Winogradsky review

Investigating eco-evolutionary processes of microbial community assembly in the wild using a model leaf litter system

Kristin Barbour and Jennifer B.H. Martiny*

Dept. of Ecology and Evolutionary Biology, University of California, Irvine, Irvine, CA 92697

*corresponding author: 321 Steinhaus Hall, University of California, Irvine, Irvine, CA 92697-2525, USA, jmartiny@uci.edu

Running head: Assembly of leaf litter communities

Key words: community assembly; eco-evolutionary processes; microbial communities; soil; microbiomes; decomposition; *Curtobacterium*

Study funding information:

This work was supported by the U.S. National Science Foundation and a U.S. National Institute of Allergy and Infectious Diseases Training Grant.

Abstract

Microbial communities are not the easiest to manipulate experimentally in natural ecosystems. However, leaf litter – topmost layer of surface soil – is uniquely suitable to investigate the complexities of community assembly. Here, we reflect on over a decade of collaborative work to address this topic using leaf litter as a model system in southern California ecosystems. By leveraging a number of methodological advantages of the system, we have worked to demonstrate how four processes – selection, dispersal, drift, and diversification – contribute to bacterial and fungal community assembly and ultimately, impact community functioning. Although many dimensions remain to be investigated, our initial results demonstrate that both ecological and evolutionary processes occur simultaneously to influence microbial community assembly. We propose that the development of additional and experimentally tractable microbial systems will be enormously valuable to test the role of eco-evolutionary processes in natural settings and their implications in the face of rapid global change.

Introduction

Community assembly describes the processes that shape the identity and abundance of organisms in ecological communities [1]. These processes are key to understanding foundational principles of ecology including biogeographic patterns, community responses to environmental change, and the relationship between biodiversity and ecosystem functioning [2-4]. Understanding the assembly of microbial communities (or microbiomes) specifically can also facilitate our ability to modify or engineer them to improve human and environmental health [5, 6].

Four processes influence the assembly of ecological communities, microbial or otherwise: selection, dispersal, ecological drift, and diversification [7-9]. Ideally, one would like to manipulate the influence of each process separately and in combination while allowing community assembly to proceed over many generations, but such experiments are often impractical for plant and animal systems. Some clever experiments have been conducted that, for instance, modify dispersal or drift [10-13], but investigating diversification (i.e., evolution) during community assembly is particularly difficult [8, 14, 15]. Thus, most support for how these four interacting processes come together to shape the composition of ecological communities are derived from observed patterns, theoretical models, or laboratory studies [16-19].

Microbial communities have been useful for testing community assembly theory in the lab [e.g., 20, 21, 22], but they are not the most obvious system for testing these theories in natural ecosystems. Unlike plants, microbes are not easily "seeded" into plots, and unlike animals, they cannot be marked and recaptured. Microbial communities are also orders of magnitude more diverse than their plant and animal counterparts, and many taxa have yet to be cultured and described. At the same time, microbial communities can be easy to replicate and manipulate. Their relatively fast generation times allow for experiments to take place over many generations. And for studying community assembly in particular, an underappreciated advantage of microbial communities is that the ecological and evolutionary processes shaping them often occur simultaneously [23]. With new methods for genome-resolved sequencing, it is therefore possible to track both the ecological dynamics of a diverse microbiome and, simultaneously, the evolution of many "species" within it.

Here, we review more than a decade of collaborative efforts to study microbial community assembly in the field. We first summarize some of the benefits of our model system, the leaf litter layer of surface soil in southern California ecosystems. Our field methods are easily deployable (involving nylon mesh, duct tape, hair straighteners, and coffee grinders!) and repeatable, as confirmed by trial and error over many years. Then, we present evidence for each of the four community assembly processes working alone to influence microbial composition and, sometimes, in concert (Figure 1A). Finally, we discuss the implications of these results for ecosystem functioning and suggest future directions for research. We hope that these initial results provide inspiration for possibilities in other systems with their own unique advantages, while recognizing that parallel efforts by other researchers are already ongoing. After all, we will need a variety of experimental systems where eco-evolutionary processes can be iteratively examined and manipulated to develop a general understanding of microbial community assembly and its implications in the face of global environmental change.

Leaf litter microbiome as a model for community assembly

Decomposition of leaf litter is an essential component of terrestrial carbon and nutrient cycling largely governed by microorganisms. The leaf litter layer is the collection of dead and decaying plant biomass (leaves, shoots, and woody debris) that makes up the topmost layer of soil. Leaf litter influences the bulk (mineral) soil below, altering abiotic properties such as light, moisture, and temperature. During decomposition, microorganisms both mineralize carbon compounds in leaf litter producing CO_2 and contribute to the production of stable, recalcitrant soil organic

88 matter through plant biomass processing and necromass formation [24, 25]. Microbial
89 degradation also releases nutrients into the surrounding bulk soil, altering resource availability
90 for plants and soil fauna and mediating the flow of carbon and nutrients from the surface into
91 deeper soil [26]. In temperate ecosystems where we work, the litter layer is seasonally dynamic.
92 A large pulse of litter accumulates at the end of the wet season and then slowly decays
93 throughout the rest of the year. The physical architecture of the leaf litter layer varies greatly
94 across ecosystem types. In forests and shrublands, fallen leaves constitute a large portion of the
95 leaf litter, whereas in grasslands and some croplands, standing dieback contributes a large
96 amount of litter mass.
97
98 Beyond its role in ecosystem functioning, leaf litter has several useful features that lend itself to
99 studying community assembly. First, it is naturally patchy, allowing the easy application of a
100 metacommunity framework to the system [1]. A community can be defined by a single leaf or
101 patches of leaves connected by dispersal. Second, the leaf litter layer experiences more
102 environmental variation than the bulk soil below. At our field site, and in many locations, the soil
103 surface can be quite hostile in terms of UV and moisture stress, and temperature and moisture
104 fluctuate daily and seasonally. Thus, leaf litter communities are likely more sensitive to
105 environmental change and experimental treatments than are communities deeper in the soil
106 profile [27]. Third, the leaf litter layer is readily accessible and naturally replenishes. Repeatedly
107 sampling leaf litter is less destructive than taking soil cores, allowing for longitudinal sampling
108 in the same locations without disrupting the bulk soil structure. Finally, microbial diversity in
109 leaf litter is high, but manageable. Both the bacterial and fungal communities are more diverse
110 than laboratory consortia, while less diverse (both in richness and evenness) than those in bulk

111 soil or sediments. Consequently, there is hope of attaining a detailed understanding of the
112 biology of the most abundant members of the community, while studying complex community
113 dynamics in the environment.
114
115 We have further developed and refined several methods that make leaf litter a practical field
116 system. First, it is relatively easy to measure key ecological metrics of leaf litter compared to
117 bulk soil. For instance, quantifying microbial abundance by microscopy (for fungi) or flow
118 cytometry (for bacteria) is easier than in bulk soil [28]. It is also straightforward to assess
119 decomposition by measuring mass loss from the litter bags over time [29] and to measure
120 potential extracellular enzyme activity and leaf litter chemistry [30, 31]. We have also isolated
121 many of the most abundant bacterial and fungal taxa from our local leaf litter by culturing them
122 on media made from litter leachate. Hence, we can create ecologically relevant consortia [32, 33]
123 and investigate the phenotypic diversity of these taxa [34, 35].
124
125 Most importantly for studying community assembly, however, the microbial community in leaf
126 litter can be manipulated separately from the abiotic environment and the litter substrate (which
127 may differ in carbon and nutrient resources, pH, and moisture retention). To do this, we reduce
128 the abundance of the resident community using gamma irradiation and/or autoclaving and then
129 re-inoculate the litter with a small amount (1% w/w litter) of an intact field community.
130 Although completely sterilizing the litter is unlikely (and nearly impossible to demonstrate), the
131 procedure successfully "grafts" the inoculum community onto the original litter substrate such
132 that the new community closely matches the inoculum community and not the original
133 community [36]. The community is then enclosed in a mesh litterbag that allows moisture and

nutrients to flow through and, depending on the membrane pore size, either blocks or allows dispersal of bacteria, fungi, soil fauna, and larger animals (Figure 1B,C). These microbial "cages" provide a way to replicate a homogenized inoculum community into replicate patches and to transplant them into different environments (treatments or sites) [37].

There are also caveats to using these cages for manipulating the leaf litter community. Although microbial composition within the bags is similar to the surrounding leaf litter, nylon mesh blocks sunlight and may trap moisture, altering the abiotic environment compared to the surrounding area. In addition, viruses and very small cells may still get into the "closed" cages that aim to exclude microbial dispersal. Similarly, there may be undetectable damage to the integrity of the cages in the field that allows for mixing with nearby communities. For these reasons, we always include controls, such as litterbags that are open to dispersal and/or bags inoculated with the local community, to account for these potential issues.

Evidence of the four assembly processes at work

Leveraging the methodological advantages of leaf litter, we have conducted a variety of field (and lab) experiments on their microbial communities. Below we summarize these studies and synthesize key outcomes from this system. Although the assembly processes are highly intertwined, we discuss them separately for organizational purposes.

Selection

Evolutionary biologists define selection as shifts in allele frequencies within a population due to differential fitness of individuals. However, this definition of selection can be expanded to the community level whereby shifts in the frequencies of species (or taxa or other units) reflect fitness differences among phenotypes [8]. Thus, the effect of selection by abiotic or biotic factors on the relative abundance of microbial taxa, also known as species sorting [38], can be assessed within an entire community.

In leaf litter, we have focused on how selection by environmental change may influence microbial community assembly. Our main site for studying this has been the Loma Ridge Global Change Experiment (LRGCE) in Irvine, California. This experiment was established in 2007 to simulate the increased frequency of drought and nitrogen availability in two dominant ecosystem types (a semi-arid grassland and coastal sage scrubland, CSS) in the area [39]. We have characterized the effects of the experimental treatments on the bacterial and fungal communities in leaf litter from these plots for over a decade (Figure 2A).

