environmental microbiology

Environmental Microbiology (2019) **00**(00), 00–00 doi:10.1111/1462-2920.14707

Dispersal alters soil microbial community response to drought

S. E. Evans,[1*] **L. P. Bell-Dereske**,[2] **K. M. Dougherty**[2] **and H. A. Kittredge**[1]

[1]*Kellogg Biological Station; Ecology, Evolutionary Biology, and Behavior Program, Department of Integrative Biology, 3700 E. Gull Lake Dr. Hickory Corners, MI, 49060, USA.*

[2]*Kellogg Biological Station; Ecology, Evolutionary Biology, and Behavior Program, 3700 E. Gull Lake Dr. Hickory Corners, MI, 49060, USA.*

Summary

Microbial communities will experience novel climates in the future. Dispersal is now recognized as a driver of microbial diversity and function, but our understanding of how dispersal influences responses to novel climates is limited. We experimentally tested how the exclusion of aerially dispersed fungi and bacteria altered the compositional and functional response of soil microbial communities to drought. We manipulated dispersal and drought by collecting aerially deposited microbes after precipitation events and subjecting soil mesocosms to either filter-sterilized rain (no dispersal) or unfiltered rain (dispersal) and to either drought (25% ambient) or ambient rainfall for 6 months. We characterized community composition by sequencing 16S and ITS rRNA regions and function using community-level physiological profiles. Treatments without dispersal had lower soil microbial biomass and metabolic diversity but higher bacterial and fungal species richness. Dispersal also altered soil community response to drought; drought had a stronger effect on bacterial (but not fungal) community composition, and induced greater functional loss, when dispersal was present. Surprisingly, neither immigrants nor drought-tolerant taxa had higher abundance in dispersal treatments. We show experimentally that natural aerial dispersal rate alters soil microbial responses to disturbance. Changes in dispersal rates should be considered when predicting microbial responses to climate change.

Received 13 July, 2018; revised 4 June, 2019; accepted 5 June, 2019. *For correspondence. E-mail evanssa6@msu.edu; Tel. (+00) 256 337 2482; Fax (269) 671-2351.

Introduction

Microbial communities will be subject to novel disturbances in the future due to climate change. Understanding how and when these disturbances influence microbial composition and microbial processes is critical; microbes drive biogeochemical cycles and impact plant and animal hosts. Dispersal is now well acknowledged as an important mechanism in microbial community assembly (Martiny *et al*., 2006; Bell, 2010; Lindström and Östman, 2011; Peay *et al*., 2012; Albright and Martiny, 2018), influencing microbial diversity, composition and function. Dispersal rate can also play a large role in a community's response to disturbance (Leibold *et al*., 2004; Hufbauer *et al*., 2015), but most of our knowledge of these dynamics comes from studies of macro-organisms.

Dispersal alters community assembly directly and through interactions with drift and selection (Vellend, 2010), or species sorting. The outcomes of these processes will influence a microbial community's response to disturbance. Most directly, dispersal can introduce (or re-introduce) taxa, increasing the abundance of those taxa, independent of their competitive ability. This could buffer the effect of disturbance or intensify it. For instance, Lawrence *et al*. (2016) suggested that microbial dispersal buffered the community's loss of function under warming stress through direct re-seeding of populations; communities that received immigrants better maintained function under warming, regardless of whether the immigrants were pre-exposed to warming. But the introduction of maladapted immigrants could also negatively affect a community's response to disturbance. Graham and Stegen (2017) found that microbial dispersal reduced biogeochemical function in a theoretical model through the introduction of maladapted immigrants.

In addition to directly re-introducing populations, dispersal can also alter disturbance response by influencing compositional shifts. Dispersal can introduce stress-tolerant taxa that provide greater opportunities for species sorting (Eggers *et al*., 2012) and sorting toward stress-tolerant taxa overcomes stochastic community shifts due to drift. For instance, Compte *et al*. (2017) found that when immigrants included marine (salt tolerant) taxa, aquatic microbial community

function was buffered against salinity stress. Other studies suggest that dispersal can facilitate such shifts, as evidenced by greater change in community composition under dispersal (compared to without dispersal) and enhanced function (Eggers *et al*., 2012; Comte *et al*., 2017; Székely and Langenheder, 2017). Together, this small body of experimental work suggests that dispersal can alter a community's response to disturbance, but the outcome can depend on immigrant traits and specific dispersal rate. Environmental conditions (e.g. terrestrial or aquatic) are also likely to affect the outcome, but we still lack studies that examine microbial dispersal in natural communities, particularly in terrestrial systems.

Soils in terrestrial systems host biogeochemical transformations of important greenhouse gases, and modulate primary production. Soils will be drier in the coming decades as warming increases evaporative water loss, and rainfall becomes more variable (Dai, 2013), posing threats to agricultural production. Aerial dispersal is a major pathway of microbial immigration into and across soils and may change in the future with shifts in land use (Bell and Tylianakis, 2016). Dispersal to soils primarily occurs passively through wind and rain (Nemergut *et al*., 2013). Although these communities are orders of magnitude smaller than soil communities, changes in rates of aerial dispersal can still shape soil microbial diversity and influence local functions, like plant growth (Peay *et al*., 2010; Peay, 2018). Aerially dispersed microbial communities also have a greater proportion of desiccation and UV-tolerant species (Kellogg and Griffin, 2006). Thus, aerial dispersal could alter microbial community response to drought by introduction and selection of drought-tolerant taxa (De Vries *et al*., 2012; Evans and Wallenstein, 2012). However, the importance of aerial dispersal to soil community disturbance response has never been tested experimentally.

We experimentally reduced aerial dispersal in soil mesocosms to test three hypotheses about the role of dispersal in microbial disturbance response. First, based on previous empirical and theoretical studies (Mouquet and Loreau, 2003; Cadotte, 2006a), we hypothesized that reduced dispersal would decrease species richness via inherent reductions in aerially dispersed colonizers. Second, we hypothesized that dispersal would increase the available species pool, increasing potential for species sorting. Thus, we expected dispersal to cause larger shifts in community composition in response to drought. Third, we hypothesized that dispersal would enhance community functioning under drought through increased biomass and increases in drought-tolerant taxa. In this case, soils with dispersal would have greater biomass, host more immigrant taxa, and show smaller changes in functional profiles under drought, compared to soils without dispersal.

Results

Microbial biomass, richness and metabolic diversity

Across drought treatments, dispersal more than doubled microbial biomass carbon compared to no dispersal treatments. Microbial biomass generally increased over the course of the experiment, relative to time 0, in all treatments except No Dispersal-Drought (Fig. 1A). Across dispersal treatments, biomass in Ambient treatments was 1.6 times higher than that of Drought (p = 0.028). Drought caused a greater reduction in biomass when dispersal was absent (difference of 34.4 μg C/g soil compared to 26.4 μg C/g soil with dispersal), but there was not a significant Dispersal × Drought, interaction (p = 0.41; Table 1).

