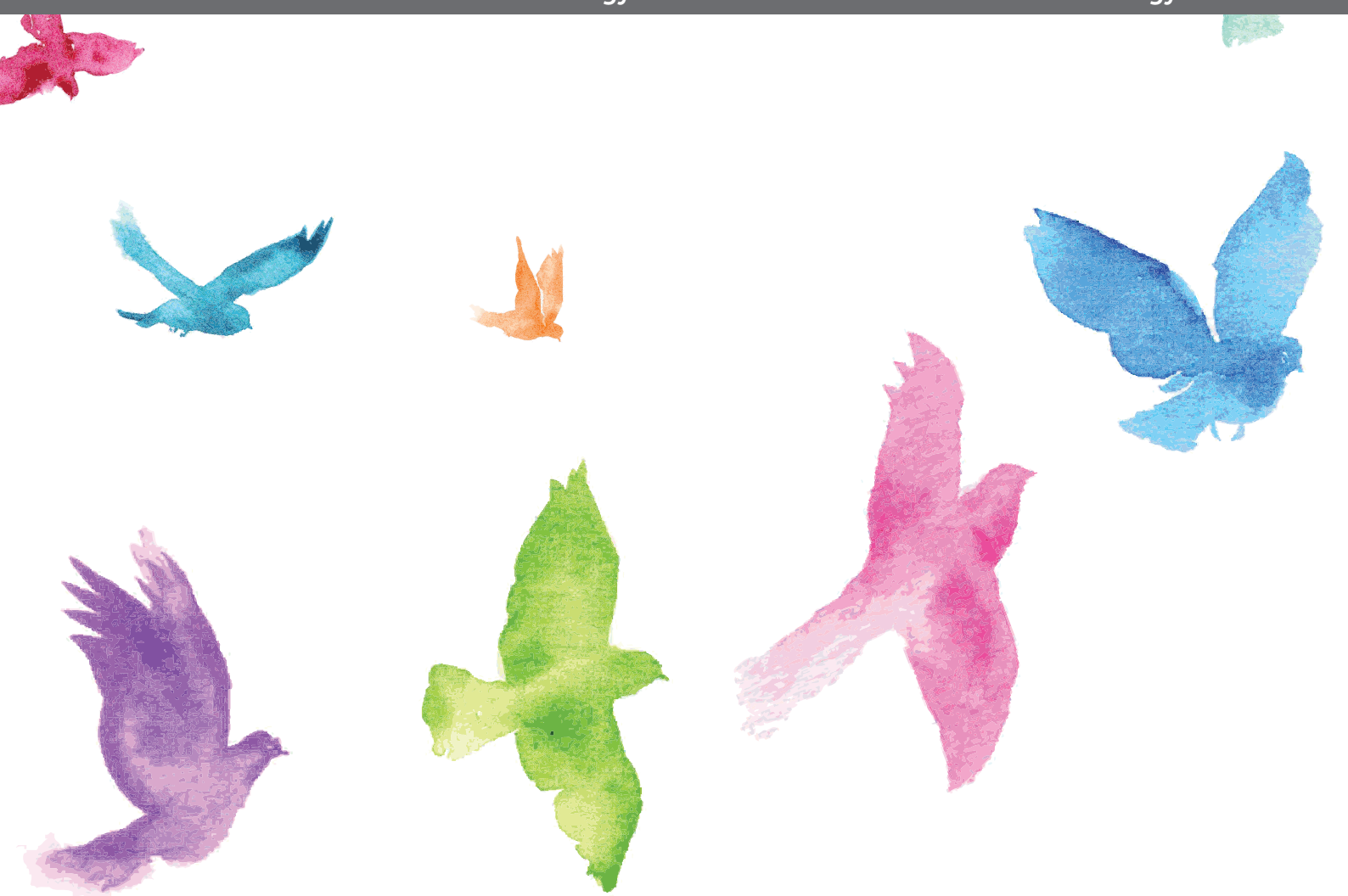
MICROBIAL LANDSCAPE ECOLOGY: HIGHLIGHTS ON THE INVISIBLE CORRIDORS

EDITED BY: Cendrine Mony, Brendan J. M. Bohannan, Kabir Peay, Philippe Vandenkoornhuyse and Mathew A. Leibold

PUBLISHED IN: Frontiers in Ecology and Evolution and Frontiers in Microbiology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88971-703-3
DOI 10.3389/978-2-88971-703-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

MICROBIAL LANDSCAPE ECOLOGY: HIGHLIGHTS ON THE INVISIBLE CORRIDORS

Topic Editors:
Cendrine Mony, University of Rennes 1, France
Brendan J. M. Bohannan, University of Oregon, United States
Kabir Peay, Stanford University, United States
Philippe Vandenkoornhuyse, University of Rennes 1, France
Mathew A. Leibold, University of Florida, United States

Citation: Mony, C., Bohannan, B. J. M., Peay, K., Vandenkoornhuyse, P., Leibold, M. A., eds. (2021). Microbial Landscape Ecology: Highlights on the Invisible Corridors. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-703-3

Table of Contents

04 ***Editorial: Microbial Landscape Ecology: Highlights on the Invisible Corridors***
Cendrine Mony, Brendan J. M. Bohannan, Mathew A. Leibold, Kabir Peay and Philippe Vandenkoornhuyse

07 ***Life Between Patches: Incorporating Microbiome Biology Alters the Predictions of Metacommunity Models***
Elizabeth T. Miller and Brendan J. M. Bohannan

20 ***A Metabarcoding Analysis of the Mycobiome of Wheat Ears Across a Topographically Heterogeneous Field***
Gabriele Schiro, Pierluigi Colangeli and Marina E. H. Müller

32 ***Effects of Past and Present-Day Landscape Structure on Forest Soil Microorganisms***
Sophie Mennicken, Floriane Kondratow, Florian Buralli, Sophie Manzi, Emilie Andrieu, Mélanie Roy and Antoine Brin

45 ***Different Roles of Environmental Selection, Dispersal, and Drift in the Assembly of Intestinal Microbial Communities of Freshwater Fish With and Without a Stomach***
Yinghua Zha, Eva S. Lindström, Alexander Eiler and Richard Svanbäck

60 ***Evaluating Alternative Metacommunity Hypotheses for Diatoms in the McMurdo Dry Valleys Using Simulations and Remote Sensing Data***
Eric R. Sokol, J. E. Barrett, Tyler J. Kohler, Diane M. McKnight, Mark R. Salvatore and Lee F. Stanish

78 ***A Landscape of Opportunities for Microbial Ecology Research***
Cendrine Mony, Philippe Vandenkoornhuyse, Brendan J. M. Bohannan, Kabir Peay and Mathew A Leibold

94 ***Evolutionary Rescue Is Mediated by the History of Selection and Dispersal in Diversifying Metacommunities***
Louise M. J. O'Connor, Vincent Fugère and Andrew Gonzalez

109 ***Geographical Variability of Mineral Elements and Stability of Restrictive Mineral Elements in Terrestrial Cyanobacteria Across Gradients of Climate, Soil, and Atmospheric Wet Deposition Mineral Concentration***
Weibo Wang, Hua Li, René Guénon, Yuyi Yang, Xiao Shu, Xiaoli Cheng and Quanfa Zhang

121 ***Comparative Study of the Rhizosphere and Root Endosphere Microbiomes of Cholistan Desert Plants***
Salma Mukhtar, Samina Mehnaz and Kauser Abdulla Malik

135 ***Resistance, Resilience, and Recovery of Dryland Soil Bacterial Communities Across Multiple Disturbances***
Blaire Steven, Michala L. Phillips, Jayne Belnap, La Verne Gallegos-Graves, Cheryl R. Kuske and Sasha C. Reed

147 ***Short-Term Effect in Soil Microbial Community of Two Strategies of Recovering Degraded Area in Brazilian Savanna: A Pilot Case Study***
Priscila Jane Romano Gonçalves Selari, Luiz Ricardo Olchanheski, Almir José Ferreira, Tiago do Prado Paim, Guido Calgaro Junior, Flavio Lopes Claudio, Estenio Moreira Alves, Darliane de Castro Santos, Welington Luiz Araújo and Fabiano Guimarães Silva

frontiers in Ecology and Evolution

EDITORIAL
published: 01 October 2021
doi: 10.3389/fevo.2021.753213

Editorial: Microbial Landscape Ecology: Highlights on the Invisible Corridors

Cendrine Mony[1], Brendan J. M. Bohannan[2], Mathew A. Leibold[3], Kabir Peay[4] and Philippe Vandenkoornhuyse[1]*

[1] Université de Rennes 1, CNRS, UMR6553 ECOBIO, Rennes, France, [2] Center for Ecology and Evolutionary Biology, University of Oregon, Eugene, OR, United States, [3] Department of Biology, University of Florida, Gainesville, FL, United States, [4] Department of Biology, Stanford University, Stanford, CA, United States

Keywords: landscape ecology, spatial ecology, metacommunity, assembly rule, microorganisms

Editorial on the Research Topic

Microbial Landscape Ecology: Highlights on the Invisible Corridors

INTRODUCTION

Understanding community assembly is a key question in ecology (Kraft and Ackerly, 2014). The first integration of spatial processes at large spatial scales was introduced *via* the island biogeography theory of MacArthur and Wilson (1967), while landscape ecology emerged in the early 1990's as a new way for analyzing the causes and consequences of spatial patterns across landscapes (Wiens et al., 1993; Turner et al., 2001). In parallel, the consideration of species dynamics as resulting from the interrelationships among local populations, and individual fluxes among them led to the metapopulation concept (Hanski, 1994), extended 20 years ago to the metacommunity concept (Leibold et al., 2004). This framework based on a more mechanistic understanding of species distribution does not integrate space in an explicit manner, but considers dispersal fluxes as a proxy for population isolation. These two main conceptual streams have converged recently to allow better prediction of community assembly at the landscape level.

The application of these concepts to microbes has been slow to develop, due to our limited understanding of microbial habitat requirements, the species concept used, and our limited capacity for spatially extensive surveys of microbial distributions. In addition, there is a long-held assumption that microbial taxa have no meaningful dispersal limits because of their small size and high propagule production (Baas-Becking's hypothesis; Baas Becking, 1934). If unlimited passive dispersion of microorganisms is occurring, biotic and abiotic environmental filters would explain the observed high heterogeneity in the microbial communities in many (all) ecosystems. These views have been challenged by more recent evidence demonstrating strong dispersal limitation, and biogeography has consequently become more prominently used for describing and understanding large-scale spatial patterns (Martiny et al., 2006; Hanson et al., 2012; Donaldson et al., 2016).

Understanding the landscape-level drivers of microbiota complexity is important in a broader ecological context because of the tremendous role these microorganisms play in many ecological functions (e.g., carbon and nutrient cycles, resistance of organisms to stresses, behaviors, and reproduction, etc.). Despite the limitation of mass sequencing approaches in species detection, their application has resulted in the observation that microbial communities encompass tremendous taxonomic diversity, including bacteria, archaea, fungi, and protozoa. The microbial world is also characterized by a large range of life-styles, from free-living organisms to microorganisms associated with plant, animal, and human hosts. Landscape drivers may shape community assembly

OPEN ACCESS

Edited and reviewed by:
Peter Convey,
British Antarctic Survey (BAS),
United Kingdom

****Correspondence:***
Cendrine Mony
cendrine.mony@univ-rennes1.fr

Specialty section:
This article was submitted to Biogeography and Macroecology, a section of the journal Frontiers in Ecology and Evolution

Received: *04 August 2021*
Accepted: *30 August 2021*
Published: *01 October 2021*

Citation:
Mony C, Bohannan BJM, Leibold MA, Peay K and Vandenkoornhuyse P (2021) Editorial: Microbial Landscape Ecology: Highlights on the Invisible Corridors. Front. Ecol. Evol. 9:753213. doi: 10.3389/fevo.2021.753213

either directly through metapopulation and metacommunity processes or indirectly through host-distribution, although these relationships and their related mechanisms are still poorly characterized, and have mostly focused on pathogens.

Overall the present topic addressed how landscape ecology including metapopulation and metacommunity frameworks can apply successfully to microorganisms. Through a large range of microorganism types and ecosystems considered, we provide a set of papers in this emerging field of microbial landscape ecology. These are categorized in three main topics: biogeography, landscape ecology, and metacommunity ecology. A review of this existing state of the art and introduction to main concepts are presented in this topic in Mony et al.

BIOGEOGRAPHY

Microorganisms are highly heterogeneous in space from large to small spatial scales (e.g., Bahram et al., 2015). Biogeography analyzes the spatial patterns at large scale and aims at determining the environmental factors shaping these spatial patterns. The development of biogeography for microorganisms is recent (e.g., Martiny et al., 2006), applying both to terrestrial microbes and aquatic microbes mostly at large spatial scales of continents or regions. Drivers underlying spatial patterns are investigated, and generally correspond to changes in abiotic conditions (i.e., habitat characteristics). In the present topic, Mukhtar et al. demonstrate strong environmental filtration of microorganisms associated with plants suggesting a patchiness being strongly dependent on selection processes. Integrating space into account, Steven et al. demonstrated patchy distribution of bacterial communities in the soil due to local habitat distribution but also as a result of past disturbance. This article nicely demonstrates the scale of response of these communities both in space and time, and proposes that soil edaphic factors, climatic disturbances, and physical trampling are drivers of these biogeographical patterns. This article also provides an interesting example of integrating drivers occurring in the past for taking into account time-lags in species responses observed. One key question related to understanding species patterns concerns the possible local adaptation to patchy environments. Wang et al. reported here geographical variation of mineral elements of one cyanobacteria developing in the soil. This article proposes to study both the geographical variation of one microorganism species, and the related biochemical composition and mineral element contents. This original work helps to explore how the geographical variation of mineral content promotes the ecological adaptation of soil cyanobacteria. In most existing literature, biogeography has been applied at a large spatial scale, Schiro et al. present here a demonstration that biogeography can apply a very small spatial scale (scale of a field). They demonstrated the small-scale heterogeneity in fungi occurring in plant phyllosphere (and especially pathogens). This heterogeneity was described by local taxon-level selection to variable canopy environmental conditions, especially linked to climatic conditions, but also to a lesser extent by geographical position.

LANDSCAPE ECOLOGY

Landscape ecology provides a range of theories for understanding how landscape structure affects species coexistence. Methods specific to landscape ecology have been also developed to precisely characterize landscape metrics, analyze how landscape shapes species distribution and dispersal. Applications to microorganisms have mostly focused on one species of interest at a time, primarily pathogens (leading to the field of "landscape epidemiology"). Landscape epidemiology has been developed in all ecosystems on plant, animal or human pathogens, and even within the human body for predicting the development of infectious agents [see reviews of Holdenrieder et al. (2004) and Suzan et al. (2012)]. Use of landscape ecology concepts and methods in microbiology is at an early stage (see review of Mony et al.). In this topic, Mennicken et al. proposed an interesting study analyzing a recent theory of landscape ecology, the habitat amount hypothesis (Fahrig, 2013), on a large set of microorganism guilds and demonstrated for some of them the importance of forest cover in the landscape. Additionally, this article brings original evidence of time-lagged dispersal processes, by demonstrating that patch age affects the response of microorganisms to habitat amount. Integrating time into landscape studies offers new promising prospects in microbial ecology.

METACOMMUNITY ECOLOGY

Understanding the mechanisms of community assembly at landscape spatial scales is a key issue. The metacommunity framework assumes that community assembly results from four key processes: dispersal, species sorting, patch dynamics, and drift (Leibold et al., 2004). Metacommunity theory is a useful framework for predicting species coexistence, as illustrated in two articles in the topic. Zha et al. analyze how environmental selection, dispersal, and drift explain variation in composition of gut microbiota in fishes. In another study, Sokol et al. analyzed the metacommunity functioning of diatoms in Antarctica, and similarly demonstrated the effect of dispersal and species sorting in community assembly. Because many microorganisms have very short generation times, they are possibly experiencing more rapid evolutionary processes than macroorganisms. Integrating evolutionary processes in metacommunity models, O'Connor et al. analyze evolutionary rescue within a metacommunity framework and demonstrate that the history of antibiotic selection and dispersal modes influence diversification in microbial metacommunities. Metacommunity and landscape ecology are generally poorly linked (Almeida-Gomes et al., 2020), even in macroorganisms. Miller and Bohannan propose an adaptation of the metacommunity model that takes into account the permeability of the matrix to dispersal movement. Using this adapted model, they analyzed how species traits in microorganisms might promote species persistence in the matrix, with possible adaptation to human and animal-associated microbiomes. This study is a pioneering attempt to go beyond the patch-matrix binary vision of landscape and to integrate landscape heterogeneity into the metacommunity framework.

PERSPECTIVES

Integrating landscape-scale drivers in community assembly for microorganisms is an important step toward a better understanding of species distribution, although the application of landscape ecology is still in its infancy. The application of landscape ecology to microorganisms has a number of potential applications, including in environmental restoration (such as that described by Gonçalves Selari et al. in this special feature).

The Mony et al. review provides examples of how theories and methods from landscape ecology can be applied successfully to microbes; importantly, it also highlights the knowledge gaps that interfere with such application, including our ignorance of many ecological processes operating at the landscape scale, such as microbial dispersal. It also stresses the need for adaptation of this framework to take into account microorganism characteristics and better integration of their real dispersion rates and realized niches. Landscape-microbe relationships are more complex, and less well-understood, than for macroorganisms involving a large range of response scales—from the classical landscape scale to micro landscapes–, nested spatial and temporal scales, and a unique case of biotic landscapes for host-associated microorganisms. Microbial community composition and distribution are also often driven directly by their host distribution, and feedback loops between micro- and macro-organisms likely occur. If the landscape ecology framework in its broad sense (i.e., including biogeography and metacommunity concepts) provides a new avenue for testing ecological hypotheses on microbial community assembly, along with eco-evolutionary processes shaping communities, microbes provide also a unique and original case study that might considerably enrich the existing theoretical backgrounds and methods. Within a more holistic framework of understanding the application of landscape ecology to the microbial world is susceptible to modify our understanding not solely about the rules of microbial community assembly, but also for instance (i) eco-evolutionary processes and dynamics structuring microbial communities, (ii) macro-ecological processes resulting from changes of microbial communities, (iii) links between host and symbionts, all resulting from/to a better interpretation to which extend the neutral model of assembly is applicable rather than non-random influences of niche differentiation to the considered microbial community. The landscape of opportunities for future research in this field is wide.

AUTHOR CONTRIBUTIONS

CM wrote the first draft. All authors reviewed the text and gave final approval.

FUNDING

This research was funded by U.S. NSF award 2025118 (to ML), CNRS EC2CO (to PV), and l'agence Française pour la Biodiversité project AGRIM (to CM).

REFERENCES

Almeida-Gomes, M., Valente-Neto, F., Pacheco, E. O., Ganci, C. C., Leibold, M. A., Melo, A. S., et al. (2020). How does the landscape affect metacommunity structure? A quantitative review for lentic environments. *Curr. Landsc. Ecol. Rep.* 5, 68–75. doi: 10.1007/s40823-020-00049-6

Baas Becking, L. G. M. (1934). *Geobiologie of Inleiding Tot de Milieukunde*. The Hague: W. P. Van Stockum and Zoon.

Bahram, M., Peay, K. G., and Tedersoo, L. (2015). Local-scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. *New Phytol.* 205, 1454–1463. doi: 10.1111/nph.13206

Donaldson, G. P., Lee, S., and Mazmanian, S. K. (2016). Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* 134, 20–32. doi: 10.1038/nrmicro3552

Fahrig, L. (2013). Rethinking patch size and isolation effects: the habitat amount hypothesis. *Ann. J. Biogeogr.* 40, 1649–1663. doi: 10.1111/jbi.12130

Hanski, I. (1994). A practical model of metapopulation dynamics. *J. Anim. Ecol.* 63, 151–162. doi: 10.2307/5591

Hanson, C. A., Fuhrman, J. A., Horner-Devine, C., and Martiny, J. B. H. (2012). Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.* 10, 497–506. doi: 10.1038/nrmicro2795

Holdenrieder, O., Pautasso, M., Weisberg, P. J., and Lonsdale, D. (2004). Tree diseases and landscape processes: the challenge of landscape pathology. *Trends Ecol. Evol.* 19, 446–452. doi: 10.1016/j.tree.2004.06.003

Kraft, N. J. B., and Ackerly, D. D. (2014). "Assembly of plant communities," in *Ecology and the Environment*, ed R. K. Monson (New York, NY: Springer-Verlag), 67–88. doi: 10.1007/978-1-4614-7501-9_1

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. doi: 10.1111/j.1461-0248.2004.00608.x

MacArthur, R. H., and Wilson, E. O. (1967). *The Theory of Island Biogeography*. Princeton, NJ: Princeton University Press.

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. doi: 10.1038/nrmicro1341

Suzan, G., Esponda, F., Carrasco-Hernandez, R., and Alonso Aguirre, A. (2012). "Habitat fragmentation and infectious disease ecology," in *New Directions in Conservation Medicine : Applied Cases of Ecological Health*, eds A. Alonso Aguirre, R. Ostfeld, and P. Daszak (New York, NY. Owford University Press).

Turner, M. G., Gardner, R. H., and O'Neill, R. V. (2001). *Landscape Ecology in Theory and Practice*. New York, NY: Springer-Verlag.

Wiens, J. A., Stenseth, N. C., Van Horne, B., and Anker Ims, R. (1993). Ecological mechanisms and landscape ecology. *Oikos* 66, 369–380. doi: 10.2307/3544931

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 18 July 2019
doi: 10.3389/fevo.2019.00276

Life Between Patches: Incorporating Microbiome Biology Alters the Predictions of Metacommunity Models

Elizabeth T. Miller and Brendan J. M. Bohannan*

Department of Biology, Institute of Ecology and Evolution, University of Oregon, Eugene, OR, United States

Although most models conceptualize a metacommunity as a collection of habitat patches embedded in a matrix that is not hospitable to life, new applications of metacommunity theory to host-microbiome systems have shown this assumption to be flawed. Frequently the matrix is at least somewhat hospitable to the species that normally reside in the habitat patches. We modify an existing patch dynamic metacommunity model to incorporate the possibility of a hospitable matrix and expand the process of dispersal to include moving out of the patch, surviving/growing in the matrix, and moving into a new patch. With these alterations, we find that it is substantially harder for a dispersal specialist to persist in a system with a hospitable matrix compared to an inhospitable matrix. In addition, we find that the traits required to successfully disperse in the hospitable system are different from those required in the inhospitable system. The difference in dispersal traits in a hospitable environment vs. an inhospitable one could be especially of interest to host-microbiome systems where manipulation of the matrix is common practice. For example, ventilation or disinfection of built environments is a common way to change the matrix properties for metacommunities of human or animal-associated microbiomes. We conclude that the qualities of the matrix can have important effects on community assembly, and that relaxing matrix assumptions broadens the range of applications for metacommunity ecology, including its use for host-microbe systems.