Simulated global changes select for distinct bacterial and fungal communities in these ecosystems, as observed previously in many other experiments [40, 41]. Drought, nitrogen addition, and their interaction alter microbial community composition in the leaf litter (Figure 2B) as well as the bulk soil [27, 42, 43], even when controlling for differences in litter substrate and its successional stage. Further, the responses of the microbial community to global change depend on the plant community [43]. Drought does not select for the same microbial taxa within the grassland as it does within the CSS, although these ecosystems are immediately adjacent to one another and experience the same climate. This interactive effect thus indicates that the

response to drought is mediated by biotic resources including, for instance, the chemical composition of the leaf litter [44, 45]. As a result, some global change responses may be difficult to transfer between ecosystems [46]. In contrast, bacterial community assembly after a wildfire at the LRGCE did not depend on the ecosystem or precipitation history [47]. Instead, wildfire selected for known, fire-loving taxa including the bacterial genus *Massilia* [48, 49].

We have further sought to understand the importance of global change relative to other factors that influence microbial community assembly. In fact, a large amount of community variation within our site can be attributed to seasonal or interannual variation (~10-39%) that is likely driven by a combination of fluctuations in temperature, moisture, UV, and the successional stage of the leaf litter (Figure 2C) [27, 42]. In comparison, simulated global changes have a more modest effect on microbial community composition in the leaf litter. For instance, across our studies, drought consistently explains about 4% of variation in bacterial composition, although the strength of this effect increases to 10% if interactive effects (i.e., drought x time and drought x ecosystem) are also considered [27, 43, 50]. Temporal variability in aquatic systems has long been recognized [51, 52], yet such high intra- and inter-annual variability in surface soils has been less studied. This bias may be partly due to the disruptive nature of taking soil cores from the same plot over time, leading soil researchers to be cautious about the number of samples they collect. Thus, another benefit of the leaf litter system is that its regenerative nature lends itself to long-term longitudinal sampling.

This large "background" of temporal variability in our system has several important implications for investigating community assembly processes. First, it means that selection by a treatment can

be missed without enough replicates and/or longitudinal sampling due to a lack of statistical power. Second, it suggests that the effect of global change factors may vary over time. For instance, the timing of a drought – whether it occurs during a wet or dry year or season – may alter its effect on community assembly. Indeed, we often detect an interactive effect between drought and sampling time on the composition of the leaf litter community [42, 43]. Third, this background variation is itself context dependent. The impact of environmental change on the soil communities at our site – whether drought, wildfire, and more generally, temporal variability – is strongest in the leaf litter layer and weakens with soil depth (Figure 2C) [27]. Together, these results highlight the context dependency of selective forces on community assembly.

Moving forward, a goal is to understand the effects of selection on microbial community composition in a more mechanistic way: can we predict the results of community assembly under different abiotic and biotic conditions? Although we typically measure composition in terms of taxa or other units of biodiversity, selection ultimately increases or decreases the abundance of a taxon because of its traits, or characteristics. Thus, one approach that may provide a more predictive understanding of community responses to particular conditions is to identify the key traits under selection [53-55]. Unfortunately, it is not a simple task to identify which traits matter under a particular selection regime [55]. Towards this end, we have focused on an abundant leaf litter bacterium, *Curtobacterium* (family Microbacteriaceae, phylum Actinomycetota), that is globally distributed [56] and easily cultured. Sequencing of our isolates revealed extensive genomic diversity that clusters into clades and subclades within those clades [57]. Yet traditional classification methods fail to capture this diversity; all *Curtobacterium* genomes would collapse into two OTUs (defined at 97% 16S rRNA gene sequence similarity) or four exact sequence

variants (ESVs) [58]. Physiological assays also revealed that clades within the genus could be distinguished by their ability to degrade carbon sources, form biofilms, and grow under different temperatures [59]. We therefore hypothesized that these traits would relate to the ability of the strains to survive and reproduce on leaf litter across a range of temperatures and moisture stress.

The combination of genomic and physiological data allowed us to designate *Curtobacterium* ecotypes [59], defined as highly similar genotypic and phenotypic strains that occupy the same ecological niche [60, 61], a concept somewhat comparable to a eukaryotic species. The ecotypes further vary in their biogeographic distribution in sites along a climate gradient (Figure 2D) [59]. After controlling for the litter substrate using our microbial cages, *Curtobacterium* traits correlate with the climate gradient, indicating that both climate conditions and litter substrate select for the composition of ecotypes present at a site [58]. Thus, even though predicting the selective effects on microbial community composition is still overwhelming, it is not inexplicable. A focus on a subset of diversity, combined with experimental manipulation, allowed us to identify traits that underlie climatic responses.

Dispersal

Compared to selection, the role of dispersal in microbial community assembly remains less clear [22, 62]. Biogeographic patterns provide indirect evidence that dispersal – defined broadly as the movement of organisms across space – might shape microbial composition [9, 63, 64], but more direct evidence is desirable. Given their ease of manipulation, microorganisms have been used extensively in lab experiments to test the influence of dispersal on community assembly,

249 microbial or otherwise. Indeed, many studies demonstrate that dispersal has the potential to be a
250 powerful force in community assembly [e.g., 65, 66]. However, the details of these experiments,
251 including the rate and composition of the individuals dispersing, are somewhat arbitrary [67].
252
253 Experiments are thus needed to assess the impact of microbial dispersal in natural systems. Leaf
254 litter is a particularly interesting system to study dispersal given the regular inputs of new
255 resources (freshly fallen plant biomass) and its location at the soil-atmosphere interface.
256 Moreover, a first step to measuring the impact of dispersal on a community is to test the effect of
257 removing it [e.g., 68, 69]. In leaf litter, we can accomplish this by altering the mesh size of the
258 litter bag to compare microbial community assembly in closed litter bags (0.22 μm pores) versus
259 open litter bags (window screen or 18 μm pores to exclude some fungi). Using this approach, we
260 find that dispersal consistently alters the richness, evenness, and composition of the leaf litter
261 microbial community (Figure 3A) [37, 70]. Further, dispersal and selection can interact to alter
262 leaf litter composition [37]. For instance, dispersal significantly contributed to the re-assembly of
263 bacterial and fungal communities after a wildfire, but this effect depended on the ecosystem [47].
264
265 Although these results provide *in situ* evidence that dispersal contributes to microbial assembly,
266 they do not consider which microbes are dispersing, from where, and how fast. Details of these
267 rates and routes are needed to develop a deeper understanding of microbial dispersal and how it
268 interacts with other assembly processes. Given that tracking the movement of individual
269 microbes in the field is impractical, we deployed sterile glass slides as microbial "traps" (Figure
270 3B). In this way, we can quantify the rate and composition of microorganisms landing on the
271 slides [67]. At the LRGCE, we observed an average of 7,900 bacterial cells/cm^2 immigrating

daily into the soil surface and found distinct communities dispersing via different routes, defined as a combination of the source community (e.g., air or soil) and the physical vector (e.g., rain or wind) [71]. Further, exposure to different dispersal routes altered the succession of the microbial community (Figure 3C) [71].

Together, this collection of experiments reveals that dispersal not only contributes to community assembly, but that – like selection – its effects are context dependent. For instance, dispersal into the soil surface from the bulk soil appears to be minimal at our site but became more important after wildfire removed the surface litter layer [47]. Moreover, the effects of dispersal on composition are highest during the early stages of litter succession such as after a wildfire or from a green to a senescing leaf.

Drift

Ecological drift is the process by which random changes in species relative abundances lead to diversity. Although thought of as its own process, drift is intricately connected to the processes of selection and dispersal. Specifically, the impact of drift on community assembly is thought to increase with weak selection pressures and low dispersal [8, 72]. Thus, disentangling the impact of drift from other assembly processes is a significant challenge in microbial communities [73].

Given these challenges, we first investigated the role of ecological drift on leaf litter communities using a theoretical model [74]. The Decomposition Model of Enzymatic Traits (DEMENT) simulates microbial communities that produce extracellular enzymes and

decompose decaying litter [75]. The impact of drift was assessed by quantifying the degree to which random differences in births, deaths, and dispersal affected the composition of simulated communities. Lower dispersal rates led to higher levels of stochasticity (i.e., higher compositional variation among modelled communities). However, drift also played a large role under high dispersal rates when selection pressure was also high. Communities on chemically complex litter substrate were more susceptible to drift because this highly selective environment reduced total microbial abundance (Figure 4A) [74].

To move from theory to the field, we next aimed to quantify the effect of ecological drift on leaf litter communities by eliminating the effect of other processes, specifically selection and dispersal. Just as evolutionary biologists measure the effect of genetic drift on populations using highly controlled laboratory experiments [76], we can use our litter bags to minimize confounding field variables to "isolate" the effects of stochastic variation – something that is challenging to do in most other systems [37]. To reduce biological heterogeneity, we inoculated a homogenized microbial community into multiple litter bags filled with irradiated leaf litter. Then, to reduce environmental heterogeneity, we deployed the litter bags within a small ($1m^2$) area at the LRGCE site. Parallel to the theoretical experiments described above, we further manipulated dispersal (open and closed litterbags) and the selective environment (added water versus ambient rainfall) to test whether drift interacts with dispersal and selection, as observed in the theoretical model [74].