Metabolic diversity (Shannon diversity index, based on utilization of 32 substrates) was higher in Dispersal treatments (Fig. 1B) than that of No Dispersal treatments, and the effect of drought on metabolic diversity was greater under dispersal (Dispersal × Drought interaction, p = 0.002; Table 1). We cannot rule out that this significant interaction emerged because Dispersal-Drought treatments had inherently lower dispersal than Dispersal-Ambient treatments (due to lower water volume applied), a general limitation in our experimental design. Species richness decreased throughout the experiment in all treatments, but at the end of the experiment, bacterial and fungal species richness was higher in No Dispersal treatments (Chao1 $F_{1,28}$ = 15.67; p < 0.001; Pielou $F_{1,28}$ = 35.15; p < 0.001, Table 1, Fig. 1C,D) compared to Dispersal treatments. In bacteria, the greater richness was accompanied by higher evenness (Table 1, Fig. 1E, p < 0.1).

Species composition

We characterized species composition of both aerial (immigrant) communities throughout the experiment, and soil communities after they were subject to treatments. Across all sampling dates, aerially dispersed communities were dominated by fungal Ascomycota and bacterial Proteobacteria (Fig. 2). Aerial fungal and bacterial communities were highly varied and both weakly correlated with date permutational analysis of variance (PerMANOVA, Month p < 0.01). Removing immigrant taxa that occurred in sterile water blanks did not affect our findings. Rain samples (which included cells deposited by wind prior to the rain event) contained an average of 2.7×10^4 cells/mL (cell counts on random subset of rain events, N = 7), which is within the range of previous estimates of bioaerosol abundance (Bauer *et al*., 2002; Amato *et al*., 2005). This means that on average, over 6 months, soils in Dispersal-Ambient treatments received around 1.9×10^7 immigrant cells. In contrast, cell density in soil was several orders of magnitude higher, with an average of 3.30×10^{10} cells/cm^3 soil (N = 12 soils).

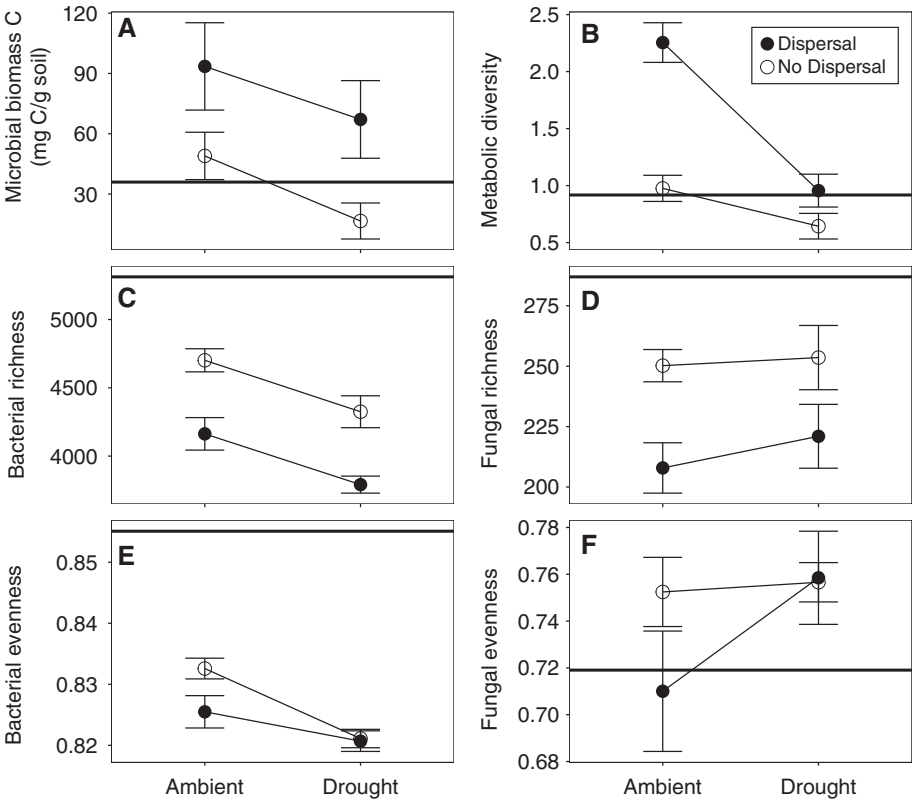

Fig. 1. Community properties after exposure to Dispersal and Drought treatments (N = 8/treatment) for 6 months. Microbial biomass carbon (A), metabolic diversity [B, (Shannon diversity index based on substrate utilization rates of 32 substrates)], bacterial (C) and fungal (D) richness (Chao1), bacterial (E) and fungal (F) evenness (Pielou's). See Table 1 for corresponding ANOVA results. Solid line represents mean values of time 0 soil cores (N = 3).

Table 1. Summary of ANOVA p-values for microbial properties exposed to 6-months of Dispersal and No Dispersal and Drought and Ambient conditions.

Factor	*p* value
Microbial biomass	
Dispersal	**0.002**
Drought	**0.028**
Dispersal × Drought	0.410
Metabolic diversity	
Dispersal	**<0.001**
Drought	**<0.001**
Dispersal × Drought	**0.002**
Community-level physiological profiles (CLPP)[a]	
Dispersal	0.653
Drought	**0.020**
Dispersal × Drought	**0.015**

	Bacteria	Fungi
Composition[a]		
Dispersal	**<0.001**	0.084
Drought	**<0.001**	**0.009**
Dispersal × Drought	**0.002**	0.807
Richness (Chao1)		
Dispersal	**<0.001**	**0.002**
Drought	**<0.001**	0.470
Dispersal × Drought	0.981	0.667
Evenness (Pielou's)		
Dispersal	0.063	0.315
Drought	**<0.001**	0.167
Dispersal × Drought	0.097	0.233

a. PerMANOVA test for beta-diversity.
Bold value indicates $p < 0.05$.

Soil communities were dominated by the bacterial phylum Proteobacteria and the fungal phylum Ascomycota (Fig. 2) and varied less across treatments than aerial communities did across sampling date (Fig. 3A,B). In soils, dispersal and drought both affected bacterial community composition (Table 1, Fig. 3C), although the proportion of variation explained by dispersal was overall low, and drought explained more variability (r^2_{Drought} = 0.36, $r^2_{\text{Dispersal}}$ = 0.13). Drought and dispersal also interacted such that drought had a stronger effect on bacterial community composition when dispersal was present, as indicated by a larger mean distance between treatments (Fig. 3C, Table 2). Fungi were generally less responsive to all experimental treatments than bacteria (Fig. 3D, Table 1) and showed a marginal change in composition with drought and no response to changes in dispersal.