Keywords: metacommunity, dispersal, patch dynamics, host-microbiome, modeling, theory

OPEN ACCESS

Edited by:
Marco A. Molina-Montenegro, University of Talca, Chile

Reviewed by:
Florence Anne Laure Gutzwiller, Center for Bioinformatics and Integrative Biology (CBIB), Chile
Sebastian Abades, Universidad Mayor, Chile

****Correspondence:***
Elizabeth T. Miller
emiller8@uoregon.edu

Specialty section:
This article was submitted to Biogeography and Macroecology, a section of the journal Frontiers in Ecology and Evolution

Received: *25 April 2019*
Accepted: *03 July 2019*
Published: *18 July 2019*

Citation:
Miller ET and Bohannan BJM (2019) Life Between Patches: Incorporating Microbiome Biology Alters the Predictions of Metacommunity Models. Front. Ecol. Evol. 7:276. doi: 10.3389/fevo.2019.00276

INTRODUCTION

The metacommunity concept has had a wide-reaching impact on ecology (Leibold et al., 2004; Logue et al., 2011). Its central contribution has been to conceive of communities as being governed by both local and regional processes, connected by dispersal. This insight has allowed the synthesis of many disparate theoretical approaches under the umbrella of metacommunity ecology (Leibold and Chase, 2018). This synthesis has had a great influence on ecology generally, and has expanded our understanding of evolution (Urban et al., 2008), coexistence (Mouquet and Loreau, 2002), and community assembly (Mittelbach and Schemske, 2015). It has been especially influential on the emerging field of microbiome science, where it has provided a theoretical foundation for understanding microbiome variation, especially in host-microbe systems (Costello et al., 2012; Burns et al., 2017; Miller et al., 2018).

A great deal of theory has been developed around understanding the patterns expected with different metacommunity regimes defined by different levels of dispersal and patch heterogeneity. Almost all of this theory treats dispersal as direct (i.e., when colonists leave one patch they arrive instantaneously in another, with possible death along the way), and the matrix as inhospitable (i.e., no colonists remain in the matrix for the next generation or dispersal time step) (Levins and Culver, 1971; Fournier et al., 2016; Shoemaker and Melbourne, 2016; Sokol et al., 2017). Despite its prevalence, the assumption of an inhospitable matrix is not met in many ecological systems. This shortcoming (especially with regard to island biogeography) has been acknowledged in the conservation literature for years (Mendenhall et al., 2014). More recently, this assumption has been challenged by the application of metacommunity ecology to host-associated communities (i.e., the microbiomes of animals and plants; Miller et al., 2018), because a hospitable matrix is especially likely in such systems. Environmental reservoirs often play important roles in the transmission of microbiome members among animal and plant hosts, and this is likely to be true of humans as well. Most humans live in built environments that harbor microbiomes that are largely influenced by the microbiomes of the human inhabitants, which can in turn be influenced by the nature [and even the architecture (Kembel et al., 2012)] of the built environment.

Ignoring the ecology of the matrix could have important implications for our understanding of community assembly and dynamics. First, it may lead to misunderstandings about the provenance of community members if we do not take into account those that came from the matrix. Second, a hospitable matrix allows dispersal to occur over a much longer and more continuous timescale, which better captures the environment's role as a reservoir, which is commonly observed for micro-organisms. Finally, dispersal through a hospitable matrix opens up more complicated life history strategies for dispersing species. Instead of merely getting out of the old patch and into a new patch in a single step, colonists could specialize on any of the steps along the way. The traits that make a species readily able to leave a patch may not be the same as those traits that might enhance survival or growth in the matrix or even entrance into a new patch. For example, traits related to seed dispersal such as dandelion parachutes or burrs that attach to animal vectors may help get out of a patch but not be much use when it comes to getting established in a new patch. Such differences are especially apparent in host-microbiome systems where emigration and immigration often happen through entirely different routes.

Here we examine what happens when we incorporate a hospitable matrix into a metacommunity model. Instead of moving directly from patch to patch, some portion of the dispersing individuals will remain in the matrix and be eligible to complete the dispersal process in the future. We find that when the assumption of an inhospitable matrix is relaxed, it becomes clear that survival or growth in the matrix complicates the process of dispersal, and it becomes substantially harder for a dispersal specialist to persist. In addition, we find that the traits required to successfully disperse in the hospitable system are different from those required in the inhospitable system.

MATERIALS AND METHODS

Patch Dynamics Framework

We set out to investigate the importance of the matrix and the strategies species employ to move through it using a simple patch dynamics model (Leibold et al., 2004). We utilize this modeling framework because it most clearly isolates the effect of dispersal on coexistence in the matrix and it is well-studied for the direct-dispersal case. The patch dynamics framework assumes identical habitat patches that are subject to random extinction events. Diversity in the system is maintained through a dispersal-competition tradeoff: one species is dominant in the patch (patch specialist) while the other species (dispersal specialist) can survive by being the first to colonize a newly empty patch. In modern coexistence parlance, this enables coexistence via the storage effect (Chesson, 2000; Shoemaker and Melbourne, 2016). Compared to other processes that promote coexistence in metacommunities, it is a weak mechanism (Shoemaker and Melbourne, 2016), but it is entirely dependent on the dispersal dynamics between patches and so it is ideal for exploring changes in dispersal within a metacommunity. There is recent evidence that a dispersal-competition tradeoff was an important factor in early microbial adaptation to dry land (Dini-Andreote et al., 2018), and it is likely to be especially important in the turbulent early days of host colonization in host-microbiome systems (Stewart et al., 2018). Therefore, we believe that this theoretical framework is ideal for examining the effect of a hospitable matrix on metacommunity processes.

To assess the effects of a hospitable matrix and an expanded range of dispersal traits on coexistence we constructed a patch-dynamic metacommunity model based on previous models that sought to understand coexistence in metacommunity processes (Shoemaker and Melbourne, 2016). This model, like the MCSim model (Sokol et al., 2017), employs a step-wise dispersal mechanism that is readily adapted to incorporate a hospitable matrix. We looked at the conditions for persistence of two competing species, one with a growth rate advantage in the patch (patch specialist) and one with a dispersal advantage (dispersal specialist). We investigated the potential for coexistence over a range of values for disturbance rate, tradeoff strength, number of patches, and mean dispersal rate in directly dispersing metacommunities, metacommunities where species could survive (but not grow) in the matrix, and metacommunities where growth was possible in the matrix.

In addition to changing the conditions of the metacommunity, we looked at the traits of the species. To be a dispersal specialist, a species must have an advantage at some point during the dispersal process to gain an overall dispersal advantage. There are three distinct methods of gaining such an advantage: a species could leave a patch at a relatively high rate compared to the patch specialist species, it could grow or survive in the matrix at a relatively high rate, or it could gain entry into a patch (from the matrix) at a relatively high rate. We call these different strategies "out trait," "matrix growth," and "in trait." We alter which of these traits provide the dispersal advantage for the dispersal specialist to see whether the point at which the advantage is applied matters to the outcome of the model.

The Model

We adapted our metacommunity model from Shoemaker and Melbourne (2016). It is a discrete-time, stepwise model that keeps track of the populations of two species as they grow in and move between identical individual habitat patches. The two microbial species are differentiated by their traits at several points: growth rate in the patch (R), growth rate in the matrix (M), dispersal rate out of the patch (d_{out}), and dispersal rate into the patch (d_{in}).

The model proceeds in a step-wise fashion: after initialization, species compete within a patch according to the Beverton-Holt model, commonly employed in metacommunity models and thus well-suited to our goal of relaxing matrix assumptions,

$$N_{i,k}\,(t+1) = \frac{R_i * N_{i,k}(t)}{1 + a * \sum_i N_{i,k}(t)}$$

Here $N_{i,k}(t)$ is the density of species i in patch k at time t, R_i indicates the growth rate of species i, and α is a parameter that sets the carrying capacity of a patch (see **Table S1** for parameters). Competition and growth (if there is any) within the matrix happens similarly to and simultaneously with growth in the patch.

$$N_{i,M}\,(t+1) = \frac{M_i * N_{i,M}(t)}{1 + a * \sum_i N_{i,M}(t)}$$

Where $N_{i,M}$ is the density of species i in the matrix and M_i is the growth rate in the matrix of species i.

After a single time step in the patch, the dispersal step takes place. Species leave the patch according to their dispersal out trait, $m_{i,k} = N_{i,k} * d_{out,i}$, where $m_{i,k}$ is the migration density of species i out of patch k, and $d_{out,i}$ is the dispersal rate of species i out of a patch. The emigrants from all patches are combined in the matrix (with the residents in the matrix, or without for the inhospitable matrix case) and then are added back into the patches proportionally to their density in the matrix and their dispersal in trait $d_{in,i}$.

Finally, some patches are chosen at random for an extinction event (set by the "Patch Disturbance Rate" parameter). If a patch is disturbed, then the densities of both species in the patch are set to zero and they remain at zero until the immigration step of the next iteration.

We ran all simulations for 2,500 time steps, and because of the fluctuations inherent in the patch dynamic model we calculated final density as the average per-patch density over the last 50 time steps. We repeated each simulation 100 times and report the proportion of times a species was present at the end of the simulation. All model simulations were done using R statistical software.

Analyses

We explored the model in three ways. First, we determined the effect of a hospitable matrix by allowing persistence or growth in the matrix between time steps. Second, we examined the effect of different dispersal traits by conferring an advantage to the dispersal specialist in either d_{out}, d_{in}, or M. Third, we confirmed that these effects were due to the matrix conditions and not merely the introduction of patch heterogeneity by adding a single matrix-like patch into a metacommunity with direct dispersal (and comparing the resulting dynamics to our original model).

In all cases, we determined the effect of altering the model by measuring persistence rates of the two species (patch specialist and dispersal specialist), across a range of number of patches and disturbance rates. There are four possible outcomes, patch specialist out-competes dispersal specialist, dispersal specialist out-competes patch specialist, coexistence, and total extinction. The prevalence of each of these outcomes is our main indicator of differential effects of survival and/or growth in the matrix.

Patch disturbance rate is a typical parameter investigated in patch-dynamic studies. It is a measure of the environmental variability and the prevalence of empty patches. In general, a higher disturbance rate should favor the dispersal specialist over the patch specialist because the dispersal specialist is better able to utilize newly emptied patches and the patch specialist is the one that primarily loses (because it is present) when a patch is destroyed. Number of patches is less well-addressed in patch dynamic studies [they often consider infinite or a very large number of patches; (Levins, 1979)] but it has the potential to greatly alter metacommunity dynamics (Burns et al., 2017).

Model Variants

We look first at the difference among a direct dispersal model, a model with a completely inhospitable matrix, a model where dispersal is indirect through a matrix where species can survive but cannot grow, and a model where all species can grow slowly and evenly in the matrix (**Figure 1**). We start by assuming that the dispersal specialist has the same advantage on the way in and on the way out (see **Table S1** for parameter values and **Figures S1–S3** for additional analyses).

We begin with two variants of an inhospitable matrix. We first construct a model with direct dispersal where the emigrants from one patch are sent to another, randomly chosen patch (**Figure 1A**). This model has no matrix; immigrants from one patch move instantly and with their cohort to another patch. Although we call this a direct dispersal model, we maintain the stepwise process that creates an emigrant pool with the d_{out} trait and then an immigrant pool with the d_{in} trait as described in section The Model. The patch connections are re-drawn each time step so the system becomes well-mixed. This one-to-one type of transmission may be analogous to vector-aided transmission in many systems: the duck foot theory of algae dispersal (Schlichting, 1960), for instance, or storm-driven dispersal between islands. Next, we look at a global dispersal model where all emigrants from all the patches are pooled and then evenly divided among all the patches (**Figure 1B**) (Shoemaker and Melbourne, 2016). The conditions of this matrix may seem somewhat artificial (global mixing, totally inhospitable matrix) but it is a commonly used approximation in the literature.

We also look at two variants of a hospitable matrix, both using the same approach as the global dispersal model (**Figure 1C**). First, we have a persistence model where some of the emigrants remain in the matrix but have no growth in the matrix between time steps. These residents of the matrix are then re-mixed with

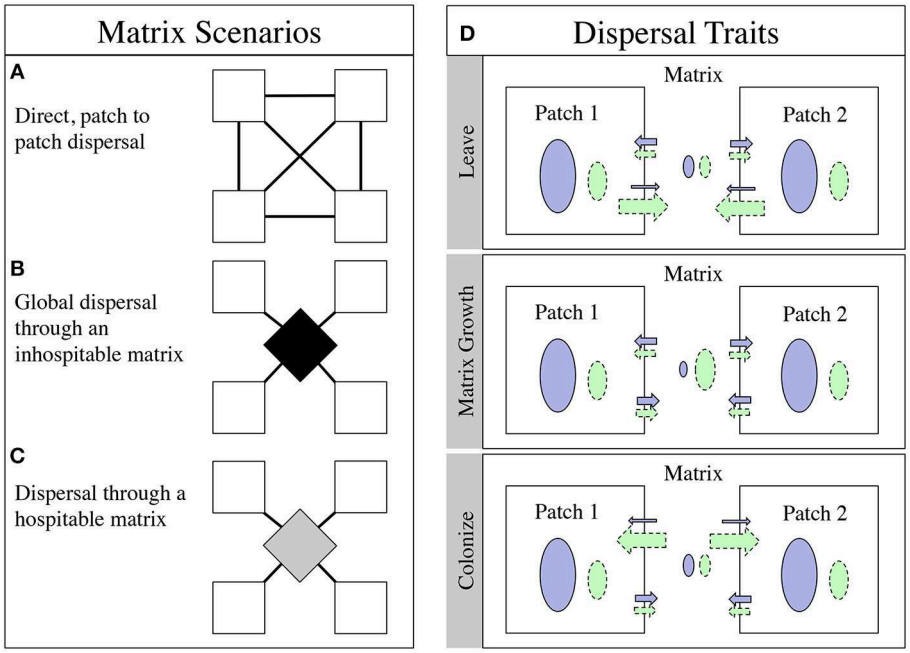


FIGURE 1 | The alternative matrix scenarios and the different dispersal regimes. **(A)** The direct dispersal model, colonists from the patches move directly into another patch, symbolized by lines directly connecting the white boxes. **(B)** The global dispersal scenario, colonists move from the patches move into a common pool (symbolized by the black diamond) and then move back out to the patches, leaving nothing behind. **(C)** The hospitable matrix, colonists move from the white patches into the matrix (now a gray diamond) and back out to the patches, but some of them stay in the gray matrix to get another chance at dispersing the next timestep. **(D)** The dispersal scenarios. The dispersal specialist (green, dashed borders) has an advantage getting out in the Leave scenario, but no advantage getting back into the patch or growing in the matrix. The dispersal specialist has an advantage in growth in the matrix in the Matrix Growth scenario (symbolized by the ovals in the matrix), but no advantage getting out of or back into the patch. Finally, in the Colonize scenario, the dispersal specialist gets back into the patch at a higher rate, but has no advantage getting out or growing in the matrix. In all scenarios the patch specialist (blue, solid borders) has a growth advantage in the patch (symbolized by the ovals in the patches).

the dispersing cohort from the following time step and have another chance to successfully disperse to a patch. Finally, we have a low growth model where the emigrants from the patches that remain in the matrix have a low growth rate of 1.2, compared to 1.4 and 1.45 in the patches.

The choice of parameter values, as with any purely theoretical study, was not designed to exactly mimic a particular system, but to illustrate the maximum range of interesting behavior. In particular, the timescale is undefined so each time step could take a second or a year. It is important, however, to note that the rates are relevant to each other: the relative speed of growth and dispersal is fixed by the particular choice of values. To put our model in terms of host-microbiome systems, we could fix the timescale at 15 min per step. This would give our two species a doubling time in the host of about 30 min (∼27 min for the patch specialist and ∼30 for the dispersal specialist), slightly slower than that of some E. coli strains (∼20 min) and about on par with other measured host-associated bacterial strains in zebrafish (∼28 min) (Robinson et al., 2018). On this same timescale, our dispersal rate results in approximately 1 unit of density of colonists on average entering a given host in 6 time steps, or 1.5 h (at equilibrium conditions). Again, this is roughly on the same scale as we see in zebrafish systems, where researchers have measured successful colonization of a population of hosts on the order of 45 min to 5 h (Robinson et al., 2018).

RESULTS

Inhospitable vs. Hospitable Matrix

Metacommunity Theory Expectations

As described above, we introduced a hospitable matrix into a patch dynamics model by allowing survival or growth in the matrix. From previous work (Levins and Culver, 1971) we expect there to be a range of parameter space where the dispersal specialist and the patch specialist can coexist. We also expect that an increase in the rate of disturbance will increase the advantage of the dispersal specialist relative to the patch specialist (Tilman et al., 1994). Finally, although patch number is not a well-studied parameter, we know from our own previous work (Burns et al., 2017) that increasing the number of patches can increase the relative advantage of the dispersal specialist. A simple argument that relies on the intermediate value theorem explains why, with only a single patch, the patch specialist by definition wins, and with multiple patches the dispersal specialist can persist, therefore there is an increasing function relating patch number to dispersal specialist persistence.

Effect of a Hospitable Matrix

The direct and global dispersal scenarios (**Figures 1A,B**) produce equivalent results (**Figures 2A,B**) and we will consider them together for the rest of the paper. Both of these models assume an

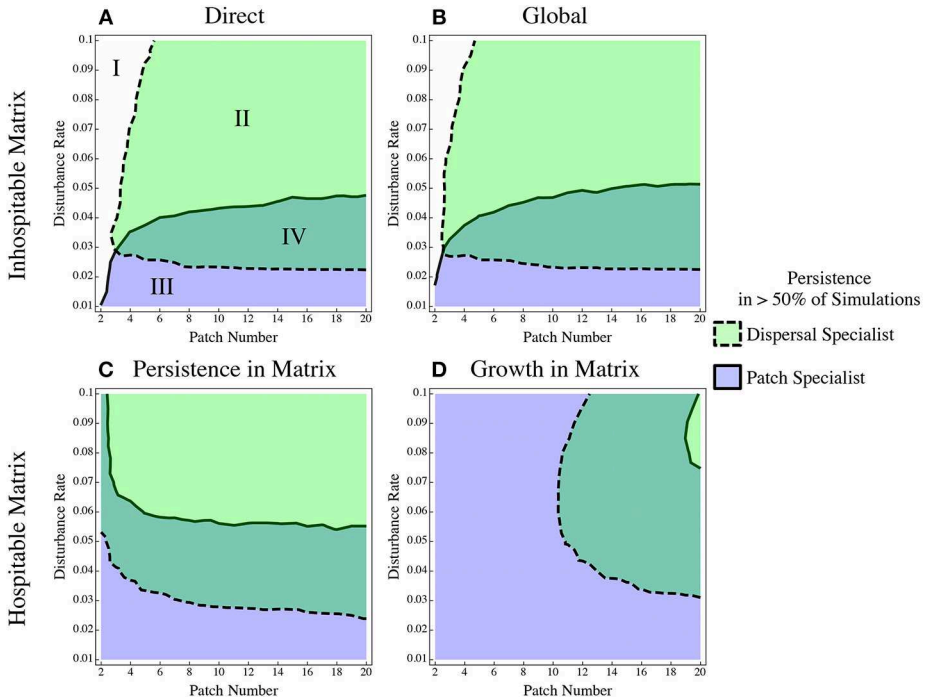


FIGURE 2 | The persistence of a dispersal specialist and patch specialist when in competition under different dispersal regimes. The four possibilities are delineated in the first panel: I Extinction, II Dispersal specialist wins, III Patch specialist wins, IV Coexistence. Two inhospitable matrix scenarios: **(A)** direct dispersal and **(B)** the Global dispersal model, and two hospitable matrix scenarios **(C)** persistence in the matrix and **(D)** growth in the matrix. The region borders the 50% chance of persistence cline, this contour is the only plotted one for simplicity, it is a very sharp change (see **Figure S4** for additional contour lines).

inhospitable matrix, and the global dispersal model is often used in patch dynamics models as a short-hand for direct dispersal models. With the chosen parameter values, there is a sizeable range of coexistence (**Figure 2A**, Region IV), as expected. We also see that increasing the disturbance rate increases the persistence of the dispersal specialist and decreases the persistence of the patch specialist. Finally, when there are very few patches and/or very high disturbance, both species go extinct. If all patches experience a disturbance effect at the same time or in quick succession, it is likely that the entire system will go extinct.

In the hospitable matrix scenarios, there are two main deviations from the inhospitable models. First, the region of extinction at low patch numbers disappears. The hospitable matrix provides a refuge that can maintain the population in the event of total extinction in the patches. The second main effect is that in both hospitable scenarios the dispersal specialist does worse than in the inhospitable matrix scenarios. The region of parameter space where it is able to coexist with or out-compete the patch specialist is smaller (**Figure 2C**). When the matrix allows growth, this effect is more pronounced (**Figure 2D**); the dispersal specialist loses out even when the disturbance rate is very high and can only out compete the patch specialist in a sliver of parameter space.

The dramatic shift in the dominance of the system is caused by the accumulation of patch specialists in the matrix (**Figure 3**). The patch specialist population in the matrix is bolstered by dispersal from patches that have not recently experienced a disturbance event. These patches have a high density of the patch specialist and nearly no dispersal specialists. Thus, even though the dispersal specialist delivers a larger proportion of its propagules to the next patch, it loses out when it comes to total biomass (**Figure 3**).