Using this highly controlled field litterbag experiment, we found that stochasticity (ecological drift, potentially amplified by priority effects) influenced bacterial community assembly,

318 contributing three times more to compositional variation than dispersal [37] (Figure 4B).
319 Contrary to our model, however, stochasticity (as quantified by beta-diversity) decreased, rather
320 than increased, with reduced dispersal. Further, the effects of drift were not restricted to
321 taxonomic composition but also permeated to impact other key aspects of the community
322 including functional potential and extracellular enzyme activity. We also found that much of the
323 measured variation among replicates could be attributed to methodological factors such as
324 technical error and spatial heterogeneity within bags; this residual variation accounted for ~75%
325 of the observed variation in community composition (Figure 4B). This result highlights that the
326 effect of drift on microbial community assembly will be overestimated if these sources of
327 variability are not quantified.
328
329 *Diversification*
330
331 The fourth process of community assembly, diversification, is often mentioned, but rarely
332 investigated within the time frame of an ecological study. Even though bacteria can evolve quite
333 rapidly, entirely new bacterial "species" (as measured by the divergence of the 16S rRNA gene)
334 will not emerge for millions of years [77]. Nonetheless, evolution may be occurring within a
335 microbial community at a finer-scale genetic resolution. However, detecting these changes
336 amongst hundreds or thousands of microbial species within a microbiome is a challenge. Thus,
337 the ability for microbes to evolve, let alone to adapt, on ecological timescales remains largely
338 unexplored in natural ecosystems.
339

To investigate the potential for rapid evolution in leaf litter microbial communities, we first asked whether we could detect the emergence of *de novo* mutations. Once again, we used our microbial cages to conduct a field experiment. Mirroring laboratory evolution experiments [76, 78], we inoculated replicate litter cages with a single isogenic *Curtobacterium* strain. We then deployed the cages across an elevational gradient of temperature and precipitation. Every 6 months, we reisolated bacterial colonies from each cage and identified a variety of nonrandom, parallel single nucleotide polymorphisms (SNPs) that we confirmed with metagenomic sequencing. SNPs were found in genes related to nutrient acquisition, stress response, and exopolysaccharide production (Figure 5A) [58]. These mutations provide a new source of genetic diversity that might allow for adaptation, but further work is needed to determine if these mutations impact organismal fitness.

Evolution occurs not only through new mutations, but also through shifts in standing genetic variation within a population. Microbial species, or ecotypes, encompass standing genetic variation that often coexists within an ecosystem [79, 80]. This so-called "microdiversity" is also observed within *Curtobacterium* in leaf litter. To track this finer diversity, we developed genus-specific primers of a protein encoding gene (*groEL*). *Curtobacterium* microdiversity – here, the relative abundance of exact sequence variants of the *groEL* gene – responded to selection by drought and the litter substrate within the global change experiment (Figure 5B) [81]. Thus, responses at this fine level of genetic resolution reflect shifts in allele frequencies, a phenomenon that, among larger organisms, would be thought of as an evolutionary process [82].

362 The evolutionary process that we arguably know least about within microbial communities is
363 recombination. This is an unfortunate gap as gene flow, or the exchange of genetic variation, is
364 what delineates populations, which are often considered the fundamental unit of evolution. Using
365 a collection of *Curtobacterium* isolates from across southern California, we identified at least
366 three recombining populations of *Curtobacterium* within one subclade of an ecotype [83]. The
367 populations were delineated using gene flow discontinuities, where we quantified signals of
368 increased "recent" recombination among strains that were clustered into discrete populations
369 (Figure 5C) [84]. Strains within a population shared more flexible genes than expected by
370 chance, and recombination of population-specific genes appeared mediated by homologous
371 recombination. Bacteria can also exchange genes via horizontal gene transfer by plasmids, a
372 pattern we also observed among our *Curtobacterium* isolates. Using long-read sequencing our
373 isolates, we identified numerous plasmids that vary greatly in their size and genetic content, even
374 among very closely related isolates [85]. The plasmids encode a diversity of traits that are not a
375 random subset of chromosomal traits, ranging from genes involved in carbon and nitrogen
376 cycling to cell motility. Yet, the time-scale upon which recombination (through homologous
377 replacement of short gene segments or transfer of an entire plasmid) contributes to
378 *Curtobacterium* diversity in leaf litter remains unclear. We do not yet know how often strains are
379 exchanging genetic information – either via plasmids or recombination – in leaf litter for the
380 observed patterns to emerge.
381
382 Overall, zooming into just a single bacterial genus allowed us to highlight the potential for rapid
383 evolution to influence genetic diversity in the leaf litter microbiome. This picture is admittedly
384 still limited. We have yet to assess the time-scale of recombination, including horizontal gene

385 transfer, within soil microbial communities despite its inferred importance for microbial
386 adaptation [86]. These details are needed to provide a holistic understanding of the potential for
387 microbial communities to adapt to future environmental change. The answer, for instance, could
388 depend on the relative importance of diversity generated by rapid evolution versus that
389 contributed through dispersal [19, 77].
390
391 **Implications for community functioning**
392
393 Our work in leaf litter illuminates how a range of ecological and evolutionary processes can
394 contribute to the assembly of environmental microbiomes. These results in themselves provide
395 useful examples for the generation and maintenance of diversity within microbiomes and
396 ecological communities more generally. Yet the question remains: does community assembly
397 lead to communities that are functionally distinct?
398
399 Thus far, we have limited ourselves to discussing the results of our experiments as they pertain to
400 community assembly. However, in many of the studies, we also measured functional metrics of
401 decomposition, allowing us to also address the idea of functional redundancy. Specifically, the
402 microbial cages allow us to disentangle the influence of the abiotic environment from the initial
403 microbial community composition on functional outcomes, which we measure later in an
404 experiment [87]. For instance, we found that drought communities (leaf litter communities
405 assembled under drought conditions at the LRGCE) altered litter decomposition rates separate
406 from the abiotic effect of drought itself (Figure 6A). Indeed, the effect of community
407 composition was as large as the abiotic effect of drought (Figure 6B) [29, 88]. Similarly, leaf

litter communities assembled along a climate gradient in Southern California decomposed litter at different rates when transplanted to a common environment along the gradient [36]. Further, the resulting chemistry of the decomposed leaf litter depended on the initial microbial inoculum, revealing that different communities utilize unique sets of compounds in the litter. Both of these studies demonstrate that selection altered community assembly that, in turn, resulted in functionally divergent communities.

The processes of dispersal and drift can also influence the functioning of leaf litter communities. Communities exposed to dispersal initially decomposed leaf litter more than twice as fast as communities closed to dispersal, an effect that dampened during later stages of leaf decomposition (Figure 6C) [71]. And, as previously mentioned, ecological drift not only impacted taxonomic composition, but permeated to impact functional potential and extracellular enzyme activity (Figure 4B) [37].

We would also like to understand the particular traits in a community that are responsible for changes in overall functioning. In the lab, leaf litter bacteria vary widely in their use of simple carbon substrates and the rate at which they decompose complex leaf litter [34]. In the field, metagenomic sequencing reveals that nitrogen cycling genes and carbohydrate degradation genes vary between the global change treatments at the LRGCE [31, 89]. In one particular experiment, we examined the composition of glycoside hydrolase (GH) genes that degrade different polysaccharides in leaf litter bags transplanted into the different LRGCE treatments. Drought, but not nitrogen addition, shifted GH gene composition, suggesting a mechanistic reason for why

decomposition was more resilient to changes in nitrogen than to changes in rainfall (Figure 6C) [88].

Moving forward

By focusing on the leaf litter system, we have derived a more detailed understanding of the drivers and context-dependency of the processes driving microbial community assembly. However, despite many years of work, there are still large gaps in our knowledge about this one system. In particular, we have focused on bacteria and (some) fungi but have neglected the impact of macro- and microfauna that breakdown larger fragments of leaf litter and potentially disperse microorganisms in the field. Some of these organisms, like nematodes, also graze on microbes and thus may considerably impact the microbial community [90, 91]. For instance, shifts in microbivore composition contributed to the differential assembly of microbial communities exposed to dispersal and those that were not [92, 93]. We have also nearly completely ignored how microbe-microbe interactions, including synergistic and antagonistic interactions between bacteria and fungi, impact community assembly [94, 95]. Similarly, bacteriophages may modify bacterial communities through predator-prey interactions and are known to be abundant and dynamic in ecosystems like ours [96].

Thus far, we have primarily focused on quantifying the effects of one process at a time, they will co-occur and likely interact. In particular, although we have only begun to explore the role of contemporary evolution for community assembly in leaf litter, it is clear that ecological and evolutionary processes occur simultaneously. Selective forces such as those imposed by global

environmental changes shift allele frequencies within *Curtobacterium* species and the frequency of other, broader taxa in the community. And, just as we have observed interactions between ecological processes [37], we expect that evolutionary and ecological processes will also interact, resulting in eco-evolutionary feedbacks [97]. Microbial communities thus offer an opportunity to test the role of eco-evolutionary feedbacks in natural settings and, with further advancement of genome-resolved tools, assess their effects at different biological scales of organization within the same community. Given the central role of microorganisms to ecosystem functioning, these dynamics may indeed be important for climate feedbacks and mitigation [98, 99].

Acknowledgements

We thank the many members of the Martiny lab that have helped to design, carry out, and publish all aspects of this work, and especially Claudia Weihe, who has been the intellectual memory and lab and field coordinator of the projects. This work is part of a larger collaboration with Steve Allison, Eoin Brodie, Adam Martiny, and Kathleen Treseder. We are grateful to Steve Allison, Alex Chase, and Alejandra Rodríguez Verdugo for providing useful comments on an earlier draft. This review was supported by the National Science Foundation (DEB-2113004 to JBHM) and a U.S. National Institute of Allergy and Infectious Diseases Training Grant (Award# AI141346 to KMB).

Competing Interests

The authors declare no competing financial interests.

References

1. Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF et al. The metacommunity concept: A framework for multi-scale community ecology. *Ecol Lett* 2004.7**:**601-613.

2. Leibold MA, Chase JM, Ernest SKM. Community assembly and the functioning of ecosystems: How metacommunity processes alter ecosystems attributes. *Ecology* 2017.98**:**909-919.

3. Mori AS, Isbell F, Seidl R. Beta-diversity, community assembly, and ecosystem functioning. *Trends Ecol Evol* 2018.33**:**549-564.