Composition of bacteria in all four experimental treatments at the end of the experiment were distinct from time 0 soil communities but those receiving ambient water levels and dispersal (i.e. closest to natural conditions) changed the least (Fig. 3; Supporting Information Table S1). Bacterial groups that respond to pulses of DOC (Acidobacteria, Firmicutes, Gammaproteobacteria, Nitrospira, Cleveland *et al*., 2007) did not change with dispersal (Supporting Information Table S2), while groups previously identified as drought tolerant [Actinobacteria and Planctomycetes, (Evans and Wallenstein, 2014; Evans *et al*., 2014; Bouskill

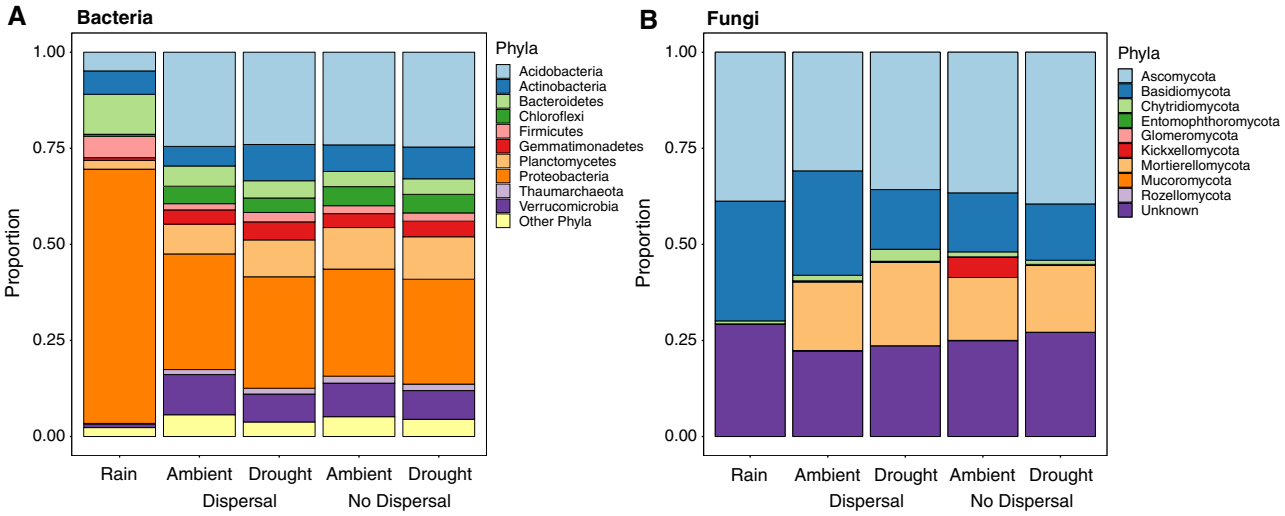

Fig. 2. Relative abundance of bacterial (A) and fungal (B) Phyla in aerial immigrant samples (summed over 37 precipitation collections) and soil treatments (N = 8 per treatment, measured at the end of the 6-month experiment). See Supporting Information Fig. S1 for changes in Actinobacteria and Planctomycetes (drought-tolerant bacterial groups) across treatments.

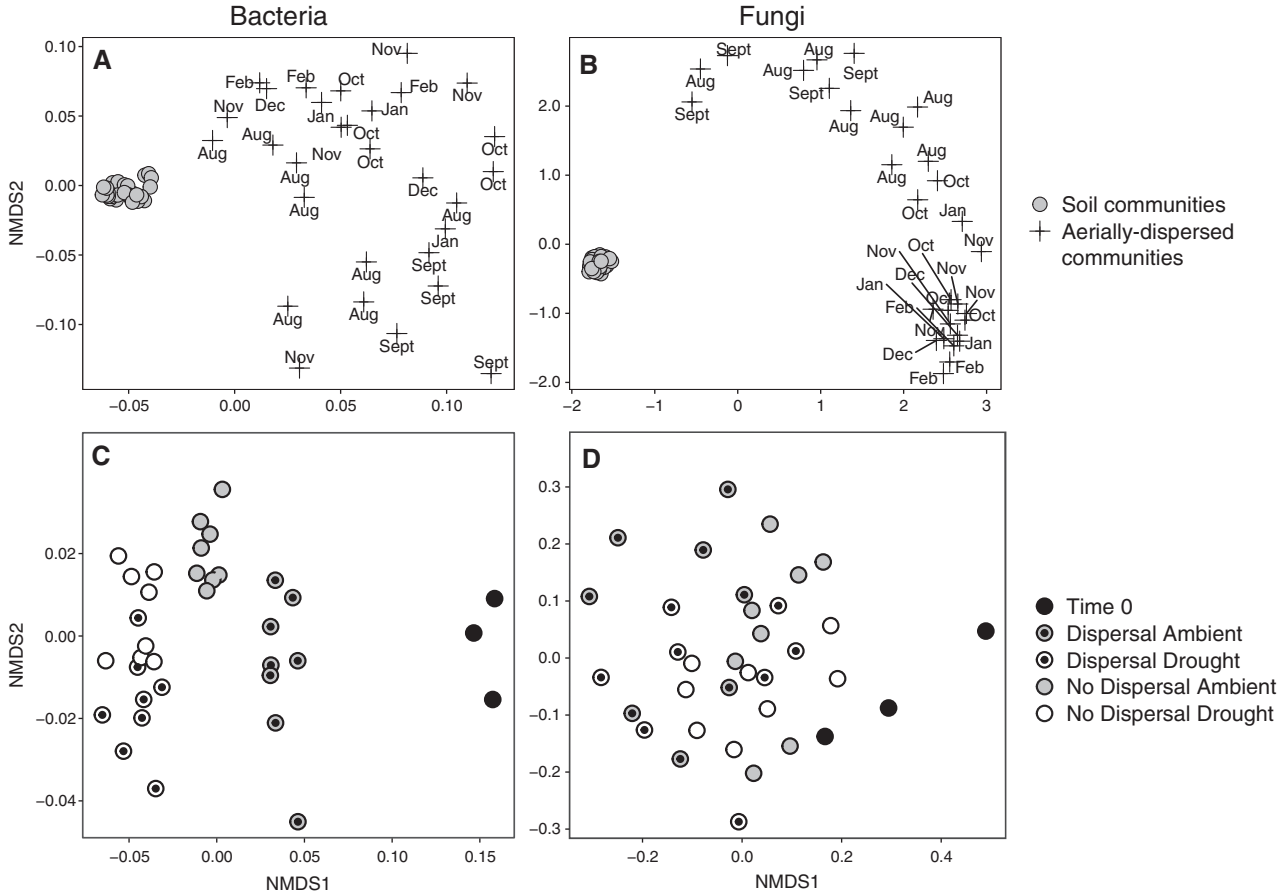

Fig. 3. Non-metric multidimensional scaling plots of bacterial (A,C) and fungal (B,D) communities. Plots A and B show communities in rain samples (+, includes air deposition) collected over the 6-month experiment, and C, D show soil communities. Soil mesocosms were experimentally altered by removing immigrants (Dispersal and No Dispersal), and/or reducing the water input (Ambient and Drought). Time 0 represents soils before treatments occurred. PerMANOVA statistics are shown in Table 1.

Table 2. Mean distance (based on Weighted Unifrac for bacteria and Bray–Curtis for fungi and commnity level physiological profiles, CLPP) between soil treatment groups (see Figs. 3c, d, and 4 in main text for ordination visual).

	Factor	Mean distance drought-ambient	SE	*p*-value[a]
Bacteria	No Dispersal	0.086	0.0023	**<0.001**
	Dispersal	0.118	0.0021	
Fungi	No Dispersal	0.584	0.0103	0.056
	Dispersal	0.630	0.0193	
CLPP[a]	No Dispersal	0.537	0.0129	**<0.001**
	Dispersal	0.671	0.0121	

a. Tests the null hypothesis that pairwise comparison within factor is equal. CLPP, commnity level physiological profiles.
b. Bold indicates $p < 0.05$.

et al., 2016; Ochoa-Hueso *et al*., 2018)] showed greater increases under drought when dispersal was present (Supporting Information Fig. S1).

