can be considered as unlikely under the fitted model. The inset plots show the overall goodness-of-fit of the respective models. The histogram shows the simulated log-likelihoods and the vertical, dashed lines given the upper and lower 2.5% and 97.5% percentiles and the vertical black line shows the log-likelihood of the observed data under the fitted model. When the black line falls outside the vertical dashed lines, the full dataset can be considered as unlikely given the fitted model.

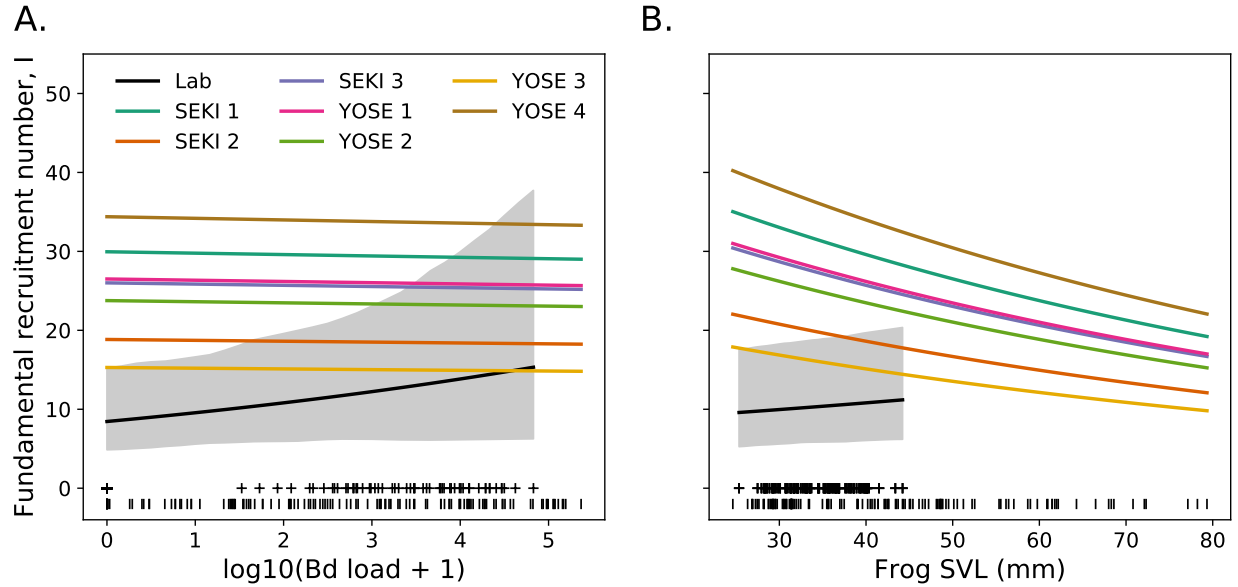


Figure 3: The predicted fixed effects of Bd load (**A.**) and host body size (**B.**) on the fundamental recruitment number  $I$  of the *Rana sierrae* microbiome in the field and the laboratory. **A.** The colored lines give the median model predictions of the Bd load effect on  $I$  for each lake in the field (Model F8). The black line gives the median model prediction from the laboratory (Model L7). The shaded region gives the 95% credible interval for the lab data. 95% CIs for the field predictions are not shown for visual clarity. There was no evidence that the main effect of Bd on  $I$  varied between lakes (adding an interaction between Lake and Bd on dispersal and drift increased model LOO-IC by 30 units). The plotted predictions are from Model F8 that did not allow the effect of Bd on  $I$  to vary between lakes, resulting in parallel lines. The crosses show the observed Bd loads from the lab experiment and the vertical lines show the observed Bd loads from the field. **B.** Similar to A., but B. shows the predicted effect of frog body size on  $I$  for the lab (black line) and field data (colored lines). Model F8 did not let the effect of body size on  $I$  vary between lakes.