4. Ackerly David D. Community assembly, niche conservatism, and adaptive evolution in changing environments. *Int J Plant Sci* 2003.164**:**S165-S184.

5. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. The application of ecological theory toward an understanding of the human microbiome. *Science* 2012.336**:**1255-1262.

6. Albright MBN, Louca S, Winkler DE, Feeser KL, Haig S-J, Whiteson KL et al. Solutions in microbiome engineering: Prioritizing barriers to organism establishment. *ISME J* 2022.16**:**331-338.

7. Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF et al. Patterns and processes of microbial community assembly. *Microbiol Mol Biol Rev* 2013.77**:**342-356.

8. Vellend M. Conceptual synthesis in community ecology. *Q Rev Biol* 2010.85**:**183-206.

9. Hanson CA, Fuhrman J, Horner-Devine MC, Martiny JBH. Beyond biogeographic patterns: Processes shaping the microbial landscape. *Nat Rev Microbiol* 2012.10**:**497-506.

10. Chase JM. Drought mediates the importance of stochastic community assembly. *Proc Natl Acad Sci USA* 2007.104**:**17430-17434.

11. Gilbert B, Levine JM. Ecological drift and the distribution of species diversity. *Proc R Soc Lond, Ser B: Biol* 2017.284**:**20170507.

12. Allbee SA, Rogers HS, Sullivan LL. The effects of dispersal, herbivory, and competition on plant community assembly. *Ecology* 2023.104**:**e3859.

13. Levine JM. Species diversity and biological invasions: Relating local process to community pattern. *Science* 2000.288**:**852-854.

14. Leibold MA, Govaert L, Loeuille N, De Meester L, Urban MC. Evolution and community assembly across spatial scales. *Annu Rev Ecol Evol Syst* 2022.53**:**299-326.

15. Urban MC, Leibold MA, Amarasekare P, De Meester L, Gomulkiewicz R, Hochberg ME et al. The evolutionary ecology of metacommunities. *Trends Ecol Evol* 2008.23**:**311-317.

16. Hendry AP (2017). Eco-evolutionary dynamics. Princeton University Press.

17. Urban MC, Strauss SY, Pelletier F, Palkovacs EP, Leibold MA, Hendry AP et al. Evolutionary origins for ecological patterns in space. *Proc Natl Acad Sci USA* 2020.117**:**17482-17490.

18. Vanoverbeke J, Urban MC, De Meester L. Community assembly is a race between immigration and adaptation: Eco-evolutionary interactions across spatial scales. *Ecography* 2016.39**:**858-870.

19. Thompson PL, Fronhofer EA. The conflict between adaptation and dispersal for maintaining biodiversity in changing environments. *Proc Natl Acad Sci USA* 2019.116**:**21061-21067.

20. Zha Y, Berga M, Comte J, Langenheder S. Effects of dispersal and initial diversity on the composition and functional performance of bacterial communities. *PLOS ONE* 2016.11**:**e0155239.

21. Zhou J, Deng Y, Zhang P, Xue K, Liang Y, Nostrand JDV et al. Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. *Proc Natl Acad Sci USA* 2014.111**:**E836-E845.

22. Langenheder S, Lindström ES. Factors influencing aquatic and terrestrial bacterial community assembly. *Environ Microbiol Rep* 2019.11**:**306-315.

23. Loreau M, Jarne P, Martiny JBH. Opportunities to advance the synthesis of ecology and evolution. *Ecol Lett* 2023.26**:**S11-S15.

24. Swift MJ, O. W. Heal, and J. M. Anderson (1979). Decomposition in terrestrial ecosystems. Blackwell Scientific Publications: Oxford, UK.

25. Whalen ED, Grandy AS, Sokol NW, Keiluweit M, Ernakovich J, Smith RG et al. Clarifying the evidence for microbial- and plant-derived soil organic matter, and the path toward a more quantitative understanding. *Global Change Biol* 2022.28**:**7167-7185.

26. Cuevas E, Lugo AE. Dynamics of organic matter and nutrient return from litterfall in stands of ten tropical tree plantation species. *For Ecol Manage* 1998.112**:**263-279.

27. Barbour KM, Weihe C, Allison SD, Martiny JBH. Bacterial community response to environmental change varies with depth in the surface soil. *Soil Biol Biochem* 2022.172**:**108761.

28. Khalili B, Weihe C, Kimball S, Schmidt KT, Martiny JBH. Optimization of a method to quantify soil bacterial abundance by flow cytometry. *mSphere* 2019.4**:**e00435-19.

29. Allison SD, Lu Y, Weihe C, Goulden ML, Martiny AC, Treseder KK et al. Microbial abundance and composition influence litter decomposition response to environmental change. *Ecology* 2013.94**:**714-725.

30. Baker NR, Khalili B, Martiny JBH, Allison SD. Microbial decomposers not constrained by climate history along a mediterranean climate gradient in southern california. *Ecology* 2018.99**:**1441-1452.

31. Nelson MB, Berlemont R, Martiny AC, Martiny JBH. Nitrogen cycling potential of a grassland litter microbial community. *Appl Environ Microbiol* 2015.81**:**7012-7022.

32. Matulich KL, Martiny JBH. Microbial composition affects a model ecosystem's functional response to environmental change. *Ecology* 2015.96**:**154-163.

33. Amend AS, Matulich KL, Martiny JBH. Nitrogen addition, not initial phylogenetic diversity, increases litter decomposition by fungal communities. *Front Microbiol* 2015.6.

34. Dolan KL, Peña J, Allison SD, Martiny JB. Phylogenetic conservation of substrate use specialization in leaf litter bacteria. *PLoS One* 2017.12**:**e0174472.

35. Scales NC, Huynh KT, Weihe C, Martiny JBH. Desiccation induces varied responses within a soil bacterial genus. *Environ Microbiol* 2023.25:3075–3086.

36. Glassman SI, Weihe C, Li J, Albright MBN, Looby CI, Martiny AC et al. Decomposition responses to climate depend on microbial community composition. *Proc Natl Acad Sci U S A* 2018.115**:**11994-11999.

37. Albright MBN, Chase AB, Martiny JBH. Experimental evidence that stochasticity contributes to bacterial composition and functioning in a decomposer community. *mBio* 2019.10**:**e00568-00519.

38. Loeuille N, Leibold M. Evolution in metacommunities: On the relative importance of species sorting and monopolization in structuring communities. *Am Nat* 2008.171**:**788-799.

39. Potts DL, Suding KN, Winston GC, Rocha AV, Goulden ML. Ecological effects of experimental drought and prescribed fire in a southern california coastal grassland. *J Arid Environ* 2012.81**:**59-66.

40. Allison SD, Martiny JBH. Resistance, resilience, and redundancy in microbial communities. *Proc Natl Acad Sci USA* 2008.105**:**11512-11519.

41. Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H et al. Fundamentals of microbial community resistance and resilience. *Front Microbiol* 2012.3**:**417.

42. Matulich KL, Weihe C, Allison SD, Amend AS, Berlemont R, Goulden ML et al. Temporal variation overshadows the response of leaf litter microbial communities to simulated global change. *ISME J* 2015.9**:**2477-2489.

43. Finks SS, Weihe C, Kimball S, Allison SD, Martiny AC, Treseder KK et al. Microbial community response to a decade of simulated global changes depends on the plant community. *Elementa-Sci Anthrop* 2021.9**:**00124.

44. Malik AA, Swenson T, Weihe C, Morrison EW, Martiny JBH, Brodie EL et al. Drought and plant litter chemistry alter microbial gene expression and metabolite production. *ISME J* 2020.14**:**2236-2247.

45. Malik AA, Martiny JBH, Brodie EL, Martiny AC, Treseder KK, Allison SD. Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *ISME J* 2020.14**:**1-9.

46. Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JAM, Cregger MA et al. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere* 2015.6**:**art130.

47. Barbour KM, Weihe C, Walters KE, Martiny JBH. Testing the contribution of dispersal to microbial succession following a wildfire. *mSystems* 2023.8**:**e00579-00523.

48. Whitman T, Whitman E, Woolet J, Flannigan MD, Thompson DK, Parisien M-A. Soil bacterial and fungal response to wildfires in the canadian boreal forest across a burn severity gradient. *Soil Biol Biochem* 2019.138**:**107571.

49. Pulido-Chavez MF, Randolph JWJ, Zalman C, Larios L, Homyak PM, Glassman SI. Rapid bacterial and fungal successional dynamics in first year after chaparral wildfire. *Mol Ecol* 2023.32**:**1685-1707.

50. Matulich K, Weihe C, Allison SD, Amend A, Berlemont R, Goulden ML et al. Temporal variation overshadows the response of leaf litter microbial communities to simulated global change. *ISME J* 2015.2477–2489.

51. Gilbert JA, Steele JA, Caporaso JG, Steinbruck L, Reeder J, Temperton B et al. Defining seasonal marine microbial community dynamics. *ISME J* 2012.6**:**298-308.

52. Fuhrman JA, Hewson I, Schwalbach MS, Steele JA, Brown MV, Naeem S. Annually reoccurring bacterial communities are predictable from ocean conditions. *Proc Natl Acad Sci U S A* 2006.103**:**13104-13109.

53. Martiny JBH, Jones SE, Lennon JT, Martiny AC. Microbiomes in light of traits: A phylogenetic perspective. *Science* 2015.350**:**aac9323.

54. Green JL, Bohannan BJM, Whitaker RJ. Microbial biogeography: From taxonomy to traits. *Science* 2008.320**:**1039-1043.

55. Guittar J, Shade A, Litchman E. Trait-based community assembly and succession of the infant gut microbiome. *Nat Comm* 2019.10**:**512.

56. Chase AB, Arevalo P, Polz MF, Berlemont R, Martiny JBH. Evidence for ecological flexibility in the cosmopolitan genus *Curtobacterium*. *Front Microbiol* 2016.7**:**1874.