To shed light on the drivers of soil community shifts when dispersal was altered, we also examined soil abundance of 'rain operational taxonomic units (OTUs)', defined as those OTUs that occurred in any rain collection over the course of the experiment. We found that on average, 35% of soil bacterial taxa (71% of reads) and 40% of soil fungi (75% of reads) were found in rain (Supporting Information Figs. S2 and S3 and Table S3). In fungi, the no dispersal treatments had fewer 'rain OTUs', while in bacteria, 'rain OTUs' did not change with dispersal treatment (Supporting Information Fig. S2).

Community-level physiological profiles

Dispersal and drought also affected community-level physiological profiles (CLPPs) of microbial communities (Fig. 4). Physiological profiles changed more over the course of the experiment in communities subject to dispersal compared to no dispersal, especially under drought (Fig. 4; Supporting Information Table S1), and dispersal treatments were more functionally different after drought than those not subject to dispersal (Table 2). The change in community physiology was most strongly driven by a reduction in the ability of Dispersal-Drought communities to metabolize the amino acid arginine ($R^2 = 0.36$, $p = 0.003$) and the sugar alcohol mannitol ($R^2 = 0.60$, $p = 0.001$) (all substrates shown in the Supporting Information Table S4).

Discussion

We quantified the effect of aerial dispersal on soil microbial communities response to drought by manipulating dispersal and drought in a 6-month soil mesocosm experiment. We first hypothesized that removing dispersal would decrease species richness. Previous studies suggest that dispersal relates positively to richness (Mouquet and Loreau 2003, Cadotte 2006a), including in modelled microbial communities (Evans *et al*. 2017). In contrast, we found that No Dispersal treatments had *higher* fungal and bacterial richness at the end of the experiment than treatments receiving dispersal. Homogenizing processes can lead to lower richness at high dispersal rates (Leibold *et al*., 2004; Graham and Stegen, 2017), but our data do not suggest ambient dispersal

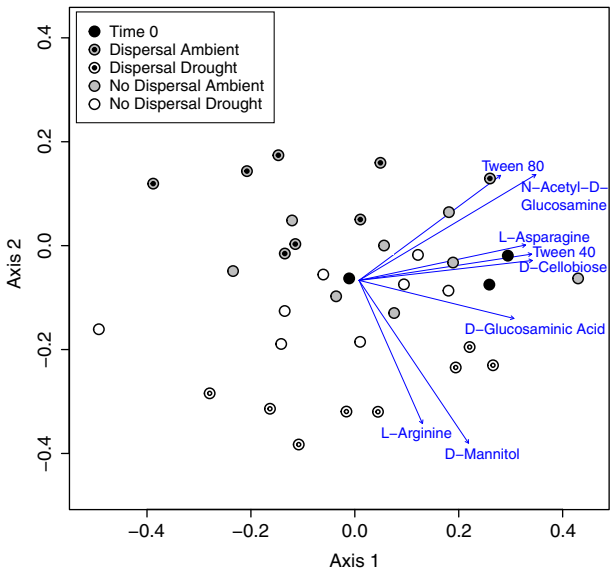

Fig. 4. Principal coordinate analysis of CLPPs (based on BIOLOG Eco plates) of microbial communities in soils at the beginning of the experiment (time 0, $N = 3$), and after dispersal and drought treatments ($N = 8$). The strongest drivers of differences among profiles are shown by vectors (top 8 shown, all $p < 0.05$, $R^2 > 0.3$, all substrates shown in the Supporting Information Table S4), the length of which correspond to R^2.

homogenized communities because communities receiving dispersal were not more similar to rain communities (or to each other compared to no dispersal treatments). Other studies have shown that biotic interactions like predator/prey dynamics can alter (Kneitel and Chase, 2004) or even reverse (Cadotte, 2006b) the positive effect of dispersal on diversity. In our study, removal of immigrants could have reduced predation (France and Duffy, 2006) or competition (Lloyd-Smith, 2013), allowing more resident taxa to grow.

Species richness changed over the course of the experiment in all treatments, as did several other microbial properties (Fig. 1). Changes over the course of the experiment (from time 0), even in communities receiving ambient rainfall and dispersal, may represent a biologically relevant process like succession or ecological drift, or an experimental artefact (e.g. transfer of soils into the greenhouse). We could not separate these in our study. Future studies should take additional measurements to help separate these possibilities, such as paired experimental samples (as an indicator of drift) or field samples (to quantify natural changes over a similar time period). Thus, we note cases where changes from time 0 may indicate biological processes but interpret it with caution, focusing on the comparison among the end treatments. In the case of species richness, we observed reductions in all treatments relative to time 0 soils, perhaps due to the removal of plants. This reduction in richness over time may have been buffered when dispersal was excluded because resident microbes (non-immigrants) grew in response to warm and humid mesocosm conditions that were not optimal for immigrants.

Our second hypothesis was that dispersal increases compositional changes in response to drought. Fungal communities shifted under drought but showed little response to dispersal. Fungi may not have had time to respond to treatments due to slower turnover times (Rousk *et al*., 2010). Fungi may also be more strongly structured by other processes, like plant dynamics (Sun *et al*., 2017) or belowground dispersal (Middleton *et al*., 2015). Fungi did shift slightly over the course of the experiment, probably as a result of changes in mesocosm conditions or a delayed response to coring. In contrast to fungi, changes in bacterial communities shifted with dispersal, and shifts under drought depended on dispersal (Figs. 3 and 4), in line with our predictions. Specifically, drought caused bigger differences in communities in treatments subject to dispersal (higher dissimilarity, Table 2), suggesting dispersal facilitated novel community responses to drought.

We hypothesized that dispersal would increase drought tolerance because aerial dispersers are desiccation tolerant (Kellogg and Griffin, 2006). Our data generally support this (although we encourage future studies to measure this directly); immigrant taxa made up a larger portion of soil community abundance under drought, regardless of dispersal (Supporting Information Fig. S3). But surprisingly, dispersal did not result in greater abundances of immigrant taxa (Supporting Information Figs. S2 and S3). Admittedly, our method for 'tracking' immigrants is imperfect – taxa we define as 'rain taxa' are common in (and many originate from) soil. However, this does highlight that the influence of dispersal on community assembly is more complex than simply increased opportunities for selection of immigrant taxa; in this case, dynamics of the 'resident' (non-immigrant) community also changed when dispersal was altered.

We cannot rule out that shifts in bacterial composition are mediated by biogeochemical or methodological artefacts, rather than community dynamics. Community composition changes with resource pulses (Cleveland *et al*., 2007), which we could have created by adding dead (or soon to be dead) immigrant cells. However, our cell counts show that the potential resource pulse would be much smaller than provided in any resource-addition studies. Also, none of the microbial taxa previously found to increase with dissolved organic carbon additions (Cleveland *et al*., 2007) were higher in dispersal treatments (Supporting Information Table S2). In addition, No Dispersal treatments could have received some immigrants that passed through filtration (e.g. viruses) or that were dispersed aerially in the greenhouse.