Trait Variation

Altering the Traits

To alter where in the dispersal process the dispersal specialist has an advantage over the patch specialist, we look at the three dispersal related traits d_{out}, d_{in}, and M. In the models described in the previous section, d_{out} and d_{in} were set so that they both conferred an equal advantage to the dispersal specialist, while M was set so that neither species had a growth advantage in the media. Now, we confer an advantage to the dispersal specialist in one of the traits at a time (**Figure 1D**). For the d_{out} and d_{in} traits, when the dispersal specialist has an advantage, it has a 6-fold advantage over the patch specialist, when there is no advantage, both the disperser and the competitor have the same trait value (either $d_{out} = 0.01$ or $d_{in} = 0.1$). By conferring the same relative advantage to each trait, we are able to directly compare the effect of specializing in one trait vs. the other. For the M trait, either the species have equal growth rates in the matrix (1 in the no growth or 1.2 in the low growth case) or the dispersal specialist has an M greater than that of the patch specialist.

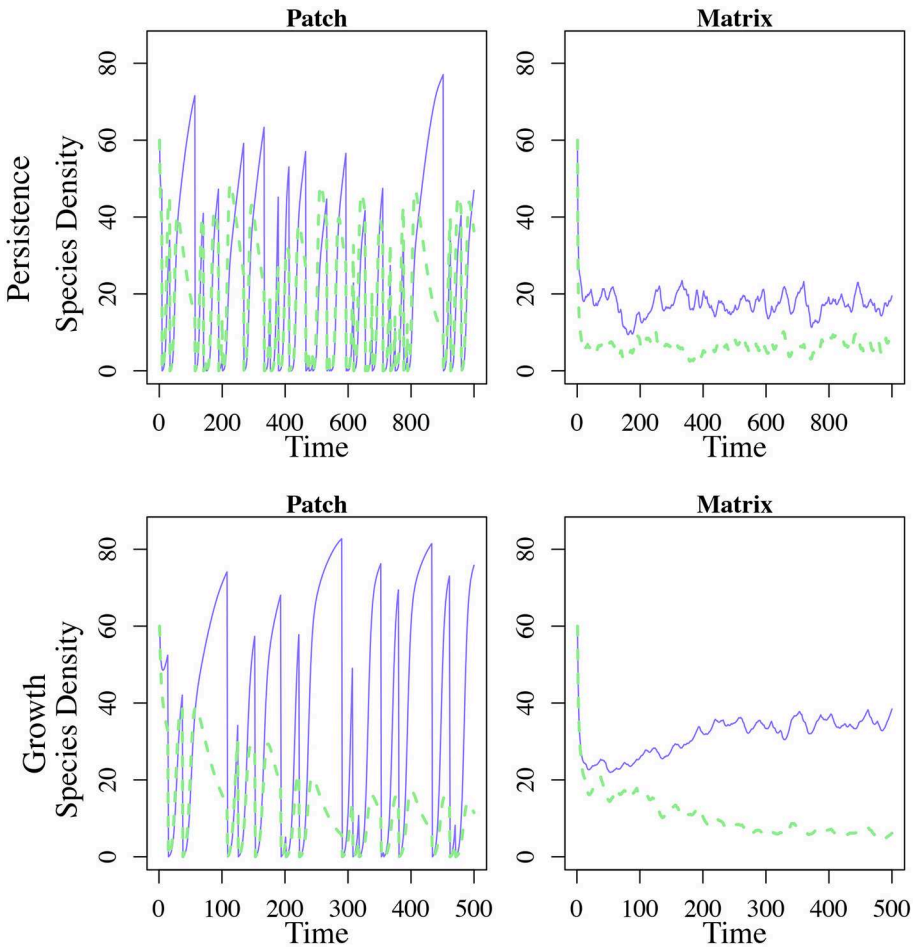


FIGURE 3 | Population dynamics of patch and matrix with 10 patches and 0.05 disturbance. Blue, solid lines indicate the patch specialist species while green dotted lines indicate the dispersal specialist. The first row is one patch and the matrix from the persistence in the matrix model while the second row is one patch and the matrix from the growth in the matrix scenario. Only one patch from each model is shown for simplicity.

d_{out} vs. d_{in}

With an inhospitable matrix, the effects of an advantage on the way in or way out were subtle. When the dispersal specialist had an advantage on the way in **Figure 4A**, it did somewhat better than when it had the advantage on the way out (**Figure 4B**). This may be due to the effect on the patch specialist: when colonists move from the patch into the inhospitable matrix, any colonists that don't make it back into the patch are lost from the system. So, when the dispersal advantage to the dispersal specialist is in the out trait, the patch specialist by definition retains more of its population in the patch, sending out fewer colonists, and losing less of its population to the inhospitable matrix. When the surrounding matrix is deadly, it makes sense either to keep your propagules close or to equip them with the ability to move through the matrix to the next patch.

The opposite occurs when the matrix is more hospitable. In the persistence model (**Figures 4C,D**), the dispersal specialist did relatively better when its advantage was applied to the out trait (**Figure 4C**). Its persistence was dramatically reduced when it held an advantage only in the in trait (**Figure 4D**). The population is no longer lost to the matrix in this model, and as shown with (**Figure 3**), the composition of the matrix can alter the composition of the colonists that get moved back into the patches. The effect is even more pronounced in the model that allows growth in the matrix (**Figures 4E,F**), When the disperser's only advantage is in the in trait, there is no region where it excludes the patch specialist (**Figure 4F**).

Growth Advantage in the Matrix

Because there is by definition no growth in the matrix for the direct and global models, we cannot compare this to the inhospitable matrix as we did with the in and out traits above. Instead, we look at how much of a growth advantage the dispersal specialist needs in the matrix to overcome the growth advantage of the patch specialist in the patch (**Figure 5**). The nature of the matrix and the number of patches both have an effect. If the matrix is very resource poor and has depressed growth rate overall, the dispersal specialist needs a larger relative

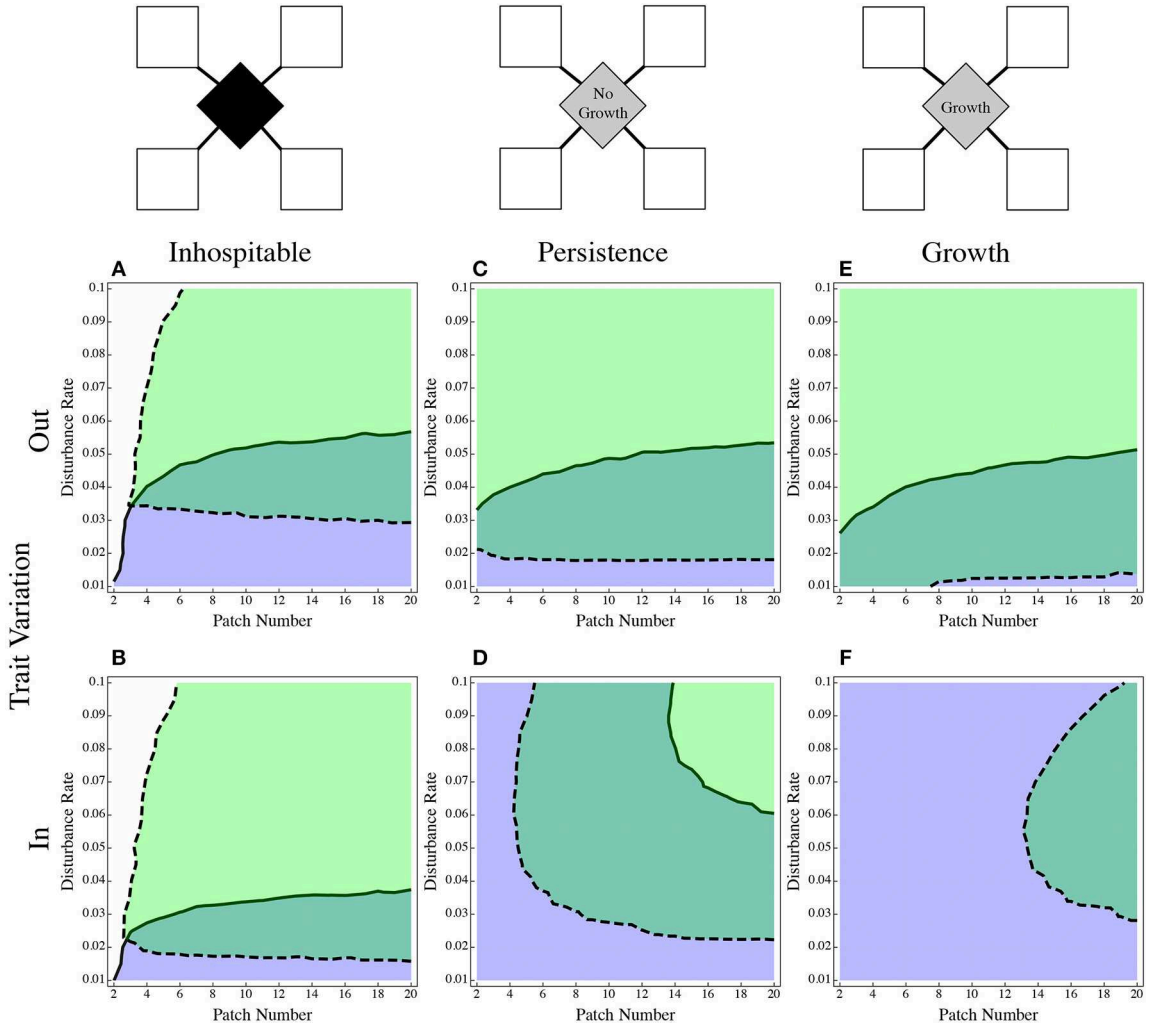


FIGURE 4 | The effect of dispersal advantage applied on the way in vs. on the way out. The direct dispersal case **(A,B)** shows that the advantage being applied on the way out **(A)** is less advantageous for the dispersal specialist than when applied on the way in **(B)**. The hospitable matrix scenarios **(C–F)** show the opposite effects, the range of the dispersal specialist is much smaller when the advantage is applied on the way into the patch **(D,F)** than it is on the way out of the patch **(C,E)**. The coloring is as in **Figure 1**.

advantage to persist, but if growth is relatively fast in the matrix, it does not need such a large advantage to compensate for the patches. The number of patches contributing to the matrix has a similar effect; if there are more patches, the population in the matrix becomes dominated by emigrants, requiring more of an advantage for the dispersal specialist in the matrix. The difference in growth rates examined translates from a 25% faster doubling time in the water (top row, **Figure 5**) to a doubling time that is roughly twice as long in the water than in the fish (bottom row, **Figure 5**). This range of growth rates is consistent with measures in the zebrafish microbiome system (J. Lebov, personal communication).

Matrix or Patch Heterogeneity?

It is possible that these effects are a result not of the matrix, but of simply introducing patch heterogeneity into the patch dynamics model. To rule this out, we re-ran the original set of models with dispersal advantage in both traits but instead of having a hospitable matrix, we simply added another patch to the direct dispersal model with the same properties (carrying capacity and growth rate) as the matrix (**Figure 6**). Either we had a patch with no growth that only collected and emitted colonists (**Figure 6B**), or a patch with the same growth rate as the matrix in the low growth model (**Figure 6C**). All patches and the matrix have the same carrying capacity (results from the direct dispersal (**Figure 6A**) and hospitable matrix (**Figures 6D,E**) included for comparison). Making the matrix into just another patch retains the effect of having a refuge from disturbance and from strong competition in the patches, but it changes the flow of dispersal. A single patch does not experience dispersal in the same way that the matrix does, since all immigrants must pass through the matrix; any difference between the two models (hospitable matrix and matrix-like patch) comes from a change in the dispersal pattern. The matrix-like patch model is similar to

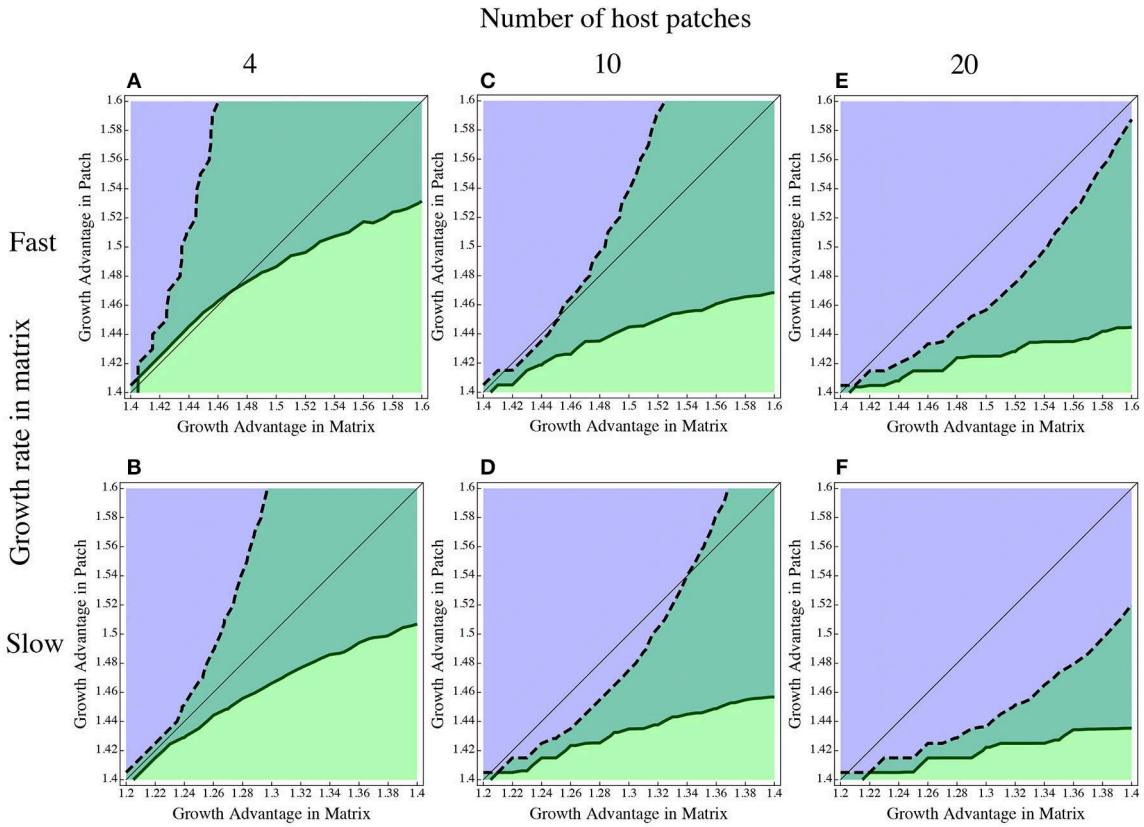


FIGURE 5 | Dispersal advantage through growth in the matrix. The growth rate of the dispersal specialist in the patch is fixed at 1.4 and the growth rate of the patch specialist in the patch is varied along the y-axis. The growth rate of the patch specialist is fixed in the matrix at either 1.4 [top row **(A,C,E)**] or 1.2 [bottom row **(B, D,F)**] while the dispersal specialist's matrix growth is varied along the x-axis. The bottom left corner of each graph has both competitors equal in both locations. The region above the thin black line indicates more relative advantage for the patch specialist in the patch and the region below that line gives the region of more relative advantage for the disperser in the matrix.

the hospitable matrix models in that the dispersal specialist has a decreased range of persistence, but the effect is much less pronounced.

DISCUSSION

Metacommunity Theory Ignores Hospitable Matrices

The dominant theoretical approaches treat dispersal in metacommunities as a direct process, assuming a totally inhospitable matrix between the patches. This assumption is likely incorrect for many ecological systems. For example, seeds can at least survive (in dormant form) outside their preferred habitat patch. This is especially true for host-microbiome systems where many of the resident species in the host can be found living and growing in the surrounding environment. This mismatch between assumption and reality is likely to lead to incorrect conclusions about the effects of dispersal between patches and about the life history strategies that species employ to move through their environment.

We use a model that explicitly tests the impact of a hospitable environment on metacommunity processes in a patch dynamic framework. Despite the current emphasis on investigating synthesis between the metacommunity archetypes (Leibold and Chase, 2018), a purely patch dynamics model is optimal here because it allows us to manipulate dispersal in relative isolation. We look both at the effect that the characteristics of the matrix have on the outcome of competition between a dispersal specialist and a patch specialist and the effect of differing dispersal strategies that the dispersal specialist could employ. In general we find that a hospitable matrix makes it more difficult to persist as a dispersal specialist relative to an inhospitable matrix. In addition, the optimal dispersal strategies are different between the two matrix types; in an inhospitable matrix, it is best to specialize on getting into a patch, while with a hospitable matrix it is better to specialize on getting out of a patch.

What Does a Hospitable Matrix Do?

In this model and in many real-world systems, a hospitable matrix acts as a reservoir for the species in the patches, removing them from the threat of extinction when a given

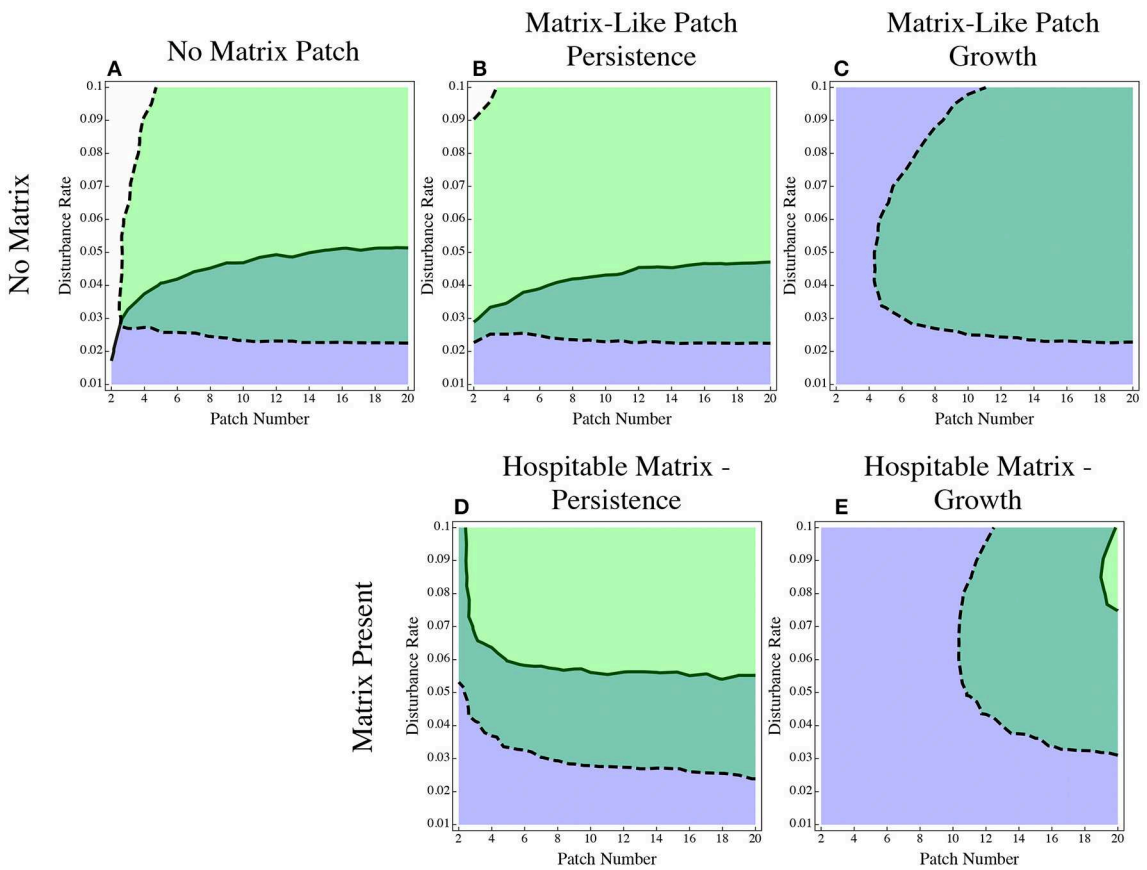


FIGURE 6 | The effect of a matrix-like patch. In **(A)** there is only direct dispersal between identical patches as before. In **(B)** an additional patch is added to the direct dispersal model with the same properties as the matrix with no growth. In **(C)** an additional patch is added to the direct dispersal model with the same properties as a matrix with low growth. **(D,E)** are reproduced from **Figure 1** for comparison. In **(D)** the matrix is the same as the matrix-like patch in **(B,E)** the matrix is the same as the matrix-like patch in **(C)**.

patch experiences disturbance. In any patchy environment where there is periodic disturbance, all populations within a patch are doomed; "dispersal is escape in space" (Levin et al., 1984). In the case of a hospitable environment, the "escape" is made by allowing individuals to leave a patch and move into the surrounding habitat. The hospitable matrix also introduces a time lag in the system: if a species goes extinct in all the patches it may still be present in the matrix at least temporarily (for an investigation of the effects of adding a time lag *within* a patch, see Wisnoski et al., 2019). A hospitable matrix also serves as a record of those communities that have come before. In host-microbe metacommunities, this property of the matrix has been used for such disparate applications as determining the previous inhabitants of a building (Hampton-Marcell et al., 2017) and designing optimal air flow in hospitals to cut down on hospital acquired infections (Arnold, 2014).

Community Effects

In general, a hospitable matrix makes it more difficult to be a dispersal specialist. The presence of an alternative patch, through which all dispersal flows, bolsters the dispersing populations of both species, decreasing the relative advantage of the dispersal specialist. The matrix population provides a stepping-stone for the patch specialist to gain early entrance into an empty patch, leading to an overall loss of dispersal advantage for the dispersal specialist. The dispersal strategy may also be more risky for a species in a hospitable matrix scenario, where there is a more complicated path to re-establishment in a new patch, decreasing the advantage of such a lifestyle. Thus, we might expect to see fewer "fugitive" species in systems with a hospitable matrix. The other main effect of a hospitable matrix is to provide a refuge against the stochastic extinction that occurs with direct dispersal at very low patch numbers. The hospitable matrix permits persistence and even coexistence with only a handful of patches where the direct dispersal model always eventually leads to total extinction.