57. Chase AB, Karaoz U, Brodie EL, Gomez-Lunar Z, Martiny AC, Martiny JBH. Microdiversity of an abundant terrestrial bacterium encompasses extensive variation in ecologically relevant traits. *mBio* 2017.8**:**e01809-01817.

58. Chase AB, Weihe C, Martiny JBH. Adaptive differentiation and rapid evolution of a soil bacterium along a climate gradient. *Proc Natl Acad Sci USA* 2021.118**:**e2101254118.

59. Chase AB, Gomez-Lunar Z, Lopez AE, Li J, Allison SD, Martiny AC et al. Emergence of soil bacterial ecotypes along a climate gradient. *Environ Microbiol* 2018.20**:**4112-4126.

60. Rocap G, Distel DL, Waterbury JB, Chisholm SW. Resolution of *Prochlorococcus* and *synechococcus* ecotypes by using 16s-23s ribosomal DNA internal transcribed spacer sequences. *Appl Environ Microbiol* 2002.68**:**1180-1191.

61. Johnson ZI, Zinser ER, Coe A, McNulty NP, Woodward EMS, Chisholm SW. Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science* 2006.311**:**1737-1740.

62. Custer GF, Bresciani L, Dini-Andreote F. Ecological and evolutionary implications of microbial dispersal. *Front Microbiol* 2022.13**:**855859.

63. Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL et al. Microbial biogeography: Putting microorganisms on the map. *Nat Rev Microbiol* 2006.4**:**102-112.

64. Stegen JC, Lin X, Fredrickson JK, Chen X, Kennedy DW, Murray CJ et al. Quantifying community assembly processes and identifying features that impose them. *ISME J* 2013.7**:**2069-2079.

65. Jones ML, Ramoneda J, Rivett DW, Bell T. Biotic resistance shapes the influence of propagule pressure on invasion success in bacterial communities. *Ecology* 2017.98**:**1743-1749.

66. Lindstrom ES, Ostman O. The importance of dispersal for bacterial community composition and functioning. *Plos One* 2011.6**:** e25883.

67. Barbour KM, Barrón-Sandoval A, Walters KE, Martiny JBH. Towards quantifying microbial dispersal in the environment. *Environ Microbiol* 2023.25**:**137-142.

68. Kaneko R, Kaneko S. The effect of bagging branches on levels of endophytic fungal infection in japanese beech leaves. *For Pathol* 2004.34**:**65-78.

69. Vannette RL, Fukami T. Dispersal enhances beta diversity in nectar microbes. *Ecol Lett* 2017.20**:**901-910.

70. Albright MBN, Martiny JBH. Dispersal alters bacterial diversity and composition in a natural community. *ISME J* 2018.12**:**296-299.

71. Walters KE, Capocchi JK, Albright MBN, Hao Z, Brodie EL, Martiny JBH. Routes and rates of bacterial dispersal impact surface soil microbiome composition and functioning. *ISME J* 2022.16**:**2295-2304.

72. Hubbell SP (2001). The unified neutral theory of biodiversity and biogeography. Princeton University Press: Princeton.

73. Zhou J, Ning D. Stochastic community assembly: Does it matter in microbial ecology? *Microbiol Mol Biol Rev* 2017.81**:**10.1128/mmbr.00002-17.

74. Evans S, Martiny JB, Allison SD. Effects of dispersal and selection on stochastic assembly in microbial communities. *ISME J* 2017.11**:**176-185.

75. Allison SD. A trait-based approach for modelling microbial litter decomposition. *Ecol Lett* 2012.15**:**1058-1070.

76. Travisano M, Mongold JA, Bennett AF, Lenski RE. Experimental tests of the roles of adaptation, chance, and history in evolution. *Science* 1995.267**:**87-90.

77. Martiny JBH, Martiny AC, Brodie E, Chase AB, Rodríguez-Verdugo A, Treseder KK et al. Investigating the eco-evolutionary response of microbiomes to environmental change. *Ecol Lett* 2023.26(Suppl. 1)**:**S81–S90.

78. Marston MF, Pierciey FJ, Shepard A, Gearin G, Qi J, Yandava C et al. Rapid diversification of coevolving marine synechococcus and a virus. *Proc Natl Acad Sci USA* 2012.109**:**4544-4549.

79. Acinas SG, Klepac-Ceraj V, Hunt DE, Pharino C, Ceraj I, Distel DL et al. Fine-scale phylogenetic architecture of a complex bacterial community. *Nature* 2004.430**:**551-554.

80. Larkin AA, Martiny AC. Microdiversity shapes the traits, niche space, and biogeography of microbial taxa. *Environ Microbiol Rep* 2017.9**:**55-70.

81. Scales NC, Chase AB, Finks SS, Malik AA, Weihe C, Allison SD et al. Differential response of bacterial microdiversity to simulated global change. *Appl Environ Microbiol* 2022.88**:**e02429-02421.

82. Barrett RDH, Schluter D. Adaptation from standing genetic variation. *Trends Ecol Evol* 2008.23**:**38-44.

83. Chase AB, Arevalo P, Brodie EL, Polz MF, Karaoz U, Martiny JBH. Maintenance of sympatric and allopatric populations in free-living terrestrial bacteria. *mBio* 2019.10**:**e02361-02319.

84. Arevalo P, VanInsberghe D, Elsherbini J, Gore J, Polz MF. A reverse ecology approach based on a biological definition of microbial populations. *Cell* 2019.178**:**820-834.e814.

85. Finks SS, Moudgalya P, Weihe C, Martiny JBH. The contribution of plasmids to trait diversity in a soil bacterium. *ISME Communications* 2024.ycae025.

86. Arnold BJ, Huang IT, Hanage WP. Horizontal gene transfer and adaptive evolution in bacteria. *Nat Rev Microbiol* 2022.20**:**206-218.

87. Reed HE, Martiny JBH. Testing the functional significance of microbial composition in natural communities. *FEMS Microbiol Ecol* 2007.62**:**161-170.

88. Martiny JBH, Martiny AC, Weihe C, Lu Y, Berlemont R, Brodie EL et al. Microbial legacies alter decomposition in response to simulated global change. *ISME J* 2017.11**:**490-499.

89. Berlemont R, Allison SD, Weihe C, Lu Y, Brodie EL, Martiny JBH et al. Cellulolytic potential under environmental changes in microbial communities from grassland litter. *Front Microbiol* 2014.5**:**639.

90. Bardgett RD, Cook R, Yeates GW, Denton CS. The influence of nematodes on below-ground processes in grassland ecosystems. *Plant Soil* 1999.212**:**23-33.

91. Thakur MP, Geisen S. Trophic regulations of the soil microbiome. *Trends Microbiol* 2019.27**:**771-780.

92. Ingham RE, Trofymow JA, Ingham ER, Coleman DC. Interactions of bacteria, fungi, and their nematode grazers: Effects on nutrient cycling and plant growth. *Ecol Monogr* 1985.55**:**119-140.

93. Crowther TW, Boddy L, Jones TH. Species-specific effects of soil fauna on fungal foraging and decomposition. *Oecologia* 2011.167**:**535-545.

94. Romdhane S, Spor A, Aubert J, Bru D, Breuil M-C, Hallin S et al. Unraveling negative biotic interactions determining soil microbial community assembly and functioning. *ISME J* 2022.16**:**296-306.

95. de Boer W. Upscaling of fungal–bacterial interactions: From the lab to the field. *Curr Opin Microbiol* 2017.37**:**35-41.

96. Santos-Medellín C, Estera-Molina K, Yuan M, Pett-Ridge J, Firestone MK, Emerson JB. Spatial turnover of soil viral populations and genotypes overlain by cohesive responses to moisture in grasslands. *Proc Natl Acad Sci USA* 2022.119**:**e2209132119.

97. Govaert L, Altermatt F, De Meester L, Leibold MA, McPeek MA, Pantel JH et al. Integrating fundamental processes to understand eco-evolutionary community dynamics and patterns. *Funct Ecol* 2021.35**:**2138-2155.

98. Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken LR, Baylis M et al. Scientists' warning to humanity: Microorganisms and climate change. *Nat Rev Microbiol* 2019.17**:**569-586.

99. ASM (2023). Microbes in models: Integrating microbes into Earth system models for understanding climate change: Report on an American Academy of Microbiology virtual colloquium held on Dec. 6 and 8, 2022. American Society of Microbiology: Washington, DC.

814 **Figure legends**

815

816 **Figure 1. Leaf litter as an experimental system in the field.** (A) An overview of the four
817 processes, their links to microbial community assembly and functioning, and the types of
818 experiments and measurements that we have used to investigate each link. (B) Leaf litter-
819 containing bags, or microbial "cages," on the soil surface of a pine-oak and (C) grassland
820 ecosystem. The cages are made from nylon mesh that prevents microbial dispersal and placed in
821 larger metal screening to prevent animal disturbance.

822

823 **Figure 2. Selection by the abiotic and biotic environment affects leaf litter community**
824 **assembly.** (A) A microbial cage experiment in the Loma Ridge Global Change Experiment
825 (LRGCE) simulated drought plots following a wildfire. Polyethylene sheets are pulled over the
826 plots during half of the annual rainfall events at the site to exclude ~50% of the annual
827 precipitation. (B) Non-metric multidimensional scaling ordination (NMDS) depicting how
828 bacterial community composition on grassland leaf litter varies across the four LRGCE treatment
829 combinations. Redrawn from elsewhere [42]. (C) Estimated percent variation explained by
830 factors significantly impacting bacterial community composition in the leaf litter layer, top 2 cm
831 of bulk soil, and top 10 cm of bulk soil at the LRGCE. Reprinted with permission from [27]. (D)
832 Absolute abundances (by cell count) of the most abundant *Curtobacterium* ecotypes (+/- 1 SD)
833 in the leaf litter layer at five sites across a climate gradient. Redrawn from elsewhere [59].