In our third hypothesis, we predicted that drought would have less effect on microbial function (biomass and metabolic capabilities) when dispersal was present due to greater representation of drought-tolerant taxa. We do find that dispersal buffers some aspects of microbial function but do not find evidence that it is through greater abundance of drought-tolerant taxa. Dispersal resulted in higher microbial biomass in both drought and ambient conditions, but dispersal and drought did not *interact* to affect microbial biomass, a relationship we might expect if buffering occurred through increased relative abundance of drought tolerant species. Dispersal also did not explain a large proportion of shifts in community composition, which we might expect if it were introducing drought-tolerant taxa. Although drought did seem to increase the abundance of some drought-tolerant taxa (as identified by the literature, Supporting Information Fig. S1), it may not have been to the extent that the community as a whole could better survive drought.

Dispersal enhanced microbial metabolic diversity, particularly in ambient conditions. Thus, dispersal may buffer functional loss (degradation of certain substrates) under disturbance, but in our experiment, much of the metabolic diversity gained under ambient conditions was lost under drought. Dispersal altered the physiological profiles that emerged under drought, but drought had a larger, not smaller, impact on functional profiles when dispersal was present. Drought-dispersal communities were more different from time 0 and ambient communities, compared with no dispersal treatments (Fig. 4 and Supporting Information Fig. S1). Dispersal also seemed to reduce, rather than

enhance function. In addition, certain functions, such as the ability to degrade L-arginine and D-Mannitol, were *lost* under drought in dispersal communities but not in those without dispersal (Fig. 4, Supporting Information Fig. S4). Overall, positive effects of dispersal on function may be more prevalent under ambient conditions.

Taken together, our findings first reinforce other studies (e.g. Albright and Martiny, 2018) that highlight the need to understand how changes in dispersal will affect terrestrial microbial diversity and function. Remarkably, we found that dispersal can have as much effect on microbial community biomass, richness and metabolic diversity as drought, but drought receives more attention. As the dispersal level we induced was extreme, future studies could examine dispersal rates intermediate to and beyond those we examined, using ever-growing characterizations of aerial communities (e.g. Barberán *et al*., 2015; Evans *et al*., 2019) to select gradients and pose more targeted questions.

Microbial communities will be increasingly subject to novel disturbances in the future. We mimicked an open soil system using soil mesocosms and found that aerial dispersal alters the compositional and functional response of communities to drought. Dispersal buffered reductions in biomass under drought but also facilitated greater loss of some metabolic functions, and reduced species richness. Drought will continue to grow as a major threat to food production, and microbial dispersal rates can change with land use fragmentation (Bell and Tylianakis, 2016) but are rarely measured (but see Bowers *et al*., 2011; Docherty *et al*., 2018). Aerial dispersal could alter the response of soil communities to global change, but predicting the direction of this response will require a greater understanding of the mechanisms at play during changes in dispersal.

Experimental procedures

Soil mesocosms

We tested how two factors, drought and dispersal, interacted to influence soil microbial communities in intact soil mesocosms (Fig. 5). We collected 32 cores (5 cm diameter by 10 cm depth) in soil core sleeves in August 2015 from a conventional agricultural plot (corn-soy-wheat rotation, corn in 2015) at the Kellogg Biological Station (KBS) Long-Term Ecological Research Main Cropping System Experiment. We kept soils in intact cores to mimic realistic moisture (and drought) conditions. We subject cores to four treatments (Drought × Dispersal factorial, Fig. 5, N = 8/treatment). We also collected a time zero control (denoted time 0, N = 3), each of which consisted of three composite cores collected from the same area.

We manipulated dispersal and watering level of the intact soil mesocosms (Fig. 5) from 10 August 2015 to

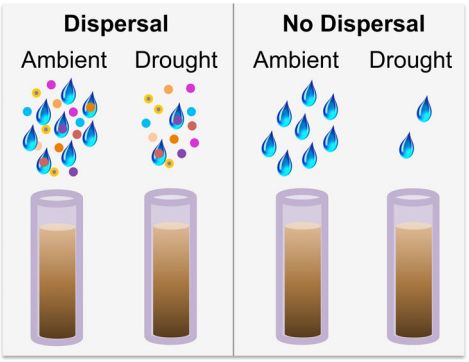

Fig. 5. Experimental design. Coloured circles represent aerial immigrant communities of bacteria and fungi. Rain was collected in a sterile container away from agricultural plots at and re-added to soil mesocosms (intact cores) either as collected (Dispersal treatment) or after filter-sterilization (No Dispersal) and at either Ambient (100%) and Drought (25%) levels.

19 February 2016. We collected naturally aerially dispersed microbial communities using a sterile autoclave bin that was attached to a funnel and sterile bottle. We set out this collector from just after a rain event until the next significant precipitation event, thus collecting microbes that were dispersed through both wind and rain during that period. We collect deposition after rain events to prevent growth of the wind- and rain-dispersed cells, and because we could manipulate it (through filtering and reduction). At each collection (37 events >1 mm; 34 rain, one fog and two snow), we filter-sterilized half of the water, and added either sterile (no dispersal) or nonsterile (dispersal) water to the intact cores, manipulating dispersal rates of aerial dispersers through the simulation of precipitation.

To induce drought, half the cores received the volume of water collected in the event (ambient treatment), while for the other half received ~25% of ambient volume (drought). This reduction level was based on the approximate severity of the 2012 drought at KBS (Robertson *et al*., 2014), which was accompanied by a significant (50%) reduction in crop yield in conventional plots. Because the surface area of each core is ~20 (19.625) cm^2, we added 20 and 5 ml (5 cm^3) of rain to the cores for every centimetre of precipitation (i.e. 10 mm and 2.5 mm event for the ambient and reduced volume cores respectively). Over the course of the ~6-month experiment, ambient rainfall cores received an equivalent of ~36 cm of rain, and reduced volume cores received ~9 cm of rain.

To exclude dispersal, we removed viable cells from collected water with a bottle-top vacuum filter system (0.22 μm Sigma) eliminating immigrants deposited by air and rain without otherwise altering water chemistry or volume. Filters containing rain microbes (used to sterilize rain for no-dispersal treatments) from 34 events were stored at −80°C for later DNA extraction. We observed minimal colony forming units on plated filtrate water and

found no difference in inorganic nitrogen content between rain and filtrate.

We performed cell counts on a subset of samples to obtain a coarse estimate of the number of immigrant cells being introduced to soils, compared to the cell density of soil communities. We did not assess differences in cell density among experimental treatments. To perform cell counts, within 24 h of collection, we fixed samples by adding PBS buffered formaldehyde to the final concentration of 2% formaldehyde and stored them at 4°C for 3 h. We stained each sample for at least 10 min at a final concentration of 5 μg/ml DAPI (4′,6-diamidin-2-phenylindol Biotium). Rain samples were filtered onto a 0.2 μm black polycarbonate membrane (Nuclepore Whatman). Membranes were mounted on a microscope slide and cells were counted by epifluorescence microscopy at 400× magnification. At least 10 different visual fields per sample were captured and saved using AxioVision. We used Image J to count the number of cells per image.