Effect of Traits

How different life history strategies (reflected in dispersal traits) play out under different matrix conditions is indicative of the role

of the hospitable matrix. As discussed above, exiting the patch is sufficient to bolster populations against patch disturbance with a hospitable matrix. In the inhospitable matrix case, leaving the patch is insufficient to ensure safety. This difference is reflected in the different effects of dispersal traits between the two cases. When the matrix is inhospitable, the best way to be a dispersal specialist is to be efficient at getting into a patch, whereas in the hospitable matrix it is better to be good at getting out of the patch. It is easy to imagine why this is so; an advantage in getting out of the patch in the inhospitable case would put a species at risk of loss to the surrounding matrix, but in the hospitable case, where loss to the matrix translates into a larger matrix population to colonize a new patch, the opposite is true. The mechanism works through an enhanced advantage to the dispersal specialist and also a cost to the patch specialist. If the competitor is equally good at getting out of the patch, then it loses population to the inhospitable matrix that might have otherwise stayed in the patch where it is more fit. Conversely, in the hospitable matrix case, the patch specialist (by virtue of its high population in the patch) can overcome its dispersal disadvantage if it can build up a large enough population in the matrix. This buildup of population in the matrix is evident in the population dynamics (**Figure 3**).

Of course, it would be possible to configure the parameters in such a way that in a hospitable system, a sufficiently high advantage in d_{in} could produce the same overall fitness advantage to the dispersal specialist as a smaller advantage in d_{out}. The result still illustrates that specializing in getting out of the patch is a more effective strategy for the dispersal specialist in a hospitable matrix than specializing in getting in.

Trait Tradeoffs

A difference in the advantage conferred by alternative dispersal traits would be particularly dramatic if there is any sort of tradeoff between these two strategies. For example, previous work suggests that there is a negative correlation between planktonic behavior and clumping behavior among bacteria in the larval zebrafish gut, behaviors that may correspond to increased "in traits" and "out traits," respectively (Wiles et al., 2016; Robinson et al., 2018; Schlomann et al., 2018). The tradeoff between seed number and seed size in plants may also reflect a trade-off between "in traits" and "out traits"; small seeds are produced in greater numbers than larger seeds, allowing more of them to get out of the patch (Smith and Fretwell, 1974), while larger seeds are superior at establishing in a new patch, giving them an advantage at getting in Westoby et al. (1996). The offspring quality-quantity tradeoff more generally might be another instance of this kind of tradeoff (Einum and Fleming, 2000). Whether it is better to simply send as many propagules as possible out into the environment or equip them more properly for the journey might depend on the hospitableness or deadliness of the surrounding matrix.

More Than Patch Heterogeneity

The comparison between the hospitable matrix and heterogeneous patches makes clear that the effect of a hospitable matrix goes beyond merely adding patch heterogeneity. Although, as expected, adding another patch where the patch specialist can avoid extinction in a patch decreases the advantage of the dispersal specialist, the results are much less pronounced than for the hospitable matrix. The implication is that the organization of the metacommunity dispersal network matters. The position of the matrix as "between" all other patches is crucial to its effect. To distinguish a hospitable matrix from already well-studied patch heterogeneity (e.g., via species sorting or mass effects), some degree of mixing in the matrix is necessary. Otherwise, the system reduces to a spatial model with two habitat types and source-sink dynamics. The mixing could be in the matrix itself, for example water may be reasonably well-mixed, or it could be a result of patch movement, as with mobile animal hosts and their associated microbiomes. The observed effect of disadvantaging the dispersing species is likely to be strongest when the matrix is more homogeneously mixed. In aquatic systems this is more likely to be the case than in terrestrial systems. But even in terrestrial systems, if the patches themselves are mobile (e.g., animals moving around, humans entering and exiting a building) the effect might be more like an aquatic than a terrestrial mixing of the matrix. Thus, we might expect to see dispersers in a built environment or in an aquatic environment at a greater disadvantage than in an explicitly spatial environment (e.g., the microbiome of downed logs in a forest or in pitcher plants).

Implications for Host-Associated Communities

Hospitable matrices are likely the norm in host-associated communities. It has long been known that human diseases can reside in the environment; such environmental reservoirs are called "fomites" in the literature (Boone and Gerba, 2007). Less is known about non-pathogenic human associated microbes, but there is evidence that our surrounding environment can be an important conduit for microbial dispersal influencing the composition of our gut microbiomes (Ruiz-Calderon et al., 2016; Stagaman et al., 2018). Houseplants, soil, and the surrounding environment have been shown to be proximal sources of the skin microbiome (Vandegrift et al., 2019). Conversely, the microbial cloud emitted from the human inhabitants of a room remain viable for long enough to be used for forensic purposes (Metcalf et al., 2017). In non-human primate systems there is evidence of microbiome members surviving for extended periods of time in the environment between hosts (Tung et al., 2015). In aquatic systems, the environment is often the means by which colonists move from host to host; research in aquaculture has shown that composition of the gut microbiome of fish is a subset of the community found in the surrounding water and sediments, implying that the environment provides a continuous reservoir for the microbiome (Wu et al., 2012) and that the conditions of this reservoir affects the function of the microbiome (Vadstein, 2018). The role of the matrix as a reservoir is even more obvious in plant-associated fungal communities, where the soil can retain microbial signatures of

former plant microbiomes for up to 80 years after the plants have been removed (Bachelot et al., 2016). All in all, for host-microbiome systems, the hospitable matrix is the rule rather than the exception.

The hospitable matrix as a reservoir unites several concepts of species pools. Often, the species pool in ecological literature is envisioned as either the sum of all the species in all the patches (as is often the case in metacommunity models) or as some external entity (MacArthur and Wilson, 1963), uninfluenced by the events in the patches (Mittelbach and Schemske, 2015). The species pool in a system with a hospitable matrix does not reflect exactly the conditions of the patches (this is its role as a reservoir), but it is affected by colonists exiting those patches. The mix of independence and feedback between the species pool and the patches may need to be considered when applying methods to assess the neutrality of a system.

Because of the prevalence of hospitable matrices in host-microbiome systems, our results regarding traits may be particularly useful. In a system with a particularly hospitable matrix we would expect particularly transmissible microbes to specialize in getting out of the host. For example, many pathogens employ this strategy, getting themselves out of the host through sneezes and coughs. Microbes that specialize on transmission may invest more resources in surviving in the environment outside of the host; spore formation, or oxygen tolerance may be examples of this strategy. Movement through a less hospitable environment would require adaptations that ensure a quick arrival in the next host: sexual transmission that eliminates the need to persist in the environment or chemotaxis to quickly find a new host.

Manipulation of the matrix to alter the transmission of microbes is an important way that humans and other animals manipulate their microbiomes. Humans alter the transmission of pathogens through changing the ventilation and cleaning of our buildings, social insects imbue their nests with antimicrobial compounds (Turnbull et al., 2011), and birds build their nests out of antimicrobial plants (Ruiz-Castellano et al., 2016). Our results suggest that such matrix management can have different results for different species. Measures that decrease the hospitality of the environment may end up selecting for transmission specialists at the expense of host-specialist species. If it is the case that pathogenic bacteria tend to be better adapted for transmission than commensal strains that specialize in playing nice with the host, our efforts at cleaning and hygiene might be counterproductive.

Future Studies

We have only scratched the surface of the implications of matrix conditions for metacommunities. We highlight three important avenues for continuing research: evolutionary dynamics, patch heterogeneity, and experimental studies with model organisms. Our observation of differential advantages of different life history strategies under hospitable and inhospitable matrix conditions raises questions about how evolutionary dynamics would proceed in these systems. Our model incorporates traits but not trait change; what would an evolutionary stable dispersal strategy look like under different matrix types? Do we see differences in average seed mass and dispersal ability across landscapes with different matrix types? The possibility of tradeoffs between d_{out} and d_{in} could lead to drastically different evolutionary endpoints.

This study also limits itself to the patch dynamic archetype; what would the role of the matrix be in a system that also included patch heterogeneity? Here, the recent work by Wisnoski et al. (2019) might provide a clue. They find that dormancy in a patch increases the overall diversity of the system; a hospitable matrix (which induces its own kind of time lag) could have a similar effect. Interest in bridging the gaps between different archetypes has been surging in metacommunity research. This could be another interesting avenue to pursue.

As always, our theoretical results must be tested empirically. In plant and animal systems, the relevant real-world tests might involve attempting to quantify species ability to survive in the matrix, and perhaps manipulating that ability. Using host-microbiome systems with model organisms might be particularly fruitful. With model organisms in laboratory settings, it would be possible to dial up or down the hospitableness of the matrix, either through sterilization or fertilization of the housing conditions. The way in which microbes move between hosts could also be manipulated: For example, one could prevent direct dispersal between hosts by physically separating the individuals, but allowing microbes to move between habitats. One could also directly manipulate the relative importance of d_{out} and d_{in}, for example by adding microbes directly to the gut (e.g., via gavage).

Finally, because matrix management is so common in human systems, microbiome surveys of built environments might be an excellent source of real world data with which to test these ideas. If changing the matrix changes the composition of human microbiomes, we would expect to see a change in composition after changes in matrix properties (for example, before and after implementation of new cleaning procedures). We might also expect to see different microbiomes in people who live in more or less easily disinfected environments.

CONCLUSION

Metacommunity ecology has proven to be a useful framework for incorporating dispersal processes into our understanding of community assembly, but it has typically assumed that patches are separated by an inhospitable matrix. In this paper we expand the usefulness of the metacommunity framework to include systems where the matrix itself is a player. We find that there is a sizeable impact of the matrix on community outcomes, both in terms of coexistence and in terms of the most advantageous traits. It is becoming increasingly clear that the matrix cannot be excluded when considering host-microbiome metacommunities, but it is also likely that many plant and animal metacommunities do not meet the assumption of an inhospitable matrix. Thus, our expansion serves not only to broaden the application of metacommunity theory (e.g., to host-microbiome systems) but also to illuminate processes common to all of ecology.

DATA AVAILABILITY

The code used to generate the model for this study can be found on github https://github.com/bethmillery/hospitable-matrix.

AUTHOR CONTRIBUTIONS

EM conducted the modeling and wrote the first draft of the manuscript. BB contributed substantially to the intellectual development of the ideas and revisions.

FUNDING

This work was supported by a grant from The National Institutes of Health #215220 and 217100.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00276/full#supplementary-material

REFERENCES

Arnold, C. (2014). Rethinking sterile: the hospital microbiome. *Environ. Health Perspect.* 122, 1–6. doi: 10.1289/ehp.122-A182

Bachelot, B., Uriarte, M., Zimmerman, J. K., Thompson, J., Leff, J. W., Asiaii, A., et al. (2016). Long-lasting effects of land use history on soil fungal communities in second-growth tropical rain forests. *Ecol. Appl.* 26, 1881–1895. doi: 10.1890/15-1397.1

Boone, S. A., and Gerba, C. P. (2007). Significance of fomites in the spread of respiratory and enteric viral disease. *Appl. Environ Microb.* 73, 1687–1696. doi: 10.1128/AEM.02051-06

Burns, A. R., Miller, E. T., Agarwal, M., Rolig, A. S., Milligan-Myhre, K., Seredick, S., et al. (2017). Inter-host dispersal overwhelms host factors in the assembly of host-associated microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 114, 11181–11186. doi: 10.1073/pnas.1702511114

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* 31, 343–366. doi: 10.1146/annurev.ecolsys.31.1.343

Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J. M., and Relman, D. A. (2012). The application of ecological theory toward an understanding of the human microbiome. *Science* 336, 1255–1262. doi: 10.1126/science.1224203

Dini-Andreote, F., van Elsas, J. D., Olff, H., and Salles, J. F. (2018). Dispersal-competition tradeoff in microbiomes in the quest for land colonization. *Sci. Rep.* 8:9451. doi: 10.1038/s41598-018-27783-6

Einum, S., and Fleming, I. A. (2000). Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature* 405, 565–567. doi: 10.1038/35014600

Fournier, B., Mouquet, N., Leibold, M. A., and Gravel, D. (2016). An integrative framework of coexistence mechanisms in competitive metacommunities. *Ecography* 40, 630–641. doi: 10.1111/ecog.02137

Hampton-Marcell, J. T., Lopez, J. V., and Gilbert, J. A. (2017). The human microbiome: an emerging tool in forensics. *Microb. Biotechnol.* 10, 228–230. doi: 10.1111/1751-7915.12699

Kembel, S. W., Jones, E., Kline, J., Northcutt, D., Stenson, J., Womack, A. M., et al. (2012). Architectural design influences the diversity and structure of the built environment microbiome. *Isme J.* 6, 1469–1479. doi: 10.1038/ismej.2011.211

Leibold, M. A., and Chase, J. M. (2018). *Metacommunity Ecology*. Princeton University Press.

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. doi: 10.1111/j.1461-0248.2004.00608.x

Levin, S. A., Cohen, D., and Hastings, A. (1984). Dispersal strategies in patchy environments. *Theor. Popul. Biol.* 26, 165–191. doi: 10.1016/0040-5809(84)90028-5

Levins, R. (1979). Coexistence in a variable environment. *Am. Nat.* 114, 765–783. doi: 10.1086/283527

Levins, R., and Culver, D. (1971). Regional coexistence of species and competition between rare species. *Proc. Natl. Acad. Sci. U.S.A.* 33, 1246–1248. doi: 10.1073/pnas.68.6.1246

Logue, J. B., Mouquet, N., Peter, H., and Hillebrand, H. (2011). Empirical approaches to metacommunities: a review and comparison with theory. *Trends Ecol. Evol.* 26, 482–491. doi: 10.1016/j.tree.2011.04.009

MacArthur, R. H., and Wilson, E. O. (1963). An equilibrium theory of insular zoogeography. *Evolution* 17, 373–387. doi: 10.1111/j.1558-5646.1963.tb03295.x

Mendenhall, C. D., Karp, D. S., Meyer, C. F. J., Hadly, E. A., and Daily, G. C. (2014). Predicting biodiversity change and averting collapse in agricultural landscapes. *Nat. Commun.* 509, 213–217. doi: 10.1038/nature13139

Metcalf, J. L., Xu, Z. Z., Bouslimani, A., Dorrestein, P., Carter, D. O., and Knight, R. (2017). Microbiome tools for forensic science. *Trends Biotechnol.* 35, 814–823. doi: 10.1016/j.tibtech.2017.03.006

Miller, E. T., Svanbäck, R., and Bohannan, B. J. M. (2018). Microbiomes as metacommunities: understanding host-associated microbes through metacommunity ecology. *Trends Ecol. Evol.* 33, 926–935. doi: 10.1016/j.tree.2018.09.002

Mittelbach, G. G., and Schemske, D. W. (2015). Ecological and evolutionary perspectives on community assembly. *Trends Ecol. Evol.* 30, 241–247. doi: 10.1016/j.tree.2015.02.008

Mouquet, N., and Loreau, M. (2002). Coexistence in metacommunities: the regional similarity hypothesis. *Am. Nat.* 159, 420–426. doi: 10.1086/338996

Robinson, C. D., Klein, H. S., Murphy, K. D., Parthasarathy, R., Guillemin, K., and Bohannan, B. J. M. (2018). Experimental bacterial adaptation to the zebrafish gut reveals a primary role for immigration. *PLoS Biol.* 16:e2006893. doi: 10.1371/journal.pbio.2006893

Ruiz-Calderon, J. F., Cavallin, H., Song, S. J., Novoselac, A., Pericchi, L. R., Hernandez, J. N., et al. (2016). Walls talk: Microbial biogeography of homes spanning urbanization. *Sci. Adv.* 2:e1501061–e1501068. doi: 10.1126/sciadv.1501061

Ruiz-Castellano, C., Tomás, G., Ruiz-Rodríguez, M., Martín-Gálvez, D., and Soler, J. J. (2016). Nest material shapes eggs bacterial environment. *PLoS ONE* 11:e0148894. doi: 10.1371/journal.pone.0148894

Schlichting, H. E. J. (1960). The role of waterfowl in the dispersal of algae. *Trans. Am. Microsc. Soc.* 79, 160–166. doi: 10.2307/3224082

Schlomann, B. H., Wiles, T. J., Wall, E. S., Guillemin, K., and Parthasarathy, R. (2018). Bacterial cohesion predicts spatial distribution in the larval zebrafish intestine. *Biophys. J.* 115, 2271–2277. doi: 10.1016/j.bpj.2018.10.017

Shoemaker, L. G., and Melbourne, B. A. (2016). Linking metacommunity paradigms to spatial coexistence mechanisms. *Ecology* 97, 2436–2446. doi: 10.1002/ecy.1454

Smith, C., and Fretwell, S. (1974). The optimal balance between size and number of offspring. *Am. Nat.* 108, 499–506. doi: 10.1086/282929

Sokol, E. R., Brown, B. L., and Barrett, J. E. (2017). A simulation-based approach to understand how metacommunity characteristics influence emergent biodiversity patterns. *Oikos* 126, 732–737. doi: 10.1111/oik.03690

Stagaman, K., Cepon-Robins, T. J., Liebert, M. A., Gildner, T. E., Urlacher, S. S., Madimenos, F. C., et al. (2018). Market Integration Predicts human gut microbiome attributes across a gradient of economic development. *mSystems* 3:e00122–17. doi: 10.1128/mSystems.00122-17

Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., et al. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nat. Commun.* 562, 583–588. doi: 10.1038/s41586-018-0617-x

Tilman, D., May, R. M., Lehman, C. L., and Nowak, M. A. (1994). Habitat destruction and the extinction debt. *Nature* 371, 65–66. doi: 10.1038/371065a0

Tung, J., Barreiro, L. B., Burns, M. B., Grenier, J.-C., Lynch, J., Grieneisen, L. E., et al. (2015). Social networks predict gut microbiome composition in wild baboons. *Elife* 4:e05224. doi: 10.7554/eLife.05224

Turnbull, C., Hoggard, S., Gillings, M., Palmer, C., Stow, A., Beattie, D., et al. (2011). Antimicrobial strength increases with group size: implications for social evolution. *Biol. Lett.* 7, 249–252. doi: 10.1098/rsbl.2010.0719

Urban, M. C., Leibold, M. A., Amarasekare, P., De Meester, L., Gomulkiewicz, R., Hochberg, M., et al. (2008). The evolutionary ecology of metacommunities. *Trends Ecol. Evol.* 23, 311–317. doi: 10.1016/j.tree.2008.02.007

Vadstein, O. (2018). K-selection as microbial community management strategy: a method for improved viability of larvae in aquaculture. *Front. Microbiol.* 9:2730. doi: 10.3389/fmicb.2018.02730

Vandegrift, R., Fahimipour, A. K., Muscarella, M., Bateman, A. C., Van Den Wymelenberg, K., and Bohannan, B. J. M. (2019). *Moving Microbes: the Dynamics of Transient Microbial Residence on Human Skin*. Available online at: https://www.biorxiv.org/content/10.1101/586008v1 (accessed April 9, 2019).

Westoby, M., Leishman, M., and Lord, J. (1996). Comparative ecology of seed size and dispersal. *Philos. Trans. R. Soc. London B: Biol. Sci.* 351, 1309–1318. doi: 10.1098/rstb.1996.0114

Wiles, T. J., Jemielita, M., Baker, R. P., Schlomann, B. H., Logan, S. L., Ganz, J., et al. (2016). Host gut motility promotes competitive exclusion within a model intestinal microbiota. *PLoS Biol.* 14:e1002517. doi: 10.1371/journal.pbio.1002517

Wisnoski, N. I., Leibold, M. A., and Lennon, J. T. (2019). Dormancy in metacommunities. *Am. Nat.* 194. doi: 10.1086/704168

Wu, S., Wang, G., Angert, E. R., Wang, W., Li, W., and Zou, H. (2012). Composition, diversity, and origin of the bacterial community in grass carp intestine. *PLoS ONE* 7:e30440–e30411. doi: 10.1371/journal.pone.0030440

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 10 September 2019
doi: 10.3389/fmicb.2019.02095

A Metabarcoding Analysis of the Mycobiome of Wheat Ears Across a Topographically Heterogeneous Field

Gabriele Schiro[1], Pierluigi Colangeli[2] and Marina E. H. Müller[1,3]**

[1] Leibniz Centre for Agricultural Landscape Research, Research Area 1 "Landscape Functioning", Müncheberg, Germany, [2] University of Potsdam, Ecology and Ecosystem Modelling, Potsdam, Germany, [3] Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany

Plant associated microbial communities have recently received a lot of attention because thought to play a fundamental role in plant health and development. Focusing on cultivated crops, optimized farming practices must consider the role of these communities when aiming at reducing the impact of pathogens and increasing yields. Typical inhabitants of plant's phyllosphere are bacteria and microscopic fungi, some of them pathogenic for the plant and dangerous for the consumers, due to the production of toxins. In order to efficiently manage the microbiome, the natural drivers regulating community assembly must be clearly understood. In our study we investigated the within field variation of the phyllosphere mycobiome of wheat ears by metabarcoding of the fungal internal transcribed sequence 1 (ITS1). We selected a field characterized by a high topographic heterogeneity, which is reflected in differences in plant productivity and fitness across it. Samples were taken from 30 sampling points laid across the field where data-loggers were placed, measuring the productivity driven under canopy microclimate. The microclimatic conditions were tested as a source of potential environmental variance. Further independent spatial structures were tested using spatial eigenvector maps (MEMs). Results show considerable differences in the phyllosphere composition across the field. The local under canopy environmental conditions at each point were strong predictors of the community composition. Independent spatial effects given by the geographical position of the sampling points showed also a weaker but significant effect. Moreover we observed different spatial responses from different fungal phyla, with results resembling those described in studies done at a regional scale. This study is the first one to investigate the spatial variation of the phyllosphere mycobiome of a commercial crop within the same field. It contributes to the study of the epidemiology and community assembly dynamics of wheat phyllosphere fungi, showing how in-field community variations are the results of different environmental and spatial processes acting simultaneously. It also shows how heterogeneous fields are a smart and useful system to investigate the ecological mechanisms regulating plant microbiome composition.