834

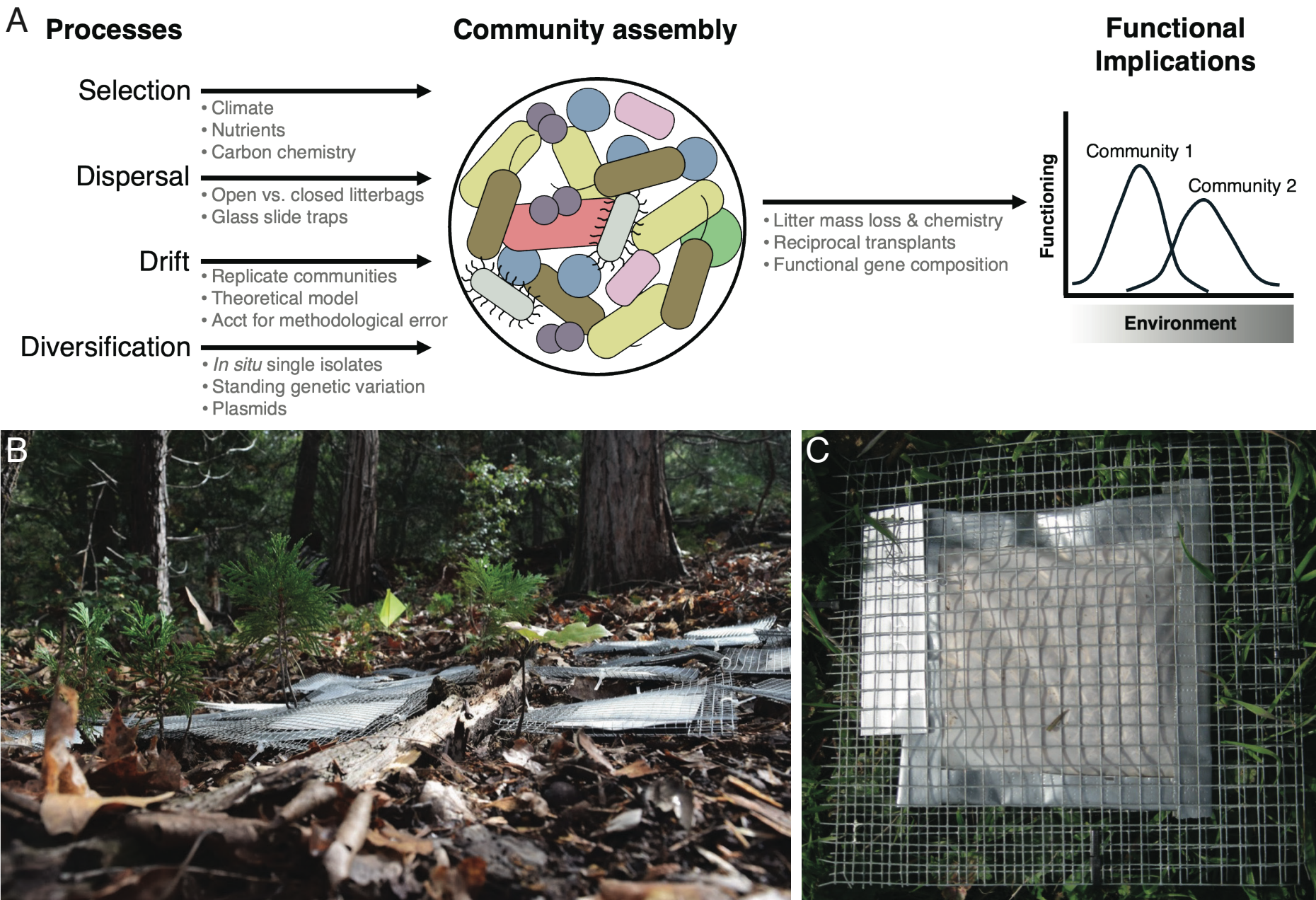
835 **Figure 3. Dispersal from different routes alters microbial communities in the field.** (A)
836 Effects of dispersal limitation on bacterial evenness from a field experiment. Line color

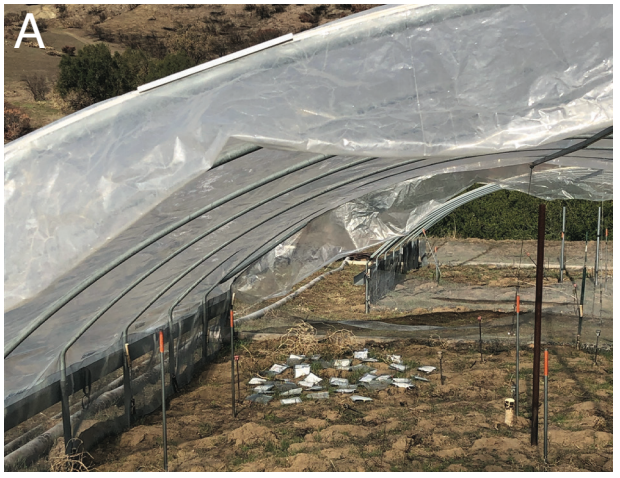
837 represents the treatment type: litterbags closed to dispersal (orange), litterbags open to dispersal
838 (purple), nylon-containing bags open to dispersal (light blue), and leaf litter collected from the
839 surrounding environment (green). Reprinted from elsewhere [70]. (B) Glass slide "trap" used to
840 capture microorganisms dispersing into the soil surface. (C) NMDS of bacterial composition in
841 litterbags exposed to different dispersal routes in a grassland (closed = no dispersal; elevated =
842 dispersal from air; overhead = dispersal from air and surrounding environmental litter; open =
843 dispersal from air, environmental litter, and bulk soil). Reprinted from elsewhere [71].
844
845 **Figure 4. *In silico* and experimental evidence that drift contributes to microbial community**
846 **assembly.** (A) Dissimilarity (within-group distance) of replicate communities within lignin/N
847 treatments that were exposed to different dispersal rates for 6 years simulated by the
848 Decomposition Model of Enzymatic Traits (DEMENT). The bottom right of each panel shows P-
849 values for the null hypothesis that within-group distances across lignin/N treatments are equal in
850 a single dispersal level. Stochasticity increases (higher within-group distance) with low dispersal
851 rates and stronger selection (higher lignin/N values). Adapted from elsewhere [74]. (B)
852 Estimated percent variation of bacterial community composition (assayed by 16S rRNA gene
853 amplicon and metagenomic sequencing) from litterbags in the field explained by a precipitation
854 treatment, dispersal, and their interaction. Within-bag variation (stochasticity) and unexplained
855 (residual) variation was also estimated for ecosystem functioning metrics including extracellular
856 enzyme assays (EEA), litter chemistry, and litter mass loss. Reprinted from elsewhere [37].
857
858 **Figure 5. Rapid evolution of a leaf litter bacterium in the field.** (A) Mutations identified in a
859 *Curtobacterium* strain that was transplanted in litterbags across a climate gradient (red = Desert,

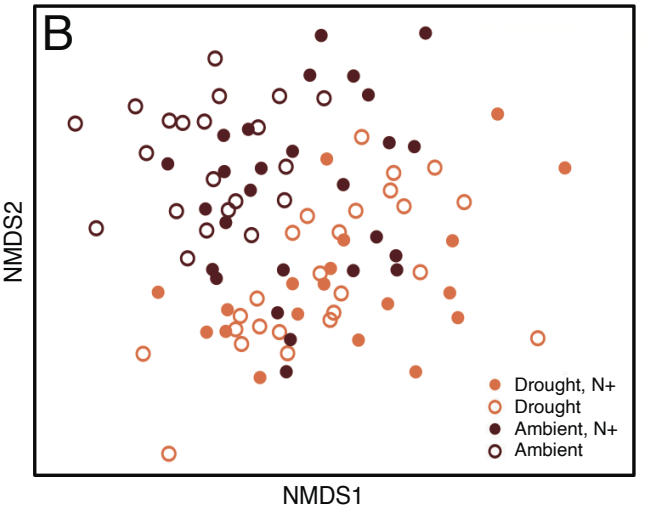
orange = Scrubland, green = Grassland, blue = Pine-Oak, and purple = Subalpine). Mutations in 112 evolved strains isolated from five sites along the climate gradient at 6 (Time point 1), 12 (T2), and 18-month (T3) intervals. Nonrandom mutations also observed in the population (metagenomic) data are denoted for synonymous (syn), nonsynonymous (nonsyn), and nonsense mutations. Reprinted from elsewhere [58]. (B) NMDS of *Curtobacterium* microdiversity (ESVs of the *groEL* gene) from grass litter collected from ambient and reduced precipitation plots in the grassland and coastal sage scrubland (CSS) at the LRGCE. Centroids of each ecosystem x precipitation treatment combination are marked by black circles, and the centroids of all samples from each ecosystem are marked by a black X. Inset indicates the direction and strength of correlation with *Curtobacterium* subclades. Reprinted from elsewhere [81]. (C) Recombination network across all pairwise combinations of 26 *Curtobacterium* strains. Thicker edges represent increased recombination between strains. Nodes are colored by population designation where populations are defined as groups with the potential to exchange genetic material. Node size indicates the number of clonal clusters (strains too closely related to differentiate recombination). Isolation sources: D, desert; Sc, scrubland; G/MMLR, grassland; SS, Salton Sea; MCBA, Boston, MA. Reprinted from elsewhere [83].