At the end of the experiment, we subcored the 5-cm-diameter core mesocosms with a 2.5 cm core to exclude core edges. We also removed the top 5 mm crust of each core with a sterilized scoopula because of visual signs of algal growth. We homogenized each subsample using a 4 mm sieve and stored at 4°C until biogeochemical assays were performed (within a week). We also stored ~5 g soil at −80°C for DNA extraction and CLPP assays, as we could not immediately process and ultrafreezing has been shown to retain enzyme activity and community structure (Wallenius *et al*., 2010).

Microbial biomass and CLPPs

We measured microbial biomass carbon (C) and nitrogen (N) in each soil sample using chloroform fumigation–extraction (Vance *et al*., 1987). Briefly, we extracted 5 g of soil with 25 ml of 0.5 M K_2SO_4 in an orbital shaker for 1 h (see Tiemann and Billings, 2012). For the fumigated extractions, we added 2 ml of chloroform to the soil in a tightly capped 50 ml centrifuge tube and fumigated the tube for 24 h. Following fumigation, we vented off chloroform prior to K_2SO_4 extraction and quantified concentrations using a Shimadzu TOC analyser.

We analysed CLPPs using Biolog-ECO plates (BIOLOG, Hayward, USA). We inoculated each well of the 96-well plates with 100 μl of a 1:100 dilution of soil suspended in PBS (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4). Briefly, we suspended 0.5 g soil in 5 ml of PBS with four 5 mm glass beads in a 15 ml centrifuge tube, vortexed each tube on high for 2 min, and further diluted soil suspension 10-fold (1 ml 1:10 dilution plus 9 ml PBS) following centrifugation at 750×*g* for 6 min to pellet soil particles. We stored the plates in the dark at room temperature for 1 week (or 10 days) and measured absorbance at both 590 and 750 nm every 24 h using a Biotek Synergy HTX (Winooski, VT) plate reader and followed calculations in the study by Rutgers *et al*. (2006) for determining metabolic profiles. Note that profiles preferentially assess bacterial communities compared to fungi due to the 31 substrates included in the Biolog-ECO plates, and the inability of some fungi to reduce the tetrazolium dye (Dobranic and Zak, 1999; Classen *et al*., 2003).

Amplicon sequencing

We used a high-throughput barcoded sequencing approach to characterize the microbial communities in both soil and rain samples using the Illumina MiSeq platform (Illumina, CA, USA) [similar to the study by Caporaso *et al*. (2012)]. For rain samples, following filtration, we aseptically removed the 0.22 μm polyethersulfone membrane from the filtration unit, suspended it in 10 ml sterile TE (10 mM Tris–HCl, 1 mM disodium EDTA, pH 8.0) and stored the filtrate at −80°C. To characterize the microbial community within most (34) rain events, we purified total genomic DNA from 1.5 ml of the rain filtrate using a modified cetyltrimethylammonium bromide (CTAB) method (see Wilson, 2001). We also included three sterile water controls in the processing of rain samples to account for contaminants often confounding results in low-biomass samples (Salter *et al*., 2014). For each soil sample (32 mesocosm samples and 3 composite samples from time 0), we purified total genomic DNA using the MoBio PowerSoil DNA extraction kit following the manufacturer's instructions with one modification. Briefly, we incubated each bead tube cell lysate for 10 min at 65°C. To ensure complete lysis of resistant cells (e.g. spores), both DNA purification methods included elevated temperature incubations (i.e. 10 min at 65°C).

We submitted DNA to the Michigan State University Core Genomics Facility for Illumina sequence library construction using the Illumina TruSeq Nano DNA Library Preparation Kit and sequencing. Following their standard protocols, bacterial 16S V4 (515f/806r) Illumina compatible libraries were prepared using primers containing both the target sequences and the dual indexed Illumina compatible adapters (see Kozich *et al*., 2013). However, Illumina compatible amplicons of the fungal ITS1 region were generated using ITS1-F/ITS2 primer sequences (see McGuire *et al*., 2013) in an initial PCR and then dual indexed Illumina library adapters were added in a subsequent PCR. Then, completed libraries were normalized using Invitrogen SequalPrep DNA Normalization plates, pooled and cleaned up using AmpureXP magnetic beads. The 16S and ITS1 amplicon pools were sequenced independently in a 2 × 250 bp paired end format using independent v2 500 cycle MiSeq reagent cartridges.

Bioinformatics

Bacterial and fungal bioinformatics pipelines are fully described in the Supporting Information Appendix S1. Briefly, reads were quality filtered and merged using the USEARCH pipeline (http://drive5.com/usearch/). Primers and adapter bases were removed using cutadapt (Martin, 2011). Bacterial reads were filtered and truncated to 250 bp, clustered into OTUs at 97% identity level then classified against SILVAv123 rRNA database (Yilmaz *et al*., 2014). Sequences were aligned and phylogenetic tree was built using PASTA (Mirarab *et al*., 2015). OTUs classified to chloroplast, mitochondria, or with less than two reads across all samples were filtered out to avoid over splitting (Thiéry *et al*., 2012) and sequencing errors (Dickie, 2010) resulting in soil samples having 12 863 OTUs and 2 575 563 reads and rain samples having 4954 OTUs and 1 205 396 reads. We identified 110 taxa in sterile water samples. As many were common laboratory contaminants [e.g. *Acinetobacter*, (Salter *et al*., 2014)], we checked to see whether removing these samples affected our conclusions, and it did not.

Fungal sequences with <225 bp were filtered but not truncated because the high variability in the length of the ITS1 (Nilsson *et al*., 2008). The reads were then clustered into OTUs at 97% identity level and classified against the UNITE 7.2 reference database (UNITE Community, 2017). All non-fungal OTUs and those with less than two reads were filtered from the community matrix resulting in soil samples having 1374 OTUs and 1 084 332 reads and rain samples having 2480 OTUs and 752 459 reads. Finally, as above, we performed the same analyses with OTUs found in the Power Water kit blanks (29 fungal taxa) were filtered from the rain samples, but found again that it did not alter our conclusions.

Statistical analysis

We analysed univariate data (e.g. microbial biomass) using two-factor ANOVA (Drought, Dispersal, and their interaction) with Type 3 sum of square [package car, (Fox and Weisberg, 2011)] and least squared means test for multiple comparisons, with a Tukey's adjustment [package lsmeans (Lenth, 2016)]. We assessed community structure in the same statistical model using PerMANOVA on the weighted Unifrac and Bray–Curtis distance matrices for bacterial and fungal communities, respectively, using phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen *et al*., 2017) in R (R Core Team, 2017). We also used Bray–Curtis for CLPP analysis. A two-factor ANOVA (with treatments as categorical variables) was necessary to examine the interaction between Dispersal and Drought, but it is an imperfect test for our data set. Dispersal under drought conditions is likely lower than dispersal under ambient, creating more than two dispersal levels. To address this, we identify patterns that may have been confounded by this uneven design and present as a caveat when needed.