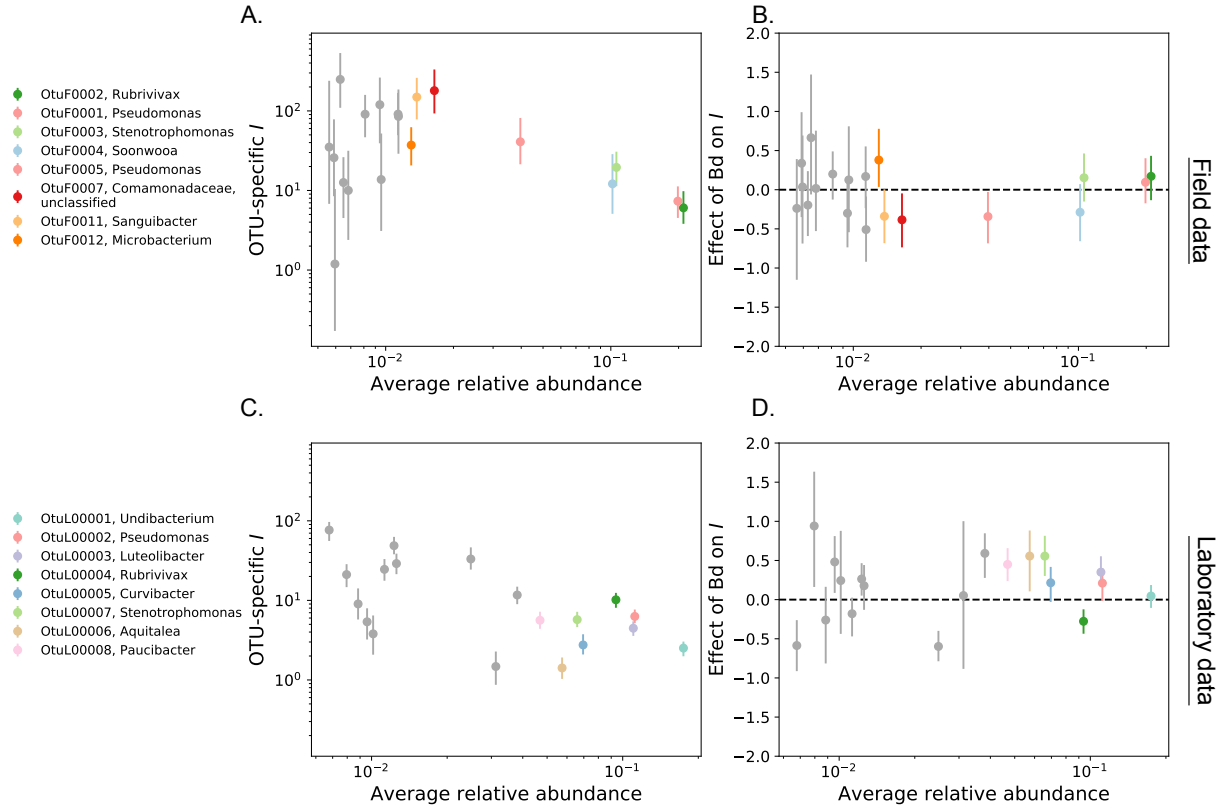


Figure 4: OTU-specific differences in dispersal and drift between OTUs in the amphibian skin microbiome for field and laboratory experiments.  $I$  is the fundamental recruitment number and should be interpreted as dispersion in abundance for this plot. **A.**, **C.** There were significant among-OTU differences in  $I$ , inconsistent with Hypotheses 1: Base neutral model. The eight most abundant OTUs are shown in color and the remaining OTUs of the top 19 most abundant OTUs are shown in gray. The points give the predicted median value of  $I$  when Bd load is 0 for all OTUs. Error bars give the 95% credible interval. **B.**, **D.** The effect of Bd on dispersal and drift varied by OTU in the field and the laboratory, consistent with Hypothesis 3: Dispersal and drift vary by bacterial species. The points give the median effect of Bd on  $I$  and the error bars are 95% credible intervals. The dashed black line indicates a Bd effect on  $I$  of zero.