Keywords: *Fusarium*, microclimate, canopy, fungal community, *Alternaria*, spatially induced variance

OPEN ACCESS

Edited by:
Philippe Vandenkoornhuyse, University of Rennes 1, France

Reviewed by:
Yun Chen, Zhejiang University, China
Ning Ling, Nanjing Agricultural University, China

****Correspondence:***
Gabriele Schiro
gabschiro@gmail.com;
schiro@zalf.de
Marina E. H. Müller
mmueller@zalf.de

Specialty section:
This article was submitted to Terrestrial Microbiology, a section of the journal Frontiers in Microbiology

Received: *17 April 2019*
Accepted: *26 August 2019*
Published: *10 September 2019*

Citation:
Schiro G, Colangeli P and Müller MEH (2019) A Metabarcoding Analysis of the Mycobiome of Wheat Ears Across a Topographically Heterogeneous Field. Front. Microbiol. 10:2095. doi: 10.3389/fmicb.2019.02095

INTRODUCTION

Nowadays, modern multi-omics techniques have shed new light on the associated plant microbiota and its relationship with the host plant, giving microbes a key role in plant development, stress resistance and disease prevention (Jansson and Baker, 2016; Berg et al., 2017). Plants and their associated microbes are now described as a "holobiont," a co-evolving unit based on symbiotic relationships between these two compartments, where microbial diversity and microbial interactions play a fundamental role in keeping the host plant healthy and productive (Vandenkoornhuyse et al., 2015; Berg et al., 2017). With a focus on cultivated plants, the need for a clearer picture of the dynamics shaping the community composition of the most common commercial crops has clearly emerged. Starting from the development of bio-control strategies, where non-pathogenic microbes are used as antagonists to pathogens, up to a more holistic view of breeding microbe-optimized plants, the microbial community of many economically important crops is nowadays being subject of studies (Singh and Trivedi, 2017; Syed Ab Rahman et al., 2018).

Among these crops is wheat (*Triticum aestivum*), which importance as worldwide staple food is out of doubt. Wheat production is threatened by various diseases, among those; fungi pose a serious menace (Figueroa et al., 2018). Common fungal diseases include blotches, caused by mainly by fungi of the family *Septoria* spp., rusts, usually associated to the genus *Puccinia* spp. or blights, like Fusarium head blight (FHB) caused by species complex involving up to 19 species, mainly belonging to genus *Fusarium* (Xu and Nicholson, 2009; Bockus et al., 2010). FHB fungi infect the ear, the grain bearing part of the plant. Along with pathogens, wheat ears are colonized by a wide variety of other fungi, considered less-pathogenic or non-pathogenic, such as *Alternaria* spp., *Cladosporium* spp. or *Epicoccum* spp. (Legard et al., 1994; Nicolaisen et al., 2014; Bankina et al., 2017). From an agricultural perspective, these phyllosphere organisms are of concern due to the caused productivity loss and for food security. Certain fungi, such as *Fusarium* or *Alternaria*, synthesize mycotoxins, chemical compounds with toxic effects for the end consumers (Bottalico and Perrone, 2002; Ostry, 2008; Vučković et al., 2012).

Studying the community assembly is useful for the manipulation of the phyllosphere community in order to control pathogenic populations. Previous studies have shown good potential in suppressing pathogens through the inoculation of antagonistic strains of fungi or bacteria (Schisler et al., 2002; Xue et al., 2014; Baffoni et al., 2015; Palazzini et al., 2016; El-Gremi et al., 2017). Fungal interactions seem to play a role also in causing differences in diseases symptoms and effects; e.g., mycotoxin accumulation has been shown to be influenced by the co-cultivation of different fungi in the laboratory (Korn et al., 2014; Siou et al., 2015). To further develop such concepts, it is important to understand which factors naturally influence the phyllosphere community. Such research should be contextualized in relation to potential variations that different environmental conditions, plant genotypes, physiologies, or agricultural practices could cause on the community composition. An efficient development of biocontrol strategies must start from a clear understanding of the community assembly dynamics, where the factors influencing the community variations are clearly individuated and accounted for their effects.

Recent developments in next generation DNA sequencing has emerged as a powerful tool for microbial ecologists, who started applying metabarcoding techniques to investigate the community associated to many organisms (Abdelfattah et al., 2018). To our knowledge, the first study applying such method on wheat was published in 2014 and analyzed the mycobiome of 90 wheat grain samples collected all over Denmark (Nicolaisen et al., 2014). The authors have found a "core" of operational taxonomic units representing 99% of all sequences with significant co-existence patterns among them. Studies from Karlsson et al. (2014, 2017) examined the effect of fungicide application and managing practices (organic and traditional agriculture) of samples of wheat leaves coming from different fields in Sweden. Sapkota et al. (2015), observed the effects of fungicide application host plant genotypes on the community composition. Hertz et al. (2016) observed the evolution of the mycobiome of wheat ears, along a timeline of ear development. Other investigations examined both the fungal and the bacterial community on different plants compartments. Granzow et al. (2017) examined the variation of the communities in a pot experiment simulating different crop regimes. Gdanetz and Trail (2017) used field samples, exploring the influence of field management practices on the wheat microbiome. All these studies observed variation in the microbial community, indicating how complex are the dynamics influencing the phyllosphere composition, as being affected by multiple factors simultaneously.

Many studies have also addressed the in-field epidemiology of important fungal pathogens. Taking *Fusarium* as an example, plenty of data is available with a focus on its spore dispersal and aerobiology (Del Ponte et al., 2003; Paul et al., 2004; Keller et al., 2014; Schiro et al., 2018). Nevertheless, to our knowledge, no study has so far investigated the spatial variation of the phyllosphere communities within a single field. Such an investigation could reveal spatially dependent structures hiding behind the mechanism of phyllosphere community assembly. Typically, spatial structures of ecological communities can be related to two fundamental processes: first, community variations could originate by differences in the environment across the sampled area. In this case, the spatial variation is told to be "induced" because the environment influences the community structure at each point. Secondly, spatial structures can be created by biotic processes generated by the community itself, such as dispersal and competition dynamics: These processes are observable through the analysis of the spatial autocorrelation among neighbor points; with closer points having a more similar community composition than those far apart. We will refer to it as "un-induced" spatial variance (Dray et al., 2006; Soininen, 2016).

In our study we investigated the spatial structure of the ear fungal community across a topographically heterogeneous wheat field. This approach allows us to reduce the number of potentially influential variables (such as plant variety and management practices), while relying on in-field differences related to the topographical diversity of the field as source of

induced spatial variance. The heterogeneous topography, with hilltops and depressions, causes differences in soil type, soil moisture and plant fitness, which reflect in a spatial variations of productivity. Under-canopy microclimate (air temperature, humidity and soil moisture) is strongly connected to in-field productivity, since more biomass is responsible for more canopy cover effect (as explained later in the results section). We used this sampling method in previous studies, where we observed the variation in mycotoxins, spore deposition and genetic abundance of the genera *Fusarium* and *Alternaria* to be related to our explanatory microclimatic variables (Müller and Korn, 2013; Schiro et al., 2018, 2019). Moreover, due to the fairly regular sampling design, a single field represents a good system to test for the effect of the un-induced spatial variance, or in other words, to detect spatially explicit distributional patterns independent of the environmental variables considered.

We collected samples from 30 points scattered across a wheat field, selected for its high heterogeneity, situated in the north east of Germany. Sampling was conducted in summer 2017, 2 weeks before harvest. Via metabarcoding of the fungal internal transcribed region 1 (ITS1), we obtained a picture of the ear fungal community at each point. We here provide for the first time for a quantification of the variations of the ear phyllosphere mycobiome within the same commercially cultivated field, shedding light on its in-field biogeography. We believe that future developments of this approach will be useful for investigating how dispersal and colonization dynamics influence community assembly.

MATERIALS AND METHODS

Field Work

The selected field was already used in a previous work (Schiro et al., 2018). A topographically heterogeneous field located within the AgroScapeLab Quillow (Agricultural Landscape Laboratory Quillow[1]), precisely in the proximity of the village of "Raakow, Brandenburg) in the North eastern German lowlands. The field was selected for its pronounced topographical heterogeneity and for the differences in landscape elements surrounding it. The field bordered with a forest at its northern edge, with an uncultivated meadow at its western edge, with an unpaved road and another cultivated field at its southern edge and a cultivated field at its eastern edge. The two fields bordering at the southern and eastern edge were also cultivated with wheat. The study site features small scale topographic variations, as a result of differences in Pleistocene glacial landforms (**Figure 1**). The field was cultivated for commercial purposes and samples were taken in agreement with the farmer. The wheat cultivar "Julius" (susceptibility to FHB is "5" in a scale from 1 to 9 (Federal Office of Plant Varieties, 2017) was grown, with maize as preceding crop. A conservation tillage method with disc cultivator less than 0.15 m deep was used. Further details on field management are presented in **Supplementary Table S1**. Weather data was measured by the climatic station located in the research station "Dedelow" of the Leibnitz Centre for Agricultural Landscape Research (ZALF), situated circa six km away from the research field.

Thirty sampling points were laid across the field; at each of them a set of microclimatic sensors was positioned. Sensors measured the following parameters: air temperature and humidity, soil water content and leaf wetness index (LWI). Sensors consisted of a data logger "HOBO H21-USB" recording every hour the values measured by air temperature/humidity sensor "S-THB-M002" mounted in a solar radiation shield "RS3", a soil humidity sensor "S-SMD-M005," and a leaf wetness sensor "S-LWA-M003," all of them provided by Onset Computer Corporation (Bourne, MA, USA). Air temperature/humidity sensor and LWI were measured at a height of 30 cm from the ground. The experiment took place in June and July 2017. The microclimatic stations started to record values 3 weeks before the sampling date (Zadoks scale $\pm$ 73 until 90), recording a measurement every hour. At each point, in the square meter around it, the height of 15 random plants was measured. The average height per point was then used for further analysis. At each point, 15 ears were randomly picked from the plants at growth stage $\pm$90 (Zadoks et al., 1974). The ears were placed in clean paper bags and transported to the laboratory.

DNA Extraction

DNA extraction followed the protocol already used in Schiro et al. (2019). The 15 collected ears per each point were dried at 60°C for 48 h. Once dried, they were milled using a laboratory ball-mill MM200 (Retsch, Haan, Germany) at 1000 rpm for 45 s. The milled material was carefully mixed. 200 mg of milled plant material were put in a centrifuge tube with 100 μg proteinase K (article nr. 7528.2, Carl Roth, Karlsruhe, Germany) and 1.2 mL CTAB precipitation buffer [20 g L^{-1} CTAB (article no. 9161.3, Carl Roth), 1.4 mol L^{-1} NaCl (article no. 33614, Merck, Darmstadt, Germany), 0.1 mol L^{-1} TRIS (article no. 37180, Serva, Heidelberg, Germany) and 20 mmol L^{-1} Na2EDTA (article no. 8043, Carl Roth)] and incubated in an incubator "Enviro-genie" (Scientific Industries Inc., Bohemia, NY, United States), overnight at 65°C, with 0.5 rotation s s^{-1}. After centrifugation at 10,000 g for 10 min, the supernatant was transferred to another tube and with 400 μL of chloroform (article nr. 102445, Merck). Samples were hand shaken for 30 s and then centrifuged at 12,000 g for 10 min. Circa 600 μL of the superior phase were transferred into a tube, where a double volume of CTAB precipitation buffer was added. Tubes were left resting for 1 h at room temperature and before being centrifuged at 12,000 g for 10 min. After centrifugation, the supernatant was discharged and the pellet resuspended in 350 μL NaCl 1.2 mol L^{-1}, 400 μL of chloroform were added and samples were softly hand shaken for 30 s. The superior phase was transferred to a new tube and 300 μL 4°C cold isopropanol (article no. 109634, Merck) were added. Samples were incubated at 4°C for 20 min before being centrifuged for 15 min at 12,000 g. Supernatant was discharged and the pellets were additionally washed with 500 μL 70% ethanol solution (article nr. 111727, Merck). After centrifugation for 15 min at 12,000 × g, ethanol was discharged and samples were dried with a "Speedvac DNA 110" (Thermo Fisher Scientific, Waltham, MA, United States). Pellets were

[1]www.biomove.org/about-biomove/study_area/

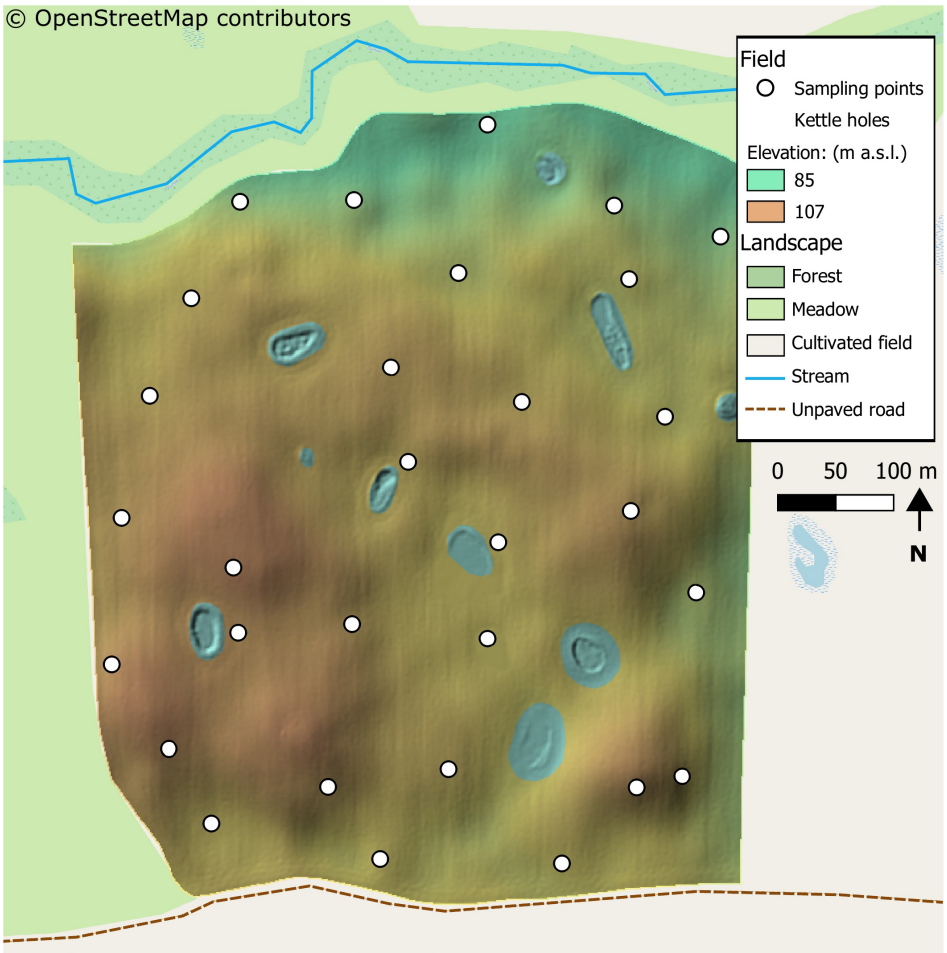


FIGURE 1 | Map of the sampled field and immediate surroundings. Within the field, a digital elevation model is shown with the resolution of one meter. The landscape features around the field are also shown, as described in the legend. The landscape details are obtained from OpenStreetMap contributors (2017). Noticeable is the heterogeneity of the field, with many hilltops depressions and kettle-holes across it. The field had also heterogeneous surroundings, with a meadow, a small river (or stream) and an unpaved road on its sides.

dissolved in 100 μL distilled sterile water and stored at −18°C until further analysis.

PCR Amplification and Amplicon Sequencing

PCR amplification and amplicon sequencing were performed by LGC Genomics (Berlin, Germany). The PCRs included about 5 ng of DNA extract, 15 pmol of each forward primer ITS1F 5′-NNNNNNNNNNTCTTGGTCATTTAGAGGAAGTAA and reverse primer ITS2R 5′-NNNNNNNNNNTGCTGCGTTCTTC ATCGATGC in 20 uL volume of 1x MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline) and 2 μl of BioStabII PCR Enhancer (Sigma). For each sample, the forward and reverse primers had the same 10-nt barcode sequence. PCRs were carried out for 35 cycles using the following parameters: 2 min 96°C pre-denaturation; 96°C for 15 s, 50°C for 30 s, 70°C for 90 s. DNA concentration of amplicons of interest was determined by gel electrophoresis. About 20 ng amplicon DNA of each sample were pooled for up to 48 samples carrying different barcodes. The amplicon pools were purified with one volume AMPure XP beads (Agencourt) to remove primer dimers and other small mispriming products, followed by an additional purification on MinElute columns (Qiagen). About 100 ng of each purified amplicon pool DNA was used to construct Illumina libraries using the Ovation Rapid DR Multiplex System 1-96 (NuGEN). Illumina libraries were pooled and size selected by preparative gel electrophoresis. Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina).

Bioinformatics

Bioinformatics analyses were performed in R software (R Core Team, 2017). Sequences have been deposited in the NCBI Sequence Read Archive, with the BioProject ID "PRJNA517107." Reads have been previously demultiplexed and barcodes removed by the sequencing provider. A clustering-free Divisive Amplicon Denoising Algorithm (DADA2) was used. This algorithm infers

error models which then use to derive amplicon sequencing variants (ASVs). ASVs are not based on arbitrary thresholds, as in the case of defining different operational taxonomic units, therefore can detect differences in sequences down to a definition of a single nucleotide (Callahan et al., 2016, 2017). Primers were removed using the Unix command line tool cutadapt (Martin, 2011). We truncated the reads at the first quality score lower or equal to two. As output, an ASV count table, which reported the number of times each ASV has been counted per point, was produced. As **Supplementary Material**, the R script used as pipeline is provided. Taxonomy was assigned using the function within the DADA2 R package "assignTaxonomy" and the Version 01.12.2017 of the UNITE general FASTA release (UNITE Community, 2017). For the 20 most abundant ASVs, a blast search in the gen-bank database was also performed (Benson, 2004). Uncultured results were not considered as well as singletons. Considering these parameters, the affiliation was given to the lowest possible level.

Statistical Analyses

Statistics was also performed using the software R (R Core Team, 2017). Due to technical problems with the data loggers, which went off during the experiment, two sampling points were excluded from the analyses. The hourly recorded values from the data loggers were averaged and scaled (with average as zero and standard deviation as unit) prior to analysis. To avoid multicollinearity between the explanatory variables, a correlation matrix was used to exclude these strongly correlated (Pearson $r \leq 0.9$). The ASV count table was Hellinger transformed; distance-based redundancy analysis (db-RDA) was performed to test the relationship between community variation represented as a Bray-Curtis dissimilarity matrix and environmental variables (Legendre and Gallagher, 2001; Borcard et al., 2018). Automatic stepwise model building for constrained ordination methods was used to select the variables; model building was run in forward direction. These operations were done using the "vegan" package in R (Oksanen et al., 2018). Spatial data was handled in R with the package "sp." (Pebesma and Bivand, 2005; Bivand et al., 2013). A spatial weighting matrix was constructed using the R package "spdep" (Bivand and Piras, 2015), row standardized (sums over all links to n), with points closer than 125 m as neighbors. This threshold was selected because it provided is the first value (at 5 m resolution), able to connect all sampling points with at least one neighbor. Moran's Eigenvector Maps (MEMs) were generated with the package "adespatial" (Dray et al., 2018). To select statistically significant MEMs, an automatic stepwise selection method as described before was used. To partition the variation of the community data between the two explanatory matrixes we used the fuction varpart of the package "vegan" (Oksanen et al., 2018) while to test their significance, a type II Anova analysis of variance was used. A negative adjusted R squared value (−0.04) for the shared fraction appeared. We corrected the negative value according to Legendre et al. (2012) using proportional apportioning. For clustering, the counting of the 20 most abundant ASVs at the different sampling points was used. Values were scaled in order to avoid biases given by the highly different abundances among ASVs. Clustering was done using Ward method using a dissimilarity matrix of Euclidean distances. The optimal number of clusters was calculated with an average silhouette method, using the package "factoextra" (Kassambara and Mundt, 2017). In order to test the effect of the two previously selected variables on the 20 most abundant ASVs, an RDA was used. Relative abundances were also in this case standardized in order to obtain a more balanced ordination. The R script for the analysis is attached as **Supplementary Material**.

RESULTS

Overview of the Climatic and Microclimatic Measurements

Summer 2017 has been humid and rainy in the region of our study. In the month of June and July (when the study took place), we recorded a total rainfall of 141.1 and 129 mm, respectively. That is above the average for the region of Brandenburg, for which for the years between 1950 and 2016 has values of 61.8 mm in June and 68.2 mm in July (Deutscher Wetterdienst, Germany). An overview of the microclimatic data recorded within the field canopy is represented in the **Supplementary Figure S2**. **Figure 2** shows how all the microclimatic conditions, besides LWI, are tightly interconnected. In **Figure 2E**, a correlation matrix of the microclimatic variables is shown. Air humidity and temperature show opposite trends, while soil humidity is less but significantly correlated to the other variables, but still showing significant correlations. Plant height is used as an indicator of field productivity. These values are explainable by considering the position of the microclimatic stations that were placed 30 cm above the ground under the plant canopy. At this height, plants shaded the sensors. Points with a higher productivity produce a higher shading effect, therefore reducing the average temperature and increasing air-humidity, while in less productive points the opposite trends happen. The results of microclimatic measurements show how air temperature, air humidity and plant height are tightly connected, with correlation coefficients same or higher than 0.9. Surprisingly, LWI does not show any correlations to the other measured parameters.

Sequencing Results and Taxonomic Affiliation

The ITS1 rRNA gene sequencing of all samples together generated 1,081,376 merged reads after quality checks, which resulted in 188 ASVs. Rarefaction curves showed that, for each sample, the method was adequate to sample the local diversity (**Supplementary Figure S3**). Of the 1,081,376 reads, 89% were classified as ascomycetes, 11% as basidiomycetes (the rest 0.02% were unclassified). The 188 ASVs were classified into 22 genera. In **Figure 3A**, the amount of detected ASVs per sampling point is represented on the plot map. On average, 43.2 ASVs were detected per sampling point. The point with the lowest number of detected ASVs had 30 ASVs, the one with the highest count had 63. The 20 most abundant ASVs counted for more than 95% of the total counts. Their taxonomic affiliation is reported in **Table 1**, with the two methods tested.