We visualized community composition using Non-metric multidimensional scaling (NMDS) using phyloseq and ggplot2 (Wickham, 2009). We visualized CLPP data using principal component analysis (as these data have fewer zeroes and do not violate the assumptions of this test). We correlated principal component analysis scores with relative optimal density readings of physiological assays to explore which metabolic functions drove differences in physiological profiles among groups (R vegan). Using the distance matrices described above, we also calculated mean distance (i) among treatment groups and (ii) between each treatment group and the initial community. Using pairwise mean distances to test differences resulted in the same conclusions as testing these differences using distance between centroids, despite $N = 3$ for initial and $N = 8$ for treatment communities, so we present only the former. Community diversity was measured using Chao1 richness (Chao, 1984) and Pielou's Evenness index (Pielou, 1975), and metabolic diversity was determined using Shannon-Wiener index (Pielou, 1975). Differences in both were assessed in the ANOVA model as described previously. Code is available at https://github.com/EvansLabMSU/PAPER_Evans_et_al_RainX.

We also examined several aspects of changes in community composition to shed light on the mechanism through which the community changed under dispersal and drought. First, to explore whether treatments were directly affecting the community composition through rain as passive dispersal mechanism, we split the soil communities into either communities containing taxa shared with those in rain or communities that did not contain taxa that occurred in rain. We then examined how treatments differed in the number of OTUs (microbial taxa, see Bioinformatics) identified as 'rain-OTUs' in soil and 'non-rain OTUs' in soil and the cumulative abundance (summed reads) of these OTUs in each treatment. We repeated analyses that included rain-OTUs using the non-filtered rain taxa to test whether filtering out taxa found in negative controls had an impact on our results. Second, we used results from previous drought studies (Evans and Wallenstein, 2014; Evans *et al*., 2014; Bouskill *et al*., 2016; Ochoa-Hueso *et al*., 2018) and identified Actinobacteria and Planctomycetes as groups that consistently have higher abundance under drought. We tested whether these groups showed greater increases under drought when dispersal was present. Third, we also examined whether compositional changes we saw might be driven by a response to DOC (necromass), so we tested whether bacterial groups that increased in response to pulses of DOC (Cleveland *et al*., 2007) also increased under dispersal.

Acknowledgements

We thank members of the Evans Lab especially Steve Gougherty, Robert Logan, Grace Keil, Tayler Chicoine and Will West for assistance with laboratory assays and analysis, and Gary Mittelbach and Nick Haddad for comments on earlier versions of this manuscript. We also thank three anonymous reviewers for thorough and constructive feedback. Funding was provided by Michigan State University and the NSF Long-term Ecological Research Program (DEB 1637653) at the Kellogg Biological Station. Author contributions: SE conceived the project and design, led the data collection and analysis. KD maintained soil mesocosms and performed laboratory analyses with help from HK. LB assisted SE with bioinformatics and statistical analysis. SE wrote the manuscript with contributions from KD, HK, and LB. This is Kellogg Biological Station contribution number 2122.

References

Albright, M.B.N., and Martiny, J.B.H. (2018) Dispersal alters bacterial diversity and composition in a natural community. *ISME J* **12**: 296–299.

Amato, P., Ménager, M., Sancelme, M., Laj, P., Mailhot, G., and Delort, A.M. (2005) Microbial population in cloud water at the Puy de Dôme: implications for the chemistry of clouds. *Atmos Environ* **39**: 4143–4153.

Barberán, A., Ladau, J., Leff, J.W., Pollard, K.S., Menninger, H.L., Dunn, R.R., and Fierer, N. (2015) Continental-scale distributions of dust-associated bacteria and fungi. *Proc Natl Acad Sci* **112**: 5756–5761.

Bauer, H., Kasper-Giebl, A., Löflund, M., Giebl, H., Hitzenberger, R., Zibuschka, F., and Puxbaum, H. (2002) The contribution of bacteria and fungal spores to the organic carbon content of cloud water, precipitation and aerosols. *Atmos Res* **64**: 109–119.

Bell, T. (2010) Experimental tests of the bacterial distance-decay relationship. *ISME J* **4**: 1357–1365.

Bell, T., and Tylianakis, J.M. (2016) Microbes in the Anthropocene: spillover of agriculturally selected bacteria and their impact on natural ecosystems. *Proc R Soc B Biol Sci* **283**: 20160896.

Bouskill, N.J., Wood, T.E., Baran, R., Ye, Z., Bowen, B.P., Lim, H.C., *et al*. (2016) Belowground response to drought in a tropical forest soil. I. Changes in microbial functional potential and metabolism. *Front Microbiol* **7**: 525.

Bowers, R.M., McLetchie, S., Knight, R., and Fierer, N. (2011) Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *ISME J* **5**: 601–612.

Cadotte, M.W. (2006a) Dispersal and species diversity: a meta-analysis. *Am Nat* **167**: 913–924.

Cadotte, M.W. (2006b) Metacommunity influences on community richness at multiple spatial scales: a microcosm experiment. *Ecology* **87**: 1008–1016.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., *et al*. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* **6**: 1621–1624.

Chao, A. (1984) Non-parametric estimation of the classes in a population. *Scand J Stat* **11**: 265–270.

Classen, A.T., Boyle, S.I., Haskins, K.E., Overby, S.T., and Hart, S.C. (2003) Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soils. *FEMS Microbiol Ecol* **44**: 319–328.

Cleveland, C.C., Nemergut, D.R., Schmidt, S.K., and Townsend, A.R. (2007) Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* **82**: 229–240.

Comte, J., Langenheder, S., Berga, M., and Lindström, E.S. (2017) Contribution of different dispersal sources to the metabolic response of lake bacterioplankton following a salinity change. *Environ Microbiol* **19**: 251–260.

Dai, A. (2013) Increasing drought under global warming in observations and models. *Nat Clim Chang* **3**: 52–58.

Dickie, I.A. (2010) Insidious effects of sequencing errors on perceived diversity in molecular surveys. *New Phytol* **188**: 916–918.

Dobranic, J.K., and Zak, J.C. (1999) A microtiter plate procedure for evaluating fungal functional diversity. *Mycologia* **91**: 756–765.

Docherty, K.M., Pearce, D.S., Lemmer, K.M., and Hale, R.L. (2018) Distributing regionally, distinguishing locally: examining the underlying effects of local land use on airborne bacterial biodiversity. *Environ Microbiol* **20**: 3529–3542.

Eggers, S.L., Eriksson, B.K., and Matthiessen, B. (2012) A heat wave and dispersal cause dominance shift and decrease biomass in experimental metacommunities. *Oikos* **121**: 721–733.

Evans, S.E., Dueker, M.E., Logan, J.R., and Weathers, K.C. (2019) The biology of fog: results from coastal Maine and Namib Desert reveal common drivers of fog microbial composition. *Sci Total Environ* **647**: 1547–1556.

Evans, S.E., and Wallenstein, M.D. (2014) Climate change alters ecological strategies of soil bacteria. *Ecol Lett* **17**: 155–164.

Evans, S.E., and Wallenstein, M.D. (2012) Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? *Biogeochemistry* **109**: 101–116.

Evans, S.E., Wallenstein, M.D., and Burke, I.C. (2014) Is bacterial moisture niche a good predictor of shifts in community composition under long-term drought? *Ecology* **95**: 110–122.