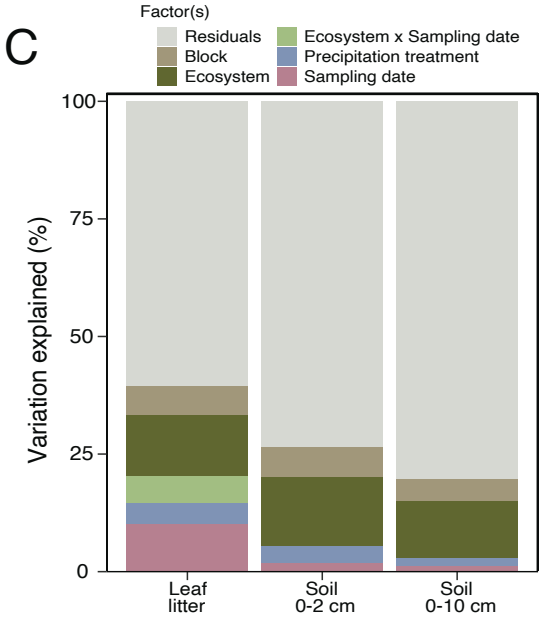
Figure 6. Differential assembly of leaf litter microbial communities impacts decomposition. Effect of (A) microbial origin and (B) contemporary plot environment on percentage mass loss in litterbags during the first year of a reciprocal transplant experiment. Microbial origin refers to leaf litter community inoculum that was exposed to either ambient (control) or reduced (drought) precipitation at the LRGCE. Adapted from elsewhere [29]. (C) Mass loss of leaf litter closed to dispersal in the field compared to litter exposed to all dispersal (Open) and litter exposed to

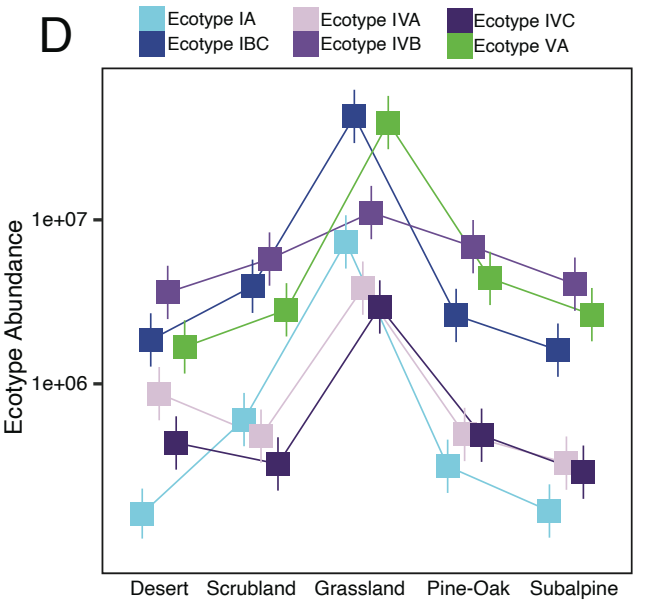
883 dispersal from above the soil surface (Overhead). Exposure to dispersal accelerated leaf litter
884 decomposition in the first month of the experiment. Reprinted from elsewhere [71]. (D) NMDS
885 depicting that the drought, but not added nitrogen, treatment altered glycoside hydrolase
886 composition of the bacterial communities on leaf litter. Redrawn from elsewhere [88].
887


A
Processes
Community assembly
Functional Implications
Selection
• Climate
• Nutrients
• Carbon chemistry
Dispersal
• Open vs. closed litterbags
• Glass slide traps
Drift
• Replicate communities
• Theoretical model
• Acct for methodological error
Diversification
• In situ single isolates
• Standing genetic variation
• Plasmids
• Litter mass loss & chemistry
• Reciprocal transplants
• Functional gene composition
Community 1
Community 2
Functioning
Environment
B
C

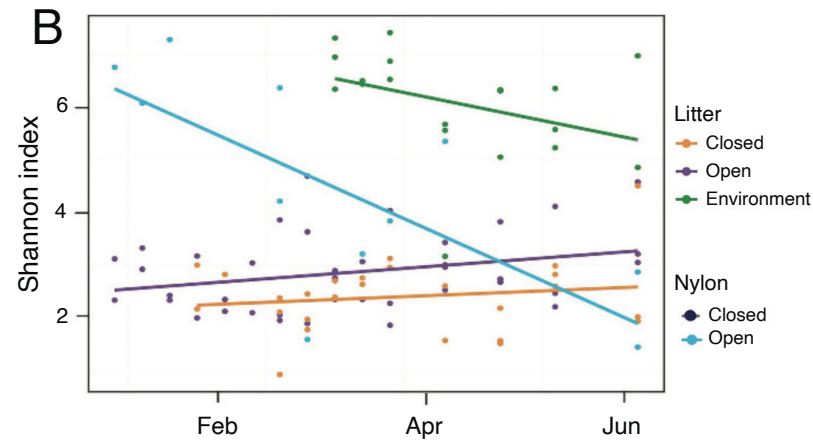

A

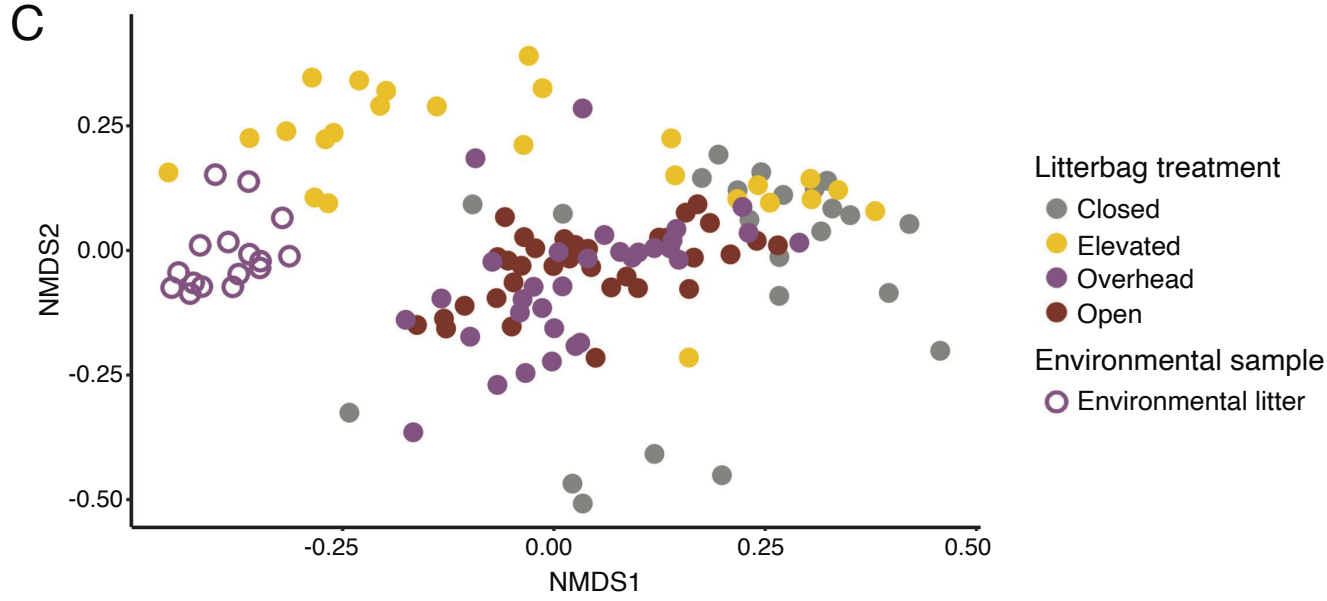

B
NMDS2
NMDS1
Drought, N+
Drought
Ambient, N+
Ambient

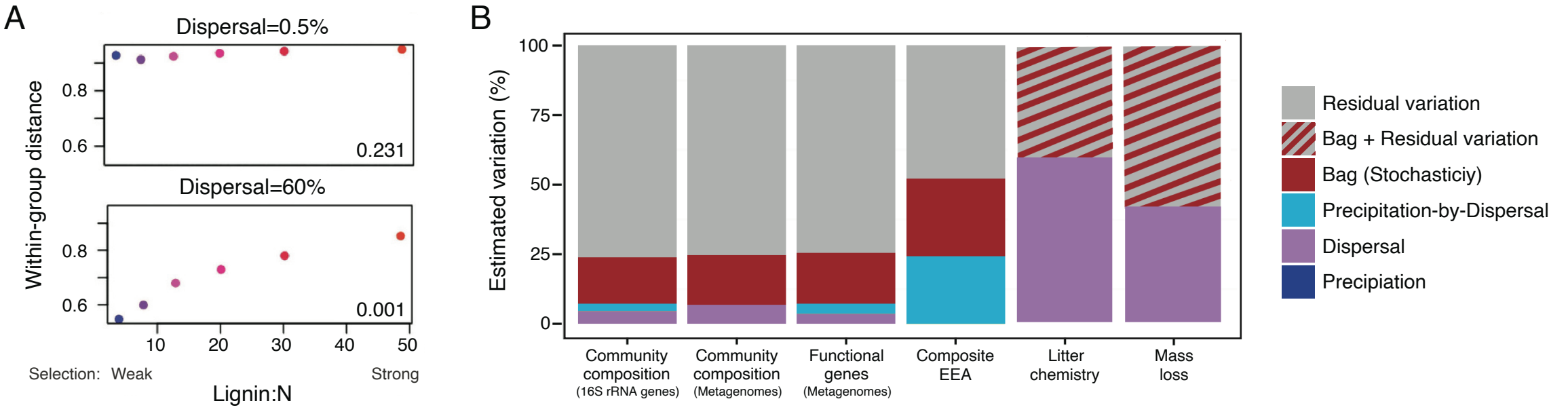

C
Factor(s)
Residuals
Block
Ecosystem
Ecosystem x Sampling date
Precipitation treatment
Sampling date
Variation explained (%)
100
75
50
25
0
Leaf litter
Soil 0-2 cm
Soil 0-10 cm