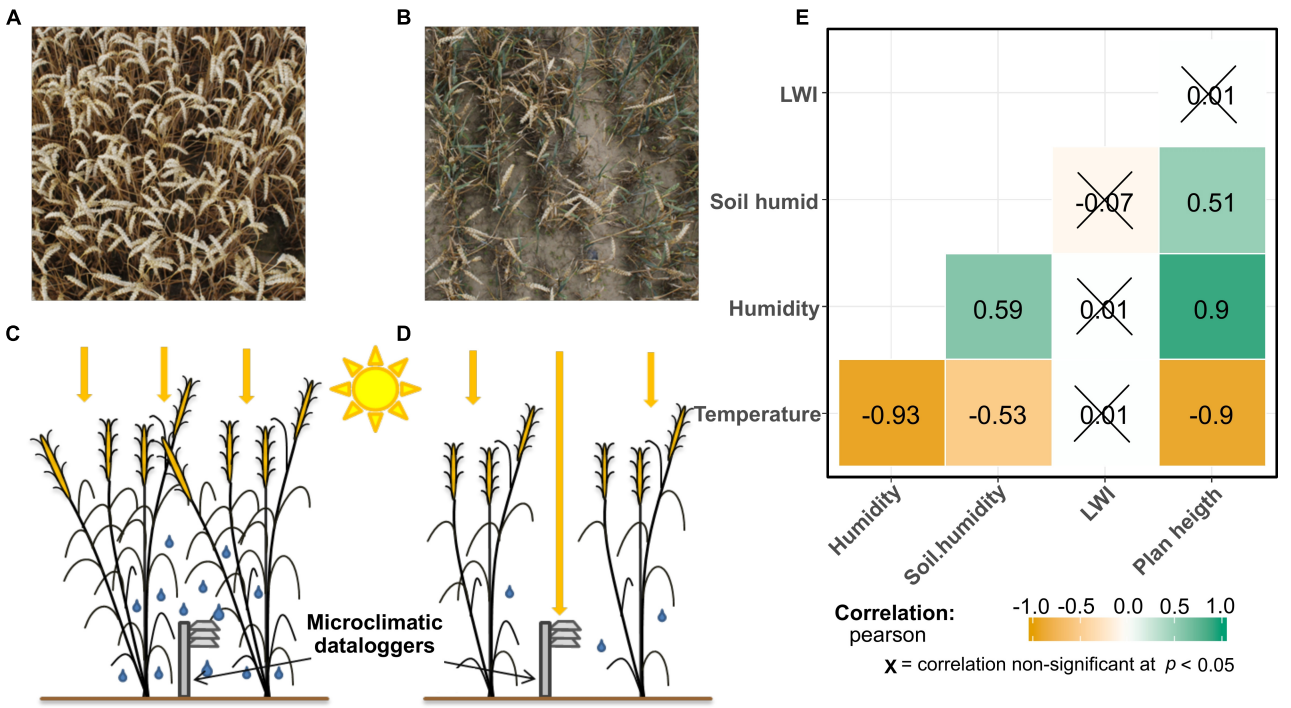


FIGURE 2 | In panel **(A)** a bird view of a field point with high plant productivity, while **(B)** shows a point with a lower plant productivity. In panel **(C,D)**, a graphical representation of the canopy effect influencing the microclimatic data-loggers is shown. In more productive points **(C)**, the sunrays do not penetrate until the data-loggers, therefore the measured microclimate is colder and more humid, compared to less productive points **(D)**. Panel **(E)** shows a correlation matrix of the environmental variables. The numbers represent the Pearson coefficient (p) between them. Plotted using the package "ggstatsplot" in R (Patil, 2018).

The two methods to assign taxonomy produced concordant results, besides for the 2nd most abundant ASV, which was assigned by the UNITE database to "*Mycosphaerella tassiana*," whereas, according to the blast on the genbank database, the sequence was affiliated to *Cladosporium* sp. In other cases, different species were assigned, but the genus was the same. For further analysis, we kept the assignments given by the GenBank blast search. *Cladosporium* was often reported as common colonizer of wheat ears, while *Mycosphaerella* are rather know to colonize ear leaves. The most abundant ASV was assigned to *Fusarium* sp., with 41% of relative abundance. All the others all showed a much lower abundance. The average composition of the whole field and the composition of the three hottest and warmest points are shown in **Figure 3B**. As we can see, in the three coldest points, the genus *Fusarium* and *Monographella* show a higher abundance compared to the average and the tree warmest points, whereas in the warmest points, *Cladosporium* and *Alternaria* represent the biggest fraction of the community.

A clustering analysis was run to identify co-occurrence patterns of the 20 most abundant ASVs (**Figure 3C**). The clustering is based on the spatial distribution of the ASVs, therefore, ASVs showing a similar distribution, are clustered together. In the first cluster, four of the five *Alternaria* are present, together with *Epicoccum*. In cluster 2, yeasts (*Vishniacozyma* and *Sporobolomyces*) and filamentous fungi, specifically *Cladosporium*, *Neoascochyta* and *Ascochyta*. Cluster 3 has the genera *Fusarium* and *Monograhella*, with one *Alternaria* ASV also present.

The Effect of Space

The raw ASV table was used as a base to calculate a Bray Curtis dissimilarity matrix. From the whole explanatory variables collected (air temperature, air humidity, soil humidity, plant height, and LWI), only air temperature, soil humidity, and LWI were kept for further analysis. Air humidity and plant height were dropped due to the high correlation coefficient they showed with air temperature, to avoid multicollinearity. These variables were tested as explanatory variables in a db-RDA using a Bray Curtis similarity matrix among point as response variable. Stepwise model building selected only temperature as explanatory variable, the spatial distribution of temperature in the field is represented in **Figure 4A**. To test for un-induced spatial variance, a Moran's eigenvector maps (MEMs) based spatial analysis found one map showing a statistically significant correlation to our dataset. The map shows a positive spatial autocorrelation, therefore it was accepted as an explanatory spatial pattern. A total model was then built with these explanatory variables. Only one MEM, represented in **Figure 4B**, together with temperature, showed a significant effect in the final model. Variation partitioning was used to distinguish the explanatory power of the two variables identified the results present in **Figure 4C**. Both variables showed a statistically significant effect, with temperature explaining 25% of the variance ($F = 11.10$, $p = 0.001$) and MEM explaining 15%

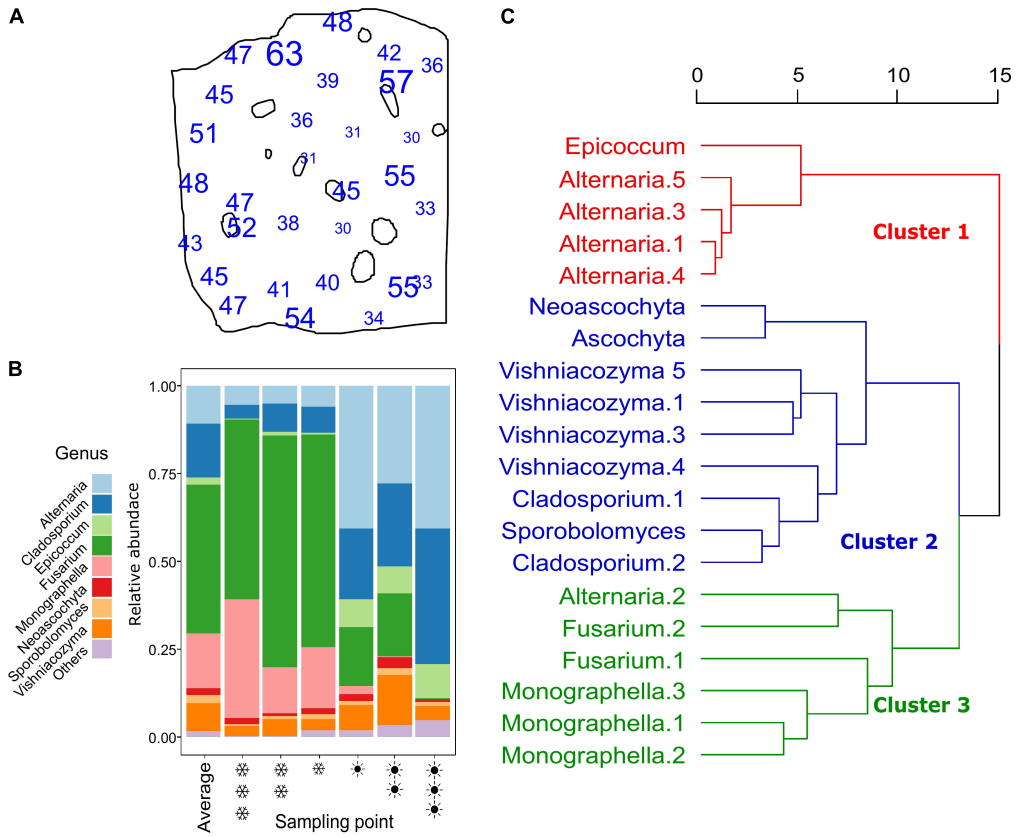


FIGURE 3 | Panel **(A)** shows the number of different ASVs counted at each point. Panel **(B)** shows stacked barplots representing the community composition, where different colors symbolize different genera (only genera with a total abundance higher than 0.5% are shown, the remaining showed as "others"). The coldest point is represented by three ❄ (snowflake) symbols, while the hottest by three ☀ (sun). In panel **(C)**, a dendrogram representing the hierarchical cluster analysis obtained using Ward on a dissimilarity matrix of Euclidean distances of the 20 most abundant ASVs. The dendrogram is divided in three clusters, as suggested by the silhouette analysis. The three clusters are shown in different colors.

of the variance ($F = 5.7$, $p = 0.001$). Variables did not show any joint effect, therefore not showing any multicollinearity.

The RDA biplot shows the relationships between the different ASVs and the two significant explanatory variables (**Figure 5**). In **Figure 5A**, ASVs respond differently to the two variables. Fusarium.1 is the ASV showing the strongest negative correlation to temperature ($R = -0.67$, $p < 0.0001$, **Figure 5B**), and positive to MEM2 ($R = 0.32$, $p = 0.01$, **Figure 5C**). The strongest positive correlation to temperature was scored by Epicoccum ($R = 0.70$, $p < 0.0001$, **Figure 5D**), while the strongest negative correlation to MEM2 was given by Vishniacozyma.3 ($R = -0.63$, $p < 0.0001$, **Figure 5E**). Generally the 20 most abundant ASVs responded, beside Fusarium.1, negatively to the MEM map selected.

DISCUSSION

Through metabarcoding of the ITS1 segment, we showed how the phyllosphere fungal community can change within the same field due to spatial structures and differences in productivity. We identified 188 ASVs, nevertheless the 20 most abundant ones accounted for more than 95% of all counts. This confirms the results of previous studies done at a regional spatial scale, which reported a "core" mycobiome accompanied by rare strains only detected occasionally at lower abundances (Nicolaisen et al., 2014). Similar results were also observed on studies targeting cereal leaves, where a core of operational taxonomic units, ubiquitously present at high proportion was described (Karlsson et al., 2014, 2017; Sapkota et al., 2015, 2017). *Fusarium* dominated the community accounting for more than 40% of the total counts. The year 2017 was very rainy and conservation tillage was applied with wheat being cultivated after maize; factors known to promote the development of Fusarium head blight (Xu, 2003; Bateman et al., 2007; Manstretta and Rossi, 2015; Müller et al., 2016). If the field work was conducted in another year in a field with a different rotational history, the community composition would probably be consistently different.

The results of the db-RDA show how under canopy temperature, used as explanatory variable, is able to explain 25% of the total variance. Temperature measured at each point is directly connected to plant productivity, since the sensors were placed below the canopy cover, and measurements were strongly influenced by the abundance of leaves above the sensors.

TABLE 1 | Taxonomic affiliation of the 20 most abundant ASVs, ranked according to their relative abundance.

Relative abundance (%)	Dada2 assigned	Genbank BLAST	ID
41	*Fusarium* sp.	*Fusarium* sp.	Fusarium.1
13.3	*Mycosphaerella tassiana*	*Cladosporium* sp.	Cladosporium.1
9.5	*Monographella nivalis*	*Monographella nivalis*	Monographella.1
5.2	*Alternaria infectoria*	*Alternaria infectoria*	Alternaria.1
4.2	*Vishniacozyma* sp.	*Vishniacozyma tephrensis*	Vishniacozyma.1
2.9	*Monographella nivalis*	*Monographella nivalis*	Monographella.2
2.7	*Monographella nivalis*	*Monographella nivalis*	Monographella.3
2.2	*Sporobolomyces roseus*	*Sporobolomyces roseus*	Sporobolomyces
2.0	*Cladosporium delicatulum*	*Cladosporium* sp.	Cladosporium.2
2.0	*Epicoccum nigrum*	*Epicoccum nigrum*	Epicoccum
1.9	*Alternaria infectoria*	*Alternaria infectoria*	Alternaria.2
1.4	*Vishniacozyma victoriae*	*Vishniacozyma victoriae*	Vishniacozyma.3
1.3	*Alternaria infectoria*	*Alternaria infectoria*	Alternaria.3
1.3	*Neoascochyta graminicola*	*Neoascochyta* sp.	Neoascochyta
1.2	*Vishniacozyma victoriae*	*Vishniacozyma victoriae*	Vishniacozyma.4
0.7	*Neoascochyta exitalis*	*Ascochyta skagwayensis*	*Ascochyta*
0.7	*Vishniacozyma victoriae*	*Vishniacozyma victoriae*	Vishniacozyma 5
0.7	*Alternaria infectoria*	*Alternaria infectoria*	Alternaria.4
0.6	*Fusarium lateritium*	*Fusarium* sp.	Fusarium.2
0.5	*Alternaria infectoria*	*Alternaria infectoria*	Alternaria.5

Two methods were used for taxonomic affiliation, the first one was integrated in the dada2 R pipeline, the second one was based on a genbank BLAST search. The "ID" column identifies the names given to the different ASVs within this study.

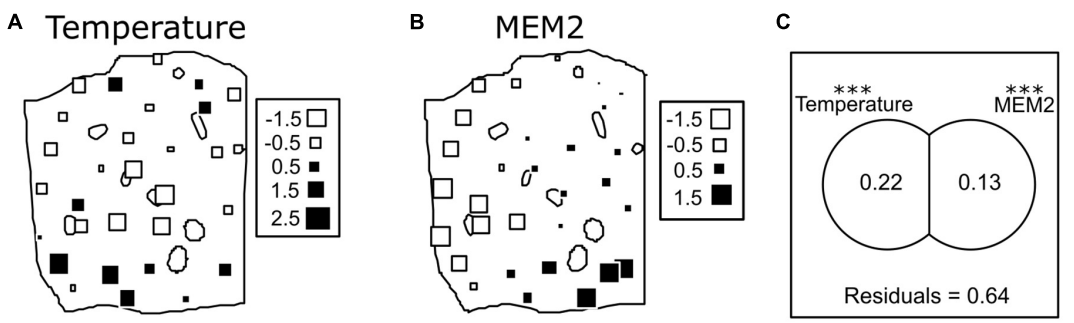


FIGURE 4 | In panel **(A)** a map showing the variations of the measured under canopy temperature is shown. The measured values are standardized with 0 as the average and the standard deviation as unity. In panel **(B)** a map of the only Eigenvector map showing a statistically significant effect is shown (named MEM2, as the generated Eigenvector map having the second highest eigenvalue). The size and color of the square indicate the values of the eigenvector assigned at each point. Panel **(C)** is a visual representation of the variation partitioning between the two explanatory variables (or maps) used, it calculates the single explanatory power of the two matrixes and calculate an eventual joint effect. Due to a negative joint effect detected, the negative values were proportionally distributed between the two matrixes, as suggested by Legendre et al. (2012). The value within the circles are adjusted R squared. With residuals it is represented the fraction of unexplained variance (Signif. codes: ***p-value < 0.001).

Points with a thicker canopy cover would be characterized by a lower temperature, while less productive points recorded a higher temperature. Therefore, plant productivity showed a significant effect on the microbial community across the field. The reason for such results might be different, and connected to a higher possibility for soil-borne pathogens to colonize the ear, given by the higher abundances of leaves, which might act as connecting "bridges" between the soil and the ear for spores carried by rain-splash (Xu, 2003; Manstretta et al., 2015; Schiro et al., 2018). A higher canopy cover effect, could also guarantee a better survival environment in the soil for the fungi, which might be less subject to temperature-humidity fluctuations and UV-light exposition, therefore having a higher amount of inoculum able to "climb up" to the ears once a rain event comes. These explanations are valid for those genera showing a negative correlation to temperature, such as *Fusarium* and *Monographella*. Genera like *Alternaria* and *Epicoccum* show a positive correlation to temperature; this might be connected to their host-preferences. They might find it easier to colonize plants in spots with a reduced productivity, since the plants are weaker and easier to colonize for saprotrophs. In this case it would be interesting to investigate the reasons for such differences in productivity. A link

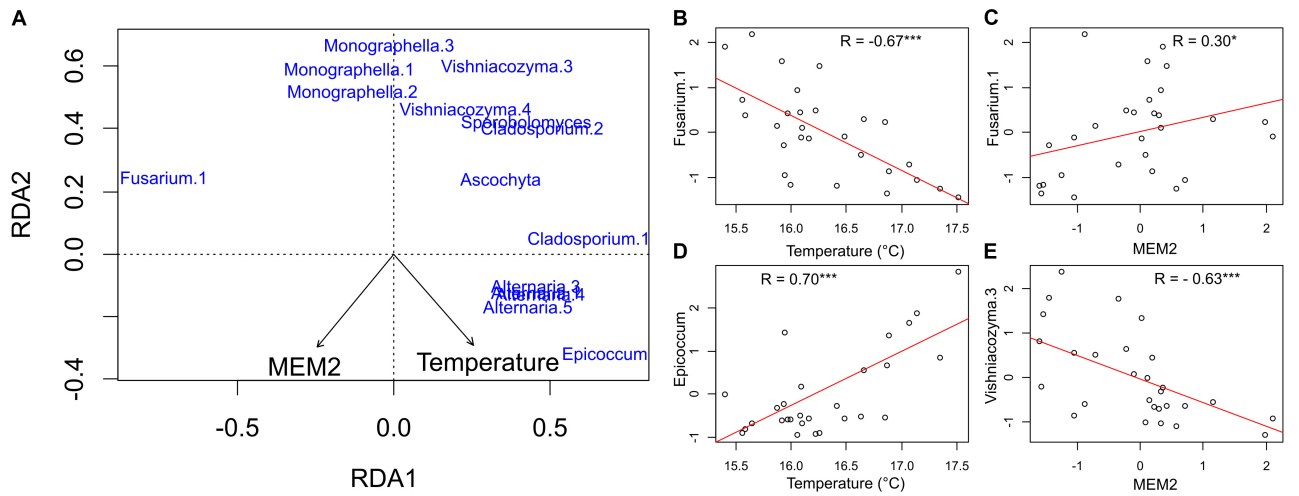


FIGURE 5 | Panel **(A)** RDA ordination plot on standardized species data representing the community composition. As for ASVs, only the 20 most abundant ASVs with more than 20% of variance explained by the two constraining axes are shown. In panels **(B–E)** linear relationships between the ASVs counts and the explanatory variables are shown. Axes represent standardized values; therefore the unit is the group's standard deviation (Signif. codes: ***p-value < 0.001, *p-value < 0.05).

to water availability, given by different topographical position has been previously described (Müller et al., 2010). Nevertheless, plants can suffer from a variety of abiotic and biotic stresses, often acting at the same time (Suzuki et al., 2014). We speculate that a combination of factors, such as lower water availability or the action of soil pathogens, weaken the plant's immune system, opening the path for the colonization of saprotrophic fungi, like members of the genera *Alternaria*, *Epicoccum* or *Cladosporium*. We did not find any study specifically addressing the effect of various plant stress conditions on the plant associated microbiota, although we are aware of the effort in trying to engineer plants microbiomes in order to enhance its stress resistance, such as drought (Coleman-Derr and Tringe, 2014; Ngumbi and Kloepper, 2016). This introduces a sort of cause-effect problem in the study of the microbiome of a plant: is the status of the plant influencing its microbiome, the microbiome regulating the status of the plant, or both phenomena are occurring at the same time? More efforts are needed to investigate what might be a sort of chained cause-effect loop.

We detected only one Eigenvector map showing a statistically significant correlation to our data. The db-RDA analysis, in this case, showed a smaller effect (15%) compared to temperature. The map shows the north-western part of the field having a different community then the south-eastern part, with all the most abundant ASVs, beside Fusarium.1, increasing toward the north-west. The reasons behind this geographical distribution are hard to predict. They could be the results of edge effects influencing the community, as the field was surrounded by different landscape components. While on the east and south side of the field there were other cultivated crops, in the west and north there were, respectively, a meadow and a forest edge. Fusarium.1 is a pathogen and its presence might be enhanced by the edges shared with other cultivated fields. On the other hand, yeasts like *Sporobolomyces* are described as naturally occurring saprophytic phyllosphere microorganisms; their presence might be boosted by the proximity to non-cultivated landscape elements, like a meadow and a forest edge in this case. Furthermore, Fusarium.1 is assigned either to *Fusarium graminearum* and/or *Fusarium culmorum;* these two species are described as very aggressive and their abundance might inhibit other fungal strains, like those showing a negative correlation to the MEM; a competitive interaction with *Monographella* was already described in the literature (Simpson et al., 2004). More efforts are necessary to clarify such patterns in relationship to the effect of different landscape structures and species interactions, studying their influence on the dispersal capacities of the members of the phyllosphere communities.