Evans, S.E., Martiny, J., and Allison, S. (2017) Effects of dispersal and selection on stochastic assemblyin microbial communities *ISME J.* https://doi.org/10.1038/ismej.2016.96

Fox, J., and Weisberg, S. (2011) *An R Companion to Applied Regression*, 2nd ed. Thousand Oaks, CA: Sage.

France, K.E., and Duffy, J.E. (2006) Diversity and dispersal interactively affect predictability of ecosystem function. *Nature* **441**: 1139–1143.

Graham, E.B., and Stegen, J.C. (2017) Dispersal-based microbial community assembly decreases biogeochemical function. *Processes* **5**: 65.

Hufbauer, R.A., Szűcs, M., Kasyon, E., Youngberg, C., Koontz, M.J., Richards, C., *et al*. (2015) Three types of

rescue can avert extinction in a changing environment. *Proc Natl Acad Sci* **112**: 10557–10562.
Kellogg, C.A., and Griffin, D.W. (2006) Aerobiology and the global transport of desert dust. *Trends Ecol Evol* **21**: 638–644.
Kneitel, J.M., and Chase, J.M. (2004) Trade-offs in community ecology: linking spatial scales and species coexistence. *Ecol Lett* **7**: 69–80.
Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Appl Environ Microbiol* **79**: 5112–5120.
Lawrence, D., Bell, T., and Barraclough, T.G. (2016) The effect of immigration on the adaptation of microbial communities to warming. *Am Nat* **187**: 236–248.
Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., *et al*. (2004) The metacommunity concept: a framework for multi-scale community ecology. *Ecol Lett* **7**: 601–613.
Lenth, R.V. (2016) Least-squares means: the R package lsmeans. *J Stat Softw* **69**: 1–33.
Lindström, E.S., and Östman, Ö. (2011) The importance of dispersal for bacterial community composition and functioning. *PLoS One* **6**: e25883.
Lloyd-Smith, J.O. (2013) Vacated niches, competitive release and the community ecology of pathogen eradication. *Philos Trans R Soc B Biol Sci* **368**: 20120150.
Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* **17**: 10.
Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R. K., Fuhrman, J.A., Green, J.L., *et al*. (2006) Microbial biogeography: putting microorganisms on the map. *Nat Rev Microbiol* **4**: 102–112.
McGuire, K.L., Payne, S.G., Palmer, M.I., Gillikin, C.M., Keefe, D., Kim, S.J., *et al*. (2013) Digging the New York City skyline: soil fungal communities in Green roofs and City parks. *PLoS One* **8**: e58020.
McMurdie, P.J., and Holmes, S. (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**: e61217.
Middleton, E.L., Richardson, S., Koziol, L., Palmer, C.E., Yermakov, Z., Henning, J.A., *et al*. (2015) Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. *Ecosphere* **6**: art276.
Mirarab, S., Nguyen, N., Guo, S., Wang, L.-S., Kim, J., and Warnow, T. (2015) PASTA: Ultra-large multiple sequence alignment for nucleotide and amino-acid sequences. *J Comput Biol* **22**: 377–386.
Mouquet, N., and Loreau, M. (2003) Community patterns in source-sink Metacommunities. *Am Nat* **162**: 544–557.
Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., *et al*. (2013) Patterns and processes of microbial community assembly. *Microbiol Mol Biol Rev* **77**: 342–356.
Nilsson, R.H., Kristiansson, E., Ryberg, M., Hallenberg, N., and Larsson, K.H. (2008) Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and ITS implications for molecular species identification. *Evol Bioinforma* **2008**: 193–201.
Ochoa-Hueso, R., Collins, S.L., Delgado-Baquerizo, M., Hamonts, K., Pockman, W.T., Sinsabaugh, R.L., *et al*. (2018) Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. *Glob Chang Biol* **24**: 2818–2827.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., et al. (2017) *Vegan: community ecology package. R Packag ver 2***4–3** 282. https://CRAN.R-project.org/package=vegan
Peay, K.G. (2018) Timing of mutualist arrival has a greater effect on *Pinus muricata* seedling growth than interspecific competition. *J Ecol* **106**: 514–523.
Peay, K.G., Garbelotto, M., and Bruns, T.D. (2010) Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree islands. *Ecology* **91**: 3631–3640.
Peay, K.G., Schubert, M.G., Nguyen, N.H., and Bruns, T.D. (2012) Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. *Mol Ecol* **21**: 4122–4136.
Pielou, E.C. (1975) Ecological Diversity Wiley, New York, NY, USA.
R Core Team. (2017) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Found. Stat. Comput.
Robertson, G.P., Gross, K.L., Hamilton, S.K., Landis, D.A., Schmidt, T.M., Snapp, S.S., and Swinton, S.M. (2014) Farming for ecosystem services: an ecological approach to production agriculture. *Bioscience* **64**: 404–415.
Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., *et al*. (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* **4**: 1340–1351.
Rutgers, M., Breure, A.M., and Insam, H. (2006) Substrate utilization in BIOLOGTM plates for analysis of CLPP. In *Microbiological Methods for Assessing Soil Quality* (pp. 212–227). Cambridge, MA: CABI Publishing. https://www.researchgate.net/profile/David_Hopkins9/publication/37628914_Defining_soil_quality/links/544233320cf2a76a3cc8b273.pdf#page=221
Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., *et al*. (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol* **12**: 87.
Sun, S., Li, S., Avera, B.N., Strahm, B.D., and Badgley, B.D. (2017) Soil bacterial and fungal communities show distinct recovery patterns during forest ecosystem restoration. *Appl Environ Microbiol* **83**: e00966-17.
Székely, A.J., and Langenheder, S. (2017) Dispersal timing and drought history influence the response of bacterioplankton to drying–rewetting stress. *ISME J* **11**: 1764–1776.
Thiéry, O., Moora, M., Vasar, M., Zobel, M., and Öpik, M. (2012) Inter- and intrasporal nuclear ribosomal gene sequence variation within one isolate of arbuscular mycorrhizal fungus, Diversispora sp. *Symbiosis* **58**: 135–147.
Tiemann, L.K., and Billings, S.a. (2012) Tracking C and N flows through microbial biomass with increased soil moisture variability. *Soil Biol Biochem* **49**: 11–22.
UNITE Community (2017) UNITE USEARCH/UTAX release.
Vance, E.D., Brookes, P.C., and Jenkinson, D.S. (1987) An extraction method for measuring soil microbial biomass-C. *Soil Biol Biochem* **19**: 703–707.
Vellend, M. (2010) Conceptual synthesis in community ecology. *Q Rev Biol* **85**: 183–206.

De Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M., and Bardgett, R.D. (2012) Land use alters the resistance and resilience of soil food webs to drought. *Nat. Clim. Chang.* **2**: 276–280.

Wallenius, K., Rita, H., Simpanen, S., Mikkonen, A., and Niemi, R.M. (2010) Sample storage for soil enzyme activity and bacterial community profiles. *J Microbiol Methods* **81**: 48–55.

Wickham, H. (2009) ggplot2: Elegant Graphics for Data Analysis. New York: Springer.

Wilson, K. (2001) Preparation of genomic DNA from bacteria. *Curr Protoc Mol Biol*. **56**: 2.4.1–2.4.5.

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., *et al*. (2014) The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. *Nucleic Acids Res* **42**: D643–D648.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting Information.