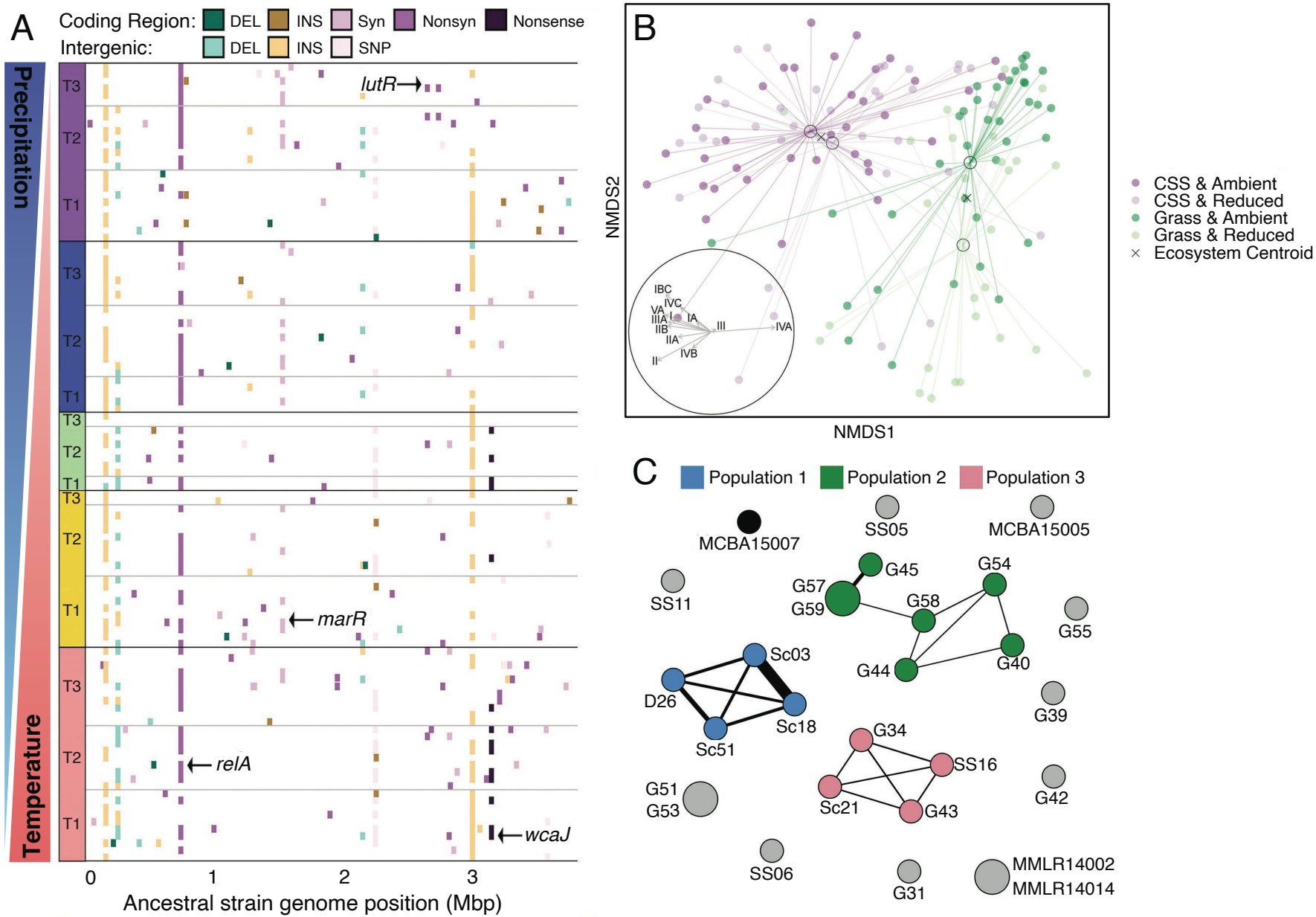

D
Ecotype IA
Ecotype IBC
Ecotype IVA
Ecotype IVB
Ecotype IVC
Ecotype VA
Ecotype Abundance
1e+07
1e+06
Desert
Scrubland
Grassland
Pine-Oak
Subalpine

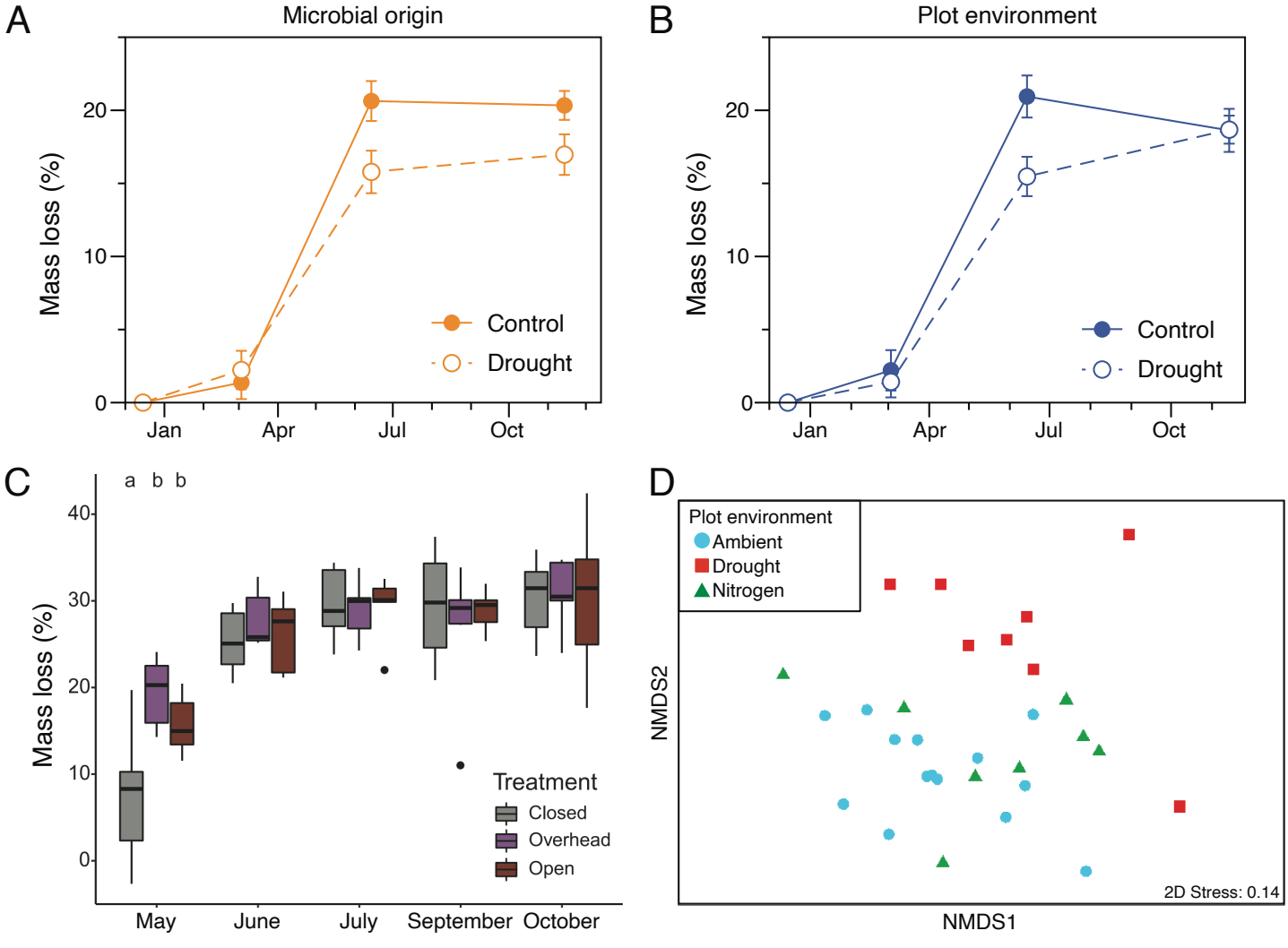

A


B
Shannon index
6
4
2
Feb
Apr
Jun
Litter
Closed
Open
Environment
Nylon
Closed
Open


C
NMDS2
0.25
0.00
-0.25
-0.50
-0.25
0.00
0.25
0.50
NMDS1
Litterbag treatment
Closed
Elevated
Overhead
Open
Environmental sample
Environmental litter


A
Dispersal=0.5%
0.231
Dispersal=60%
0.001
Within-group distance
0.8
0.6
0.8
0.6
10
20
30
40
50
Selection: Weak
Strong
Lignin:N
B
Estimated variation (%)
100
75
50
25
0
Community composition (16S rRNA genes)
Community composition (Metagenomes)
Functional genes (Metagenomes)
Composite EEA
Litter chemistry
Mass loss
Residual variation
Bag + Residual variation
Bag (Stochasticiy)
Precipitation-by-Dispersal
Dispersal
Precipiation


A
Coding Region: DEL INS Syn Nonsyn Nonsense
Intergenic: DEL INS SNP
Precipitation
Temperature
T3
T2
T1
lutR
marR
relA
wcaJ
0
1
2
3
Ancestral strain genome position (Mbp)
B
NMDS2
NMDS1
IBC
IVC
VA
IIIA
I
IA
III
IVA
IIB
IIA
IVB
II
CSS & Ambient
CSS & Reduced
Grass & Ambient
Grass & Reduced
Ecosystem Centroid
C
Population 1
Population 2
Population 3
MCBA15007
SS05
MCBA15005
SS11
G45
G54
G57
G59
G58
G55
G44
G40
Sc03
D26
Sc18
Sc51
G39
G34
SS16
G51
G53
Sc21
G43
G42
SS06
G31
MMLR14002
MMLR14014


A
Microbial origin
Mass loss (%)
20
10
0
Jan
Apr
Jul
Oct
Control
Drought
B
Plot environment
Mass loss (%)
20
10
0
Jan
Apr
Jul
Oct
Control
Drought
C
a b b
Mass loss (%)
40
30
20
10
0
May
June
July
September
October
Treatment
Closed
Overhead
Open
D
Plot environment
Ambient
Drought
Nitrogen
NMDS2
NMDS1
2D Stress: 0.14