The clustering analysis of the obtained ASVs was based on their spatial distribution; ASVs with a similar distribution would show a smaller distance and therefore cluster together. The results here presented are similar results to those of Nicolaisen et al. (2014), who analyzed samples coming from different fields in Denmark (from a regional scale). Like this study, we obtained three main clusters. In cluster 1 *Epicoccum* and *Alternaria* are present, which can be classified as saprotrophs. Clusters 2 consists of yeasts and saprotrophs, while cluster 3 consists of fungi usually classified as pathogens (*Fusarium* and *Monographella*). Interestingly, Alternaria.2 is clustered in cluster 3, away from the other members of its genus. It displays a different spatial behavior than the other ASVs assigned to *Alternaria* in our studies. This fact introduces the need for having a self-made library for the taxonomic affiliation of our ASVs, where fungal isolates from the field will be characterized in order to better understand their behavior. This would be especially useful for the genus *Alternaria*, which complicated taxonomy is traditionally based on its morphology and sporulation patterns, but not always efficient in defining its biogeographical behavior or its aggressiveness toward the host plant. We selected ITS1 as metabarcoding

marker; this method is already established and allowed us to have a comparison with previous studies (e.g., Nicolaisen et al., 2014). ITS1 is known to work well until genus level, but fails to identify species for important genera of fungi such as *Cladosporium* and *Fusarium* (Schoch et al., 2012). This is especially important for this study due to the high abundance of the genus *Fusarium*, where different species with different characteristics, e.g., *Fusarium graminearum* and *Fusarium culmorum,* cannot be distinguished. A powerful extension to our study, would involve the analysis of how different *Fusarium* species react to our explanatory variables, using a molecular approach able to reveal them (Boutigny et al., 2019).

To conclude, our results show how the phyllosphere fungal community changes within a topographically heterogeneous field. We show that these variations were related to potential explanatory variables, such as field productivity and autocorrelated spatial structures. In our case, field productivity, measured using under-canopy microclimatic variables, had a stronger explanatory effect than the geographical position of the sampling points. We also show how different fungal phyla behave differently in space, giving precious hints on their in-field epidemiology. We highlight that topographically heterogeneous fields are a valuable study system to investigate the ecology of phyllosphere fungi, showing similar dynamics observed in studies performed at a regional scale. With this study we also want to pinpoint the need for knowing more about the dispersal dynamics influencing the phyllosphere mycobiome, testing the effects of spatial structures at different scales and using different barcodes. Such observations are useful for the general challenge of understanding the drivers of wheat microbiome community assembly.

DATA AVAILABILITY

Metabarcoding data is deposited as bioproject in the NCBI GenBank database (accession number: PRJNA517107).

AUTHOR CONTRIBUTIONS

GS and MM designed the study and conducted the field work. GS conducted the laboratory work and took the leadership in the writing of the manuscript. GS and PC analyzed the data. All authors contributed to the manuscript.

FUNDING

This work was supported by the Deutsche Forschungsgemeinschaft, in the framework of the BioMove Research Training Group (DFG-GRK 2118/1).

ACKNOWLEDGMENTS

Special thanks go to Rainer Fuchs, Martina Peters, Grit v. d. Waydbrink, Thomas Müller, and Gernot Verch for fieldwork assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.02095/full#supplementary-material

REFERENCES

Abdelfattah, A., Malacrinò, A., Wisniewski, M., Cacciola, S. O., and Schena, L. (2018). Metabarcoding: a powerful tool to investigate microbial communities and shape future plant protection strategies. *Biol. Control* 120, 1–10. doi: 10.1016/j.biocontrol.2017.07.009

Baffoni, L., Gaggia, F., Dalanaj, N., Prodi, A., Nipoti, P., Pisi, A., et al. (2015). Microbial inoculants for the biocontrol of *Fusarium* spp. in durum wheat. *BMC Microbiol.* 15:242. doi: 10.1186/s12866-015-0573-7

Bankina, B., Bimšteine, G., Neusa-Luca, I., Roga, A., and Fridmanis, D. (2017). What influences the composition of fungi in wheat grains? *Acta Agrobot.* 70, 1–8. doi: 10.5586/aa.1726

Bateman, G. L., Gutteridge, R. J., Gherbawy, Y., Thomsett, M. A., and Nicholson, P. (2007). Infection of stem bases and grains of winter wheat by *Fusarium culmorum* and F. *graminearum* and effects of tillage method and maize-stalk residues. *Plant Pathol.* 56, 604–615. doi: 10.1111/j.1365-3059.2007.01577.x

Benson, D. A. (2004). GenBank. *Nucleic Acids Res.* 33, D34–D38. doi: 10.1093/nar/gki063

Berg, G., Köberl, M., Rybakova, D., Müller, H., Grosch, R., and Smalla, K. (2017). Plant microbial diversity is suggested as the key to future biocontrol and health trends. *FEMS Microbiol. Ecol.* 93, 1–9. doi: 10.1093/femsec/fix050

Bivand, R., and Piras, G. (2015). Comparing implementations of estimation methods for spatial econometrics. *J. Stat. Soft.* 63, 1–36. doi: 10.18637/jss.v063.i18

Bivand, R. S., Pebesma, E., and Gomez-Rubio, V. (2013). *Applied Spatial Data Analysis with {R}*, 2 Edn. New York, NY: Springer.

Bockus, W. W., Bowden, R. L., Hunger, R. M., Morrill, W. L., Murray, T. D., and Smiley, R. W. (2010). *Compendium of Wheat Diseases and Pests*, 3 Edn. Morgantown, WV: APS Press.

Borcard, D., Gillet, F., and Legendre, P. (2018). *Numerical Ecology with R*. Basel: Springe.

Bottalico, A., and Perrone, G. (2002). Toxigenic *Fusarium species* and mycotoxins associated with head blight in small-grain cereals in Europe. *Eur. J. Plant Pathol.* 108, 611–624. doi: 10.1023/A:1020635214971

Boutigny, A. L., Gautier, A., Basler, R., Dauthieux, F., Leite, S., Valade, R., et al. (2019). Metabarcoding targeting the EF1 alpha region to assess fusarium diversity on cereals. *PLoS One* 14:e0207988. doi: 10.1371/journal.pone.0207988

Callahan, B. J., McMurdie, P. J., and Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J.* 11, 2639–2643. doi: 10.1038/ismej.2017.119

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi: 10.1038/nmeth.3869

Coleman-Derr, D., and Tringe, S. G. (2014). Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. *Front. Microbiol.* 5:283. doi: 10.3389/fmicb.2014.00283

Del Ponte, E. M., Shah, D. A., and Bergstrom, G. C. (2003). Spatial patterns of *Fusarium* head blight in New York wheat fields suggest role of airborne inoculum. *Plant Heal. Prog.* 4:6. doi: 10.1094/PHP-2003-0418-01-RS

Dray, S., Bauman, D., Guillaume, B., Borcard, D., Clappe, S., Guenard, G., et al. (2018). *Package "adespatial"*. Available at: https://cran.r-project.org/web/packages/adespatial/ (accessed November 1, 2018).

Dray, S., Legendre, P., and Peres-Neto, P. R. (2006). Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecol. Modell.* 196, 483–493. doi: 10.1016/j.ecolmodel.2006.02.015

El-Gremi, S. M., Draz, I. S., and Youssef, W. A. E. (2017). Biological control of pathogens associated with kernel black point disease of wheat. *Crop Prot.* 91, 13–19. doi: 10.1016/j.cropro.2016.08.034

Federal Office of Plant Varieties (2017). *Beschreibende Sortenliste Getreide, Mais, Öl- und Faserpflanzen, Leguminosen, Rüben, Zwischenfrüchte*. Hanover: BSA.

Figueroa, M., Hammond-Kosack, K. E., and Solomon, P. S. (2018). A review of wheat diseases-a field perspective. *Mol. Plant Pathol.* 19, 1523–1536. doi: 10.1111/mpp.12618

Gdanetz, K., and Trail, F. (2017). The wheat microbiome under four management strategies, and potential for endophytes in disease protection. *Phytobiomes* 1, 158–168. doi: 10.1094/PBIOMES-05-17-0023-R

Granzow, S., Kaiser, K., Wemheuer, B., Pfeiffer, B., Daniel, R., Vidal, S., et al. (2017). The effects of cropping regimes on fungal and bacterial communities of wheat and faba bean in a greenhouse pot experiment differ between plant species and compartment. *Front. Microbiol.* 8:902. doi: 10.3389/fmicb.2017.00902

Hertz, M., Jensen, I. R., Jensen, L. Ø, Thomsen, S. N., Winde, J., Dueholm, M. S., et al. (2016). The fungal community changes over time in developing wheat heads. *Int. J. Food Microbiol.* 222, 30–39. doi: 10.1016/j.ijfoodmicro.2016.01.018

Jansson, J. K., and Baker, E. S. (2016). A multi-omic future for microbiome studies. *Nat. Microbiol.* 1:16049. doi: 10.1038/nmicrobiol.2016.49

Karlsson, I., Friberg, H., Kolseth, A. K., Steinberg, C., and Persson, P. (2017). Organic farming increases richness of fungal taxa in the wheat phyllosphere. *Mol. Ecol.* 26, 3424–3436. doi: 10.1111/mec.14132

Karlsson, I., Friberg, H., Steinberg, C., and Persson, P. (2014). Fungicide effects on fungal community composition in the wheat phyllosphere. *PLoS One* 9:e0111786. doi: 10.1371/journal.pone.0111786

Kassambara, A., and Mundt, F. (2017). *factoextra: Extract and Visualize the Results of Multivariate Data Analyses*. Available at: https://cran.r-project.org/package=factoextra (accessed November 1, 2018).

Keller, M. D., Bergstrom, G. C., and Shields, E. J. (2014). The aerobiology of *Fusarium graminearum*. *Aerobiologia* 30, 123–136. doi: 10.1007/s10453-013-9321-3

Korn, U., Koch, M., Urban, K., Müller, M. E. H., Siegel, D., and Köppen, R. (2014). Mycotoxins as antagonistic or supporting agents in the interaction between phytopathogenic *Fusarium* and *Alternaria* fungi. *World Mycotoxin J.* 8, 311–321. doi: 10.3920/wmj2014.1747

Legard, D. E., McQuilken, M. P., Whipps, J. M., Fenlon, J. S., Fermor, T. R., Thompson, I. P., et al. (1994). Studies of seasonal changes in the microbial populations on the phyllosphere of spring wheat as a prelude to the release of a genetically modified microorganism. *Agric. Ecosyst. Environ.* 50, 87–101. doi: 10.1016/0167-8809(94)90128-7

Legendre, P., Daniel, B., and David, W. R. (2012). Variation partitioning involving orthogonal spatial eigenfunction submodels. *Ecology* 93, 1234–1240. doi: 10.1890/11-2028.1

Legendre, P., and Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia* 129, 271–280. doi: 10.1007/s004420100716

Manstretta, V., Gourdain, E., and Rossi, V. (2015). Deposition patterns of *Fusarium graminearum* ascospores and conidia within a wheat canopy. *Eur. J. Plant Pathol.* 143, 873–880. doi: 10.1007/s10658-015-0722-8

Manstretta, V., and Rossi, V. (2015). Modelling the effect of weather on moisture fluctuations in maize stalk residues, an important inoculum source for plant diseases. *Agric. For. Meteorol.* 207, 83–93. doi: 10.1016/j.agrformet.2015.04.001

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 17:10. doi: 10.14806/ej.17.1.200

Müller, M. E. H., Brenning, A., Verch, G., Koszinski, S., and Sommer, M. (2010). Multifactorial spatial analysis of mycotoxin contamination of winter wheat at the field and landscape scale. *Agric. Ecosyst. Environ.* 139, 245–254. doi: 10.1016/j.agee.2010.08.010

Müller, M. E. H., and Korn, U. (2013). Alternaria mycotoxins in wheat - A 10 years survey in the Northeast ofGermany. *Food Control* 34, 191–197. doi: 10.1016/j.foodcont.2013.04.018

Müller, M. E. H., Koszinski, S., Bangs, D. E., Wehrhan, M., Ulrich, A., Verch, G., et al. (2016). Crop biomass and humidity related factors reflect the spatial distribution of phytopathogenic *Fusarium* fungi and their mycotoxins in heterogeneous fields and landscapes. *Precis. Agric.* 17, 698–720. doi: 10.1007/s11119-016-9444-y

Ngumbi, E., and Kloepper, J. (2016). Bacterial-mediated drought tolerance: current and future prospects. *Appl. Soil Ecol.* 105, 109–125. doi: 10.1016/j.apsoil.2016.04.009

Nicolaisen, M., Justesen, A. F., Knorr, K., Wang, J., and Pinnschmidt, H. O. (2014). Fungal communities in wheat grain show significant co-existence patterns among species. *Fungal Ecol.* 11, 145–153. doi: 10.1016/j.funeco.2014.06.002

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2018). *vegan: Community Ecology Package*. Available at: https://cran.r-project.org/package=vegan (accessed November 1, 2018).

OpenStreetMap contributors (2017). *Planet dump*. Available at: https://planet.osm.org (accessed May 1, 2018).

Ostry, V. (2008). Alternaria mycotoxins: an overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. *World Mycotoxin J.* 1, 175–188. doi: 10.3920/WMJ2008.x013

Palazzini, J. M., Alberione, E., Torres, A., Donat, C., Köhl, J., and Chulze, S. (2016). Biological control of *Fusarium graminearum* sensu stricto, causal agent of *Fusarium* head blight of wheat, using formulated antagonists under field conditions in Argentina. *Biol. Control* 94, 56–61. doi: 10.1016/j.biocontrol.2015.12.009

Patil, I. (2018). *ggstatsplot: "ggplot2" Based Plots with Statistical Details*. Available at: https://cran.r-project.org/package=ggstatsplot (accessed November 1, 2018).

Paul, P. A., El-Allaf, S. M., Lipps, P. E., and Madden, L. V. (2004). Rain splash dispersal of *Gibberella zeae* within wheat canopies in Ohio. *Phytopathology* 94, 1342–1349. doi: 10.1094/PHYTO.2004.94.12.1342

Pebesma, E. J., and Bivand, R. S. (2005). *Classes and methods for spatial data in {R}*. Available at: https://cran.r-project.org/web/packages/sp/ (accessed November 1, 2018).

R Core Team (2017). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation Statistical Computing.

Sapkota, R., Jørgensen, L. N., and Nicolaisen, M. (2017). Spatiotemporal variation and networks in the mycobiome of the wheat canopy. *Front. Plant Sci.* 8:1357. doi: 10.3389/fpls.2017.01357

Sapkota, R., Knorr, K., Jørgensen, L. N., O'Hanlon, K. A., and Nicolaisen, M. (2015). Host genotype is an important determinant of the cereal phyllosphere mycobiome. *New Phytol.* 207, 1134–1144. doi: 10.1111/nph.13418

Schiro, G., Müller, T., Verch, G., Sommerfeld, T., Mauch, T., Koch, M., et al. (2019). The distribution of mycotoxins in a heterogeneous wheat field in relation to microclimate, fungal and bacterial abundance. *J. Appl. Microbiol.* 126, 177–190. doi: 10.1111/jam.14104

Schiro, G., Verch, G., Grimm, V., and Müller, M. (2018). Alternaria and *Fusarium* fungi: differences in distribution and spore deposition in a topographically heterogeneous wheat field. *J. Fungi* 4:63. doi: 10.3390/jof4020063

Schisler, D. A., Khan, N. I., Boehm, M. J., and Slininger, P. J. (2002). Greenhouse and field evaluation of biological control of fusarium head blight on durum wheat. *Plant Dis.* 86, 1350–1356. doi: 10.1094/PDIS.2002.86.12.1350

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., et al. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. U.S.A.* 109, 6241–6246. doi: 10.1073/pnas.1117018109

Simpson, D. R., Thomsett, M. A., and Nicholson, P. (2004). Competitive interactions between Microdochium nivale var. majus, *M. nivale var. nivale* and *Fusarium culmorum* in planta and *in vitro*. *Environ. Microbiol.* 6, 79–87. doi: 10.1046/j.1462-2920.2003.00540.x

Singh, B. K., and Trivedi, P. (2017). Microbiome and the future for food and nutrient security. *Microb. Biotechnol.* 10, 50–53. doi: 10.1111/1751-7915.12592

Siou, D., Gélisse, S., Laval, V., Elbelt, S., Repinçay, C., Bourdat-Deschamps, M., et al. (2015). Interactions between head blight pathogens: consequences for disease development and toxin production in wheat spikes. *Appl. Environ. Microbiol.* 81, 957–965. doi: 10.1128/AEM.02879-14

Soininen, J. (2016). Spatial structure in ecological communities - a quantitative analysis. *Oikos* 125, 160–166. doi: 10.1111/oik.02241

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., and Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytol.* 203, 32–43. doi: 10.1111/nph.12797

Syed Ab Rahman, S. F., Singh, E., Pieterse, C. M. J., and Schenk, P. M. (2018). Emerging microbial biocontrol strategies for plant pathogens. *Plant Sci.* 267, 102–111. doi: 10.1016/j.plantsci.2017.11.012

UNITE Community (2017). *UNITE general FASTA release*. Shadwell: UNITE Community, doi: 10.15156/BIO/587475

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., and Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytol.* 206, 1196–1206. doi: 10.1111/nph.13312

Vučković, J. N., Brkljača, J. S., Bodroža-Solarov, M. I., Bagi, F. F., Stojšin, V. B., Ćulafić, J. N., et al. (2012). *Alternaria* spp. on small grains. *Food Feed Res.* 39, 79–88.

Xu, X. (2003). Effects of environmental conditions on the development of *Fusarium* ear blight. *Eur. J. Plant Pathol.* 109, 683–689. doi: 10.1023/A:1026022223359

Xu, X., and Nicholson, P. (2009). Community ecology of fungal pathogens causing wheat head blight. *Annu. Rev. Phytopathol.* 47, 83–103. doi: 10.1146/annurev-phyto-080508-081737

Xue, A. G., Chen, Y., Voldeng, H. D., Fedak, G., Savard, M. E., Längle, T., et al. (2014). Concentration and cultivar effects on efficacy of CLO-1 biofungicide in controlling *Fusarium* head blight of wheat. *Biol. Control* 73, 2–7. doi: 10.1016/j.biocontrol.2014.02.010

Zadoks, J. C., Chang, T. T., and Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Res.* 14, 415–421. doi: 10.1111/j.1365-3180.1974.tb01084.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 19 May 2020
doi: 10.3389/fevo.2020.00118

Effects of Past and Present-Day Landscape Structure on Forest Soil Microorganisms

Sophie Mennicken[1,2], Floriane Kondratow[3], Florian Buralli[3], Sophie Manzi[1], Emilie Andrieu[3], Mélanie Roy[1] and Antoine Brin[3]*

[1] Evolution et Diversité Biologique (EDB), Université Toulouse 3 Paul Sabatier, CNRS, IRD, Toulouse, France, [2] Biology of Ecosystems Department, Faculty of Science, University of South Bohemia, Ceské Budejovice, Czechia, [3] Purpan School of Engineering, University of Toulouse, Toulouse, France

Theory surrounding landscape ecology has been built on the species distribution of birds and plants, but increasing evidence now exists for below-ground organisms, whose dispersal may also be affected by above-ground landscape structures. Uncertainties remain for how communities of microorganisms respond to landscape structure over time, and whether some groups of microorganisms react more than others. Here, we investigated if fungal or bacterial diversity is driven by the amount of forest cover in the current or the past landscape. We tested the habitat amount hypothesis (HAH) on ancient forests of the Cevennes national park, that experienced increased fragmentation 150 years ago, and are today surrounded by recent forests. As ancient forests are often more diverse in plant species, we hypothesized that the higher quantity of ancient forests in the landscape, the richer local fungal and bacterial communities would be. More precisely, we expected that ectomycorrhizal fungi, and pathotrophic fungi, often indicators of mature forests, would be also more sensitive to forest history and therefore to the quantity of ancient forests than bacteria and saprotrophic fungi. We sampled 40 soil cores per 0.5 ha, pooled in 8 composite samples per plot in 27 landscapes and sequenced ITS1 and 16S markers by Illumina-Mi seq. To identify functional groups of fungi, we relied on their taxonomy and the use of public databases. Our results partly follow the HAH, as fungal richness was positively related with the quantity of ancient forests in the landscape and not by the focal patch size. Ectomycorrhizal and pathotrophic fungi were positively affected by the ancient forest cover, and so were saprotrophic ones, but not bacteria. Local factors also shaped the communities such as soil composition and elevation, confirming classical patterns in soil microbial ecology. Interestingly, past landscape structure was better at explaining fungal community richness than contemporary landscape, suggesting a time lag in the response of communities to landscape modification and a potential extinction debt. Our results reveal the importance of below-ground communities in studies of landscape and historical ecology, with their structure and functions likely to be intimately linked with soil and landscape history.

Keywords: landscape ecology, habitat amount hypothesis, fungi, bacteria, metabarcoding

OPEN ACCESS

Edited by:
Cendrine Mony, University of Rennes 1, France

Reviewed by:
Juan Ernesto Guevara Andino, Field Museum of Natural History, United States
Margaux Boeraeve, KU Leuven, Belgium

****Correspondence:***
Mélanie Roy
melanie.roy@univ-tlse3.fr

Specialty section:
This article was submitted to Biogeography and Macroecology, a section of the journal Frontiers in Ecology and Evolution

Received: *11 December 2019*
Accepted: *14 April 2020*
Published: *19 May 2020*

Citation:
Mennicken S, Kondratow F, Buralli F, Manzi S, Andrieu E, Roy M and Brin A (2020) Effects of Past and Present-Day Landscape Structure on Forest Soil Microorganisms. Front. Ecol. Evol. 8:118. doi: 10.3389/fevo.2020.00118

INTRODUCTION

Soil microorganisms have only recently been included in studies of landscape ecology (Almasia et al., 2016; Grilli et al., 2017), with the discipline focusing mainly on plant, bird or insect species distributions and dispersal. Microorganisms such as fungi and bacteria, are among the most abundant and diverse organisms in soil (Fierer et al., 2007a) and play diverse and key roles in forest soil processes (e.g., decomposition, nutrient cycles, biotic relations; Altieri, 1999; Birkhofer et al., 2012; Hobara et al., 2014; Bani et al., 2018). Soil microorganisms are also intimately linked with plant communities, and could be affected by the landscape defined for plants, such as fields, forest, or hedgerows. However, for soil microorganisms, one major challenge in landscape ecology is to delineate patches, and corridors, depending on species dispersal abilities. For fungi, dispersal can occur over long distances (Vincenot and Selosse, 2017), but is often local (Peay et al., 2012), and thus size of patches may be extremely variable. Moreover, recent studies of landscape ecology on fungi have shown highly contrasting patterns, depending on the functional community and the scale studied (Nordén et al., 2018). For example, wood-associated fungi are distinct at forest edges and are affected by forest fragmentation (Helander et al., 2007; Abrego and Salcedo, 2014). Similarly, in the lava-fragmented landscape of Hawaï, local root-associated fungal diversity increased with forest area, and fungal species composition was correlated with fragment connectivity (Vannette et al., 2016). On the contrary, fragmentation did not shape communities of arbuscular mycorrhizal fungi associated to a ruderal forb (Grilli et al., 2015) while it affected ectomycorrhizal communities associated with *Alnus*, *Crataegus* and *Corylus* (Boeraeve et al., 2018a). Overall, Grilli et al. (2017) concluded that in general, fragmentation negatively affects fungal diversity but did not discuss other landscape characteristics. Among other soil microorganisms, bacterial distribution has already been revealed to respond to landscape effects (Fierer et al., 2007b; Culman et al., 2010; de Vries et al., 2012), at least in the context of streams, alpine soils and agricultural landscapes. Under more continuous cover such as forests, the response of bacteria to landscape has not been investigated and could reveal strong spatial patterns, as suggested by their high spatial turnover in heterogeneous environments (Horner-Devine et al., 2004).

Beyond fragmentation, the landscape can shape species distributions in various ways, with for example Fahrig (2013) suggesting that the quantity of habitat in the landscape could be more important than its spatial distribution. This hypothesis, named "habitat amount hypothesis" (HAH) has been intensely debated (Fahrig, 2015; Hanski, 2015) as for some authors it ignores well-known effects of landscape configuration (Haddad et al., 2017), and may not apply to the more isolated patches (Lindgren and Cousins, 2017) nor to all organisms. For example, it was verified for small mammals (Melo et al., 2017) but not for saproxylic beetles (Seibold et al., 2017). A meta-analysis based on 13 tests of the HAH suggested that patch isolation and size, after controlling for habitat amount, have an overall weak effect (Martin, 2018). The synthesis of Martin (2018) concluded that response to landscape configuration and HAH would be taxa- and context-specific, calling for repeated studies on a broader range of organisms. We thus decided to investigate the response of fungal and bacterial communities to the amount of available habitat, which could reveal alternative hypothesis when investigating the importance of fragmentation or patch area on these organisms. More precisely, we aimed to test if ancient forest quantity could shape existing fungal and bacterial communities.

The impact of historical patterns of habitat cover on contemporary biodiversity patterns has been documented in many fragmented landscapes (Harding et al., 1998; Kuussaari et al., 2009). Indeed, as it may take many generations for populations to go extinct after habitat loss and fragmentation, it has been hypothesized that a "ghost of land-use past" (Harding et al., 1998) can be a powerful driver of present-day diversity. If so, this would imply considering landscape history in conservation planning (Schrott et al., 2005; Kuussaari et al., 2009). A few pioneer studies have already demonstrated that fungal distribution is partly explained by ancient roman occupancy (Diedhiou et al., 2009), and that ectomycorrhizal fungi functions are distinct on such sites (Diedhiou et al., 2010). Case studies in Norway also revealed that the distribution of lichens – noted slow growing organisms- was shaped by the distribution of old oaks (Ranius et al., 2008a,b). Finally, Berglund and Jonsson (2005) revealed an extinction debt (Kuussaari et al., 2009) in lichens but not for wood-decaying fungi, suggesting that fungi with distinct ecologies may react differently to landscape dynamics and forest histories. Such evidence supports the consideration of both HAH and historical delineation of landscapes when investigating current soil microorganism distribution.

In temperate forests, ancientness, defined as the "temporal continuity of wooded soil" (Cateau et al., 2015), is a major characteristic of forests. Ancient forests are habitats for numerous species of plants (Hermy et al., 1999; Bergès et al., 2016; Bergès and Dupouey, 2017), and 57 to 132 species are recognized as indicators of ancient forests in France and Europe, respectively. While fungi can be indicator of forest maturity and therefore old-growth forests (Parmasto, 2001; Christensen et al., 2005; Dvořák et al., 2017; Halme et al., 2017), their link with ancient landscapes has received much less attention (Hofmeister et al., 2014). Interestingly, ancient forests of Belgium have revealed a higher diversity of fungi as compared with fragmented recent forests (Boeraeve et al., 2018b), but similar to non-fragmented forests, which suggests that both landscape structure and forest history may shape fungal diversity. To our knowledge, no study has investigated the bacterial communities of ancient forests. A recent study showed that bacterial communities are influenced by local abiotic factors, and not by soil history (Almasia et al., 2016). These results confirmed anterior studies that showed that pH was the best predictor for soil bacteria community distribution (Fierer and Jackson, 2006; Lauber et al., 2009). Investigating both fungi and bacteria in ancient forests may therefore reveal contrasting patterns, and we could expect fungi to be more specifically associated with ancient forests.

The development of both environmental sequencing to investigate soil organisms (Taberlet et al., 2012; Zinger et al., 2016) and public databases of historical maps (Bergès and Dupouey, 2017) make such a study of soil community and landscape ecology possible. Indeed,

environmental sequencing can target all bacteria and fungi with universal markers, and enable the sequencing of both ectomycorrhizal, saprotrophic or pathotrophic ones. In parallel, public databases are now reporting functional traits for hundreds of soil taxa (Nguyen et al., 2016), and can therefore be used to derive functional diversity from environmental sequencing. Until now, wood associated species, often pathotrophs, are recognized as indicators of old-growth forests (Parmasto, 2001; Dvořák et al., 2017; Halme et al., 2017) and the question remains as to whether such species are also sensitive to the quantity of ancient forests in the landscape. Moreover, as ectomycorrhizal fungi are intimately associated with trees, we could hypothesize that they would also be favored by forest continuity and the quantity of ancient forests in the landscape. Conversely, for saprotrophic fungi, abundant in the litter, and bacteria, the question remains as to whether they would reflect differences in landscape structure in a forest context.

In Cevennes National Park, ancient forests are scattered in the landscape, and occur surrounded by conifer plantations, or recently established beech (*Fagus sylvatica*) forests. While 80% of the Park territory is represented by forests today, only 13% of the territory was covered by forests in 1850 (**Figure 1**). The difference between ancient and recent forest distribution is particularly high as compared to other forests in France (Bergès and Dupouey, 2017), and represents a study site to investigate the response of soil microorganisms to past and present landscapes, and test HAH. To better understand the effect of landscape on below-ground communities, we therefore handled a large-scale sampling of soil communities in Cevennes National Park. Our objectives were to test (1) if fungi and bacteria communities could follow the HAH, being richer in forests surrounded by higher forest cover, (2) if functional groups show distinct responses to the landscape structure, and (3) if soil microorganism communities show a response time lag to modification of landscape structure.

MATERIALS AND METHODS

Study Sites and Landscape Variables Design

The study area covers circa 15,000 hectares in the core area of Cevennes National Park (93,800 hectares). This area was mainly covered by forest (70% surface cover), dominated by European beech (*F. sylvatica* L.), mixed either with deciduous species (*Quercus* sp.) or with coniferous species (*Abies alba* Mill. and *Picea abies* (L.) Karst). We selected 27 landscapes to include a gradient of ancient forest cover (**Figure 1**). Following Fahrig (2013), we checked that the patch surface (including the sampling site) and the total surface occupied by ancient forests in the landscape were decorrelated (e.g., of patches, **Figure 2**, **Supplementary Figure S1**). Sample sites were located at the center of each landscape, in ancient forests dominated by beech, covering an area of 0.5 ha. The information on beech coverage and forest surface (both ancient and recent) were acquired from conservation maps (2011) provided by the National Park and based on digitized maps produced between 1920 and 1966 (IGN, 2011). Beech coverage was confirmed by an estimation of beech total basal area with a relascope at each site. We computed this information for buffers in a radius of 500, 1000, 1500, and 2000 meters around the central sampling patch (e.g., **Figure 1**). All landscapes were selected from the montane bioclimatic zone, between 935 and 1453 m a.s.l. and on granitic bedrock using MNT BD Alti® (IGN, 1996) and a geological map (Carte lithologique simplifiée®, BRGM, 2008). Total ancient forest area was calculated for landscapes of 78, 314, 706, and 1256 hectares.

Soil Sampling and Processing

Soil samples were collected during April 2017 (average temperature 4.4°C, average precipitation 17.6 mm from www.infoclimat.fr). At each study site, soil cores were collected along two 60 m transects, spaced 20 m apart. Every 20 m along a transect, a soil core was collected, and pooled with four soil cores collected at 3.5 m from the focal point in each cardinal direction. The soil core itself was collected with a shovel, from the first 10 cm of soil depth, targeting the A horizon and removing the larger debris from the litter. Between each soil core, a false core was sampled in a radius a 1m, to remove the debris from the previous sampling point, and prepare the next sampling point. The shovel was clean every night with 2% bleach and rinsed with tap water (i.e., every two plots). For each plot, a total of 40 soil cores were then collected and pooled into 8 composite samples, before being returned to the lab the same day. 15 g of each composite sample was isolated in clean tea-bags and dried with 50 g of silicagel (with more added if the sample was not dry the day after). Soil DNA was extracted less than 1 month later from the dry samples (circa 10 g of soil) with Macherey Nagel NucleoSpin® Soil kit (Ref 740780.250). Amplifications targeted ITS1 nuclear rDNA primers for fungi (Fwd: ITS5 GGAAGTAAAAGTCGTAACAAGG from Epp et al., 2012 and a modified version of Rev: 5.8S_Fungi CAAGAGATCCGTTGTTGAAAGTK, Taberlet et al., 2018), and 16S rDNA (V5,V6) for bacteria [Bact01 primers – Fwd: GGATTAGATACCCTGGTAGT and Rev: CACGACACGAGCTGACG (Fliegerova et al., 2014)]. Each PCR was performed with a unique set of primers, characterized by a unique set of 8 nucleotide tags added to the universal primers, to distinguish sample sequences later in the analysis (as described in Taberlet et al., 2018). Negative controls without any PCR reactants were included (representing 10% of our reactions) and all PCR reactions were replicated twice, each time with distinct primer pairs. Replicates were not amplified on the same PCR plate. PCR conditions followed Nagati et al. (2018). All PCR reactions were pooled to prepare a unique Illumina-Miseq library, as the use of 8 nucleotide tags allowed to distinguish replicates and samples sequenced within the library. Lastly, the library was sent to the GenoToul Core Facility platform for sequencing in two directions and at 250 bp (2*250 bp).

Bioinformatic and Sequence Analysis

Bioinformatic analyses were handled with OBItools (Boyer et al., 2016). First, R1 and R2 reads were "paired-end" based on a 50 bp overlap, then low quality sequences, sequences shorter than 100 bp, and non-assembled sequences were removed. Sequences were attributed to samples thanks to their unique primer and 8-nucleotide tag combinations. Non-attributed sequences were

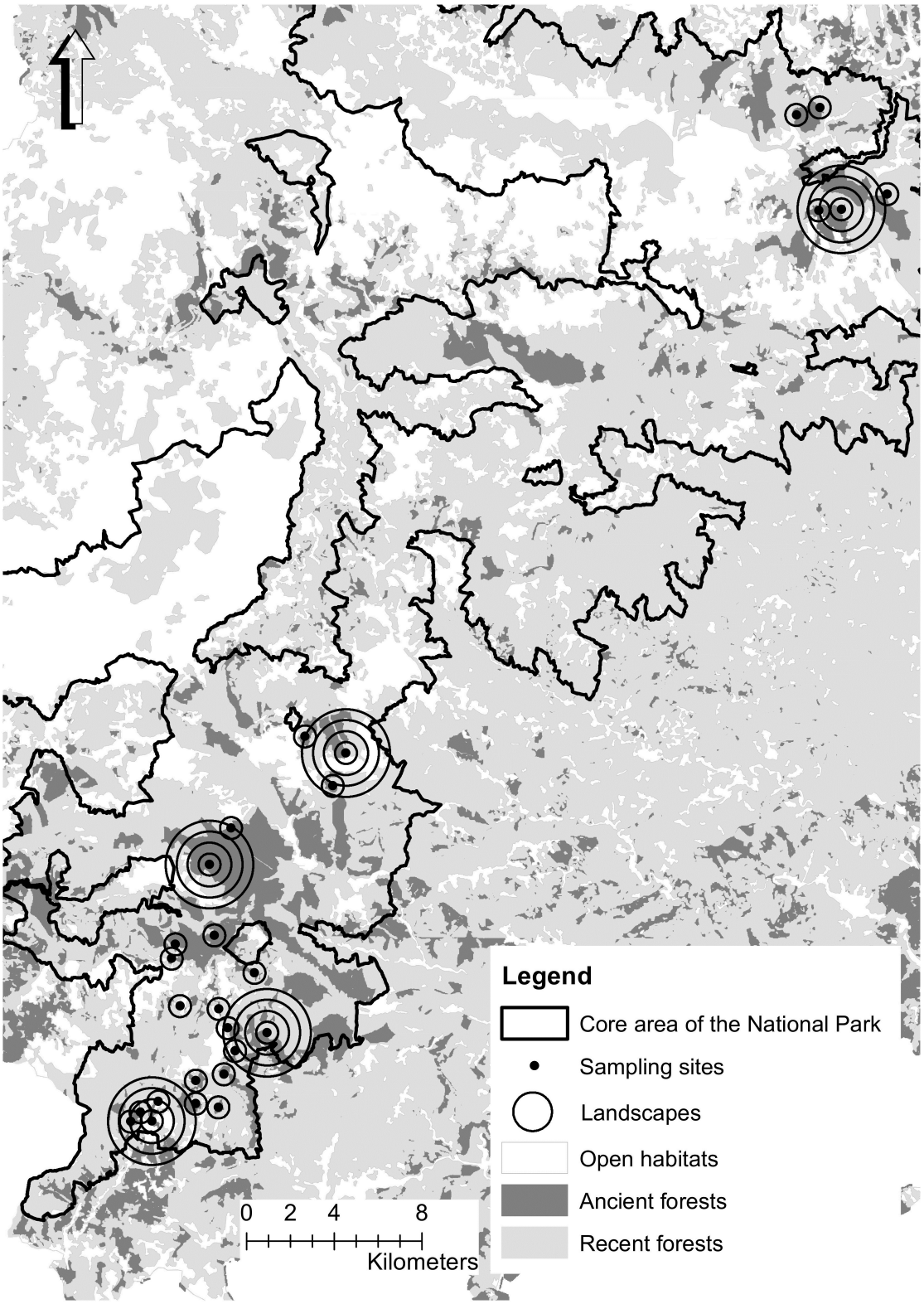


FIGURE 1 | Map of Cevennes National Park and distribution of ancient forests and sampling sites. Landscapes are illustrated as circles of different radii around sampling plots, but for more clarity, only radii of 500 m are drawn for most sites.

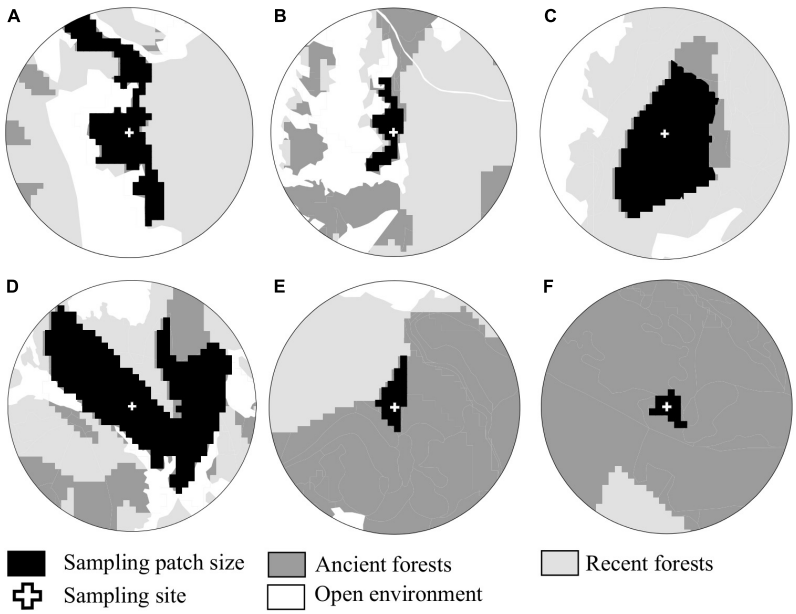


FIGURE 2 | Examples of landscape structure, illustrating gradients of habitat amount in the landscape, from **(A)** a landscape dominated by recent forest, to **(F)** a landscape dominated by ancient forest. The size of the central patch can be variable, and does not correlate with the quantity of ancient forest in the buffer. As an example, both large **(A–C)** and small patches **(B,E,F)** of ancient forests were sampled, in landscapes with a variable quantity of ancient forests (increasing from **(A–F)**).

discarded after this step. To speed up analyses, sequences were dereplicated, i.e., for each repeated sequence, one sequence was kept, and its occurrence per sample was counted. At this step sequences only occurring once in the dataset were removed, since these likely represented sequencing artifacts. Pairwise distances were then computed using *sumaclust* function to delineate sequence clusters with similarity of 97% or above (representing operational taxonomic units, OTUs, following Nilsson et al., 2008). Taxonomic assignment of OTUs was conducted using reference databases with the *ecotag* function (Boyer et al., 2016), which uses global alignment of sequences against full-length references. This function compares sampled short sequences with a database of short sequences, and returns not only the most similar sequence but also the taxonomic assignment of other closely similar sequences. With this method, low similarity sequences are assigned to "Fungi", while sequences with a high similarity to several Russulaceae are assigned to "Russulaceae," or even to an individual species. For fungi the reference database of short sequences was obtained by running an *in silico* PCR with the ecoPCR program (Ficetola et al., 2010) on Genbank (release 197;[1]) using the primer pairs employed here. Chimeras have very bad results with *ecotag*, as sequences are not assigned to a high taxonomic level. Then, taxonomic assignment yielding the highest similarity score was kept. For Bacteria, taxonomic assignment was not handled with ecotag but conducted using the SILVA database (Quast et al., 2012), with similarity parameters kept at 0.97. Subsequent dataset processing and analyses were conducted using R (R Core Team, 2019). Based on taxonomy, we removed all non-fungi and non-bacteria sequences, respectively. For bacteria, we also removed chloroplast or mitochondrial sequences. Using the sequenced negative controls, we also removed sequences abundant in the controls and rare in our samples (representing less than 5% of their counts). We had no case of sequences which were both abundant in samples and in controls. Finally, fungal guilds were assigned using FUNGUILD (Nguyen et al., 2016). Raw sequences were deposited on DRYAD[2].

Soil Sampling for Chemical Analyses

After collecting soil for the DNA extraction, the remaining 485 g of soil of each composite sample was transferred to a paper bag and dried at 50°C for three days. Before drying, pH units were obtained by pH colorimetric measurement. All composite samples were used for soil chemical analysis, resulting in 8 samples per plot. The soil physicochemical analyses were performed according to current standards by a soil testing laboratory[3]: total soil organic carbon content (NF ISO 10694), total nitrogen (NF ISO 13878), phosphorus (Duchaufour), measures of cation exchange capacity and exchangeable cations: calcium, magnesium, sodium, potassium, iron, aluminum and manganese (cobalt hexamine).

Data Analyses

All analyses were carried out with the R 3.6.1 (R Core Team, 2019) software, using the *iNext* package (Hsieh et al., 2016) to compute Hill numbers and sample coverage, the *lme4* (Bates et al., 2015)

[1] ftp://ftp.ncbi.nlm.nih.gov/genbank

[2] https://doi.org/10.5061/dryad.hmgqnk9c8

[3] https://www6.hautsdefrance.inra.fr/las/

and *MuMin* (Bartoñ, 2019) packages for the GLMMs, and the *vegan* package (Oksanen et al., 2019) for multivariate analysis.

Bacterial and Fungal Diversity and Sampling Coverage

First of all, to investigate if the NGS approach had produced comprehensive inventories, we assessed the sampling coverage at the site level using the framework proposed by Chao and Jost (2012). The sampling coverage was measured for each functional and taxonomic group.

Habitat Amount Effect on Species Diversity

To test the HAH, we investigated several indices of diversity via the Hill numbers framework (Hill, 1973; Alberdi and Gilbert, 2019) which allows the weighting of rare species through the parameter q. The larger the q value, the higher the importance attributed to abundant OTUs. We considered three values: $q = 0$ (equivalent to species richness), $q = 1$ (equivalent to Shannon index) and $q = 2$ (equivalent to the inverse of Simpson index). Diversity was computed separately for all bacteria and all fungi. For the latter, we also estimated diversity indices for each trophic guild (symbiotrophs, pathotrophs and saprotrophs).

To test if species diversity was positively correlated with ancient forest coverage in the landscape, we used GLMM with a Poisson distribution and several co-variables: the focal patch area, the first three axis of a PCA on edaphic descriptors (**Table 1**) and elevation. The site was included in our models, as a random effect, to account for spatial structure of the sampling design.

Habitat Amount Effect on Community Composition

The effects of landscape structure and local descriptors on community composition were assessed by distance-based redundancy analysis. We used the Bray-Curtis dissimilarity index as recommended by Lucas et al. (2017) to analyze microbial communities described by high-throughput molecular techniques. To partial out spatial variation in community composition, we used the vectors created by a Principal Coordinates of Neighborhood Matrix (PCNM, Dray et al., 2006). Indeed, our sampling was scattered in Cevennes National Park, and we used this technique not to test the spatial structure, but to remove the variation linked to spatial structure and focus on habitat amount variations. Permutation tests were used to assess the significance of marginal effect of each predictor on Bray-Curtis distances.

TABLE 1 | Variable contributions (%) to the first three axis of the PCA of local descriptors.

Variables	Dim 1	Dim 2	Dim 3
PCA components			
C	8.24	12.90	1.50
N	9.62	4.05	4.39
C/N	0.54	20.91	6.28
P_2O_5	0.34	1.03	45.27
CEC	13.71	1.29	1.11
Ca	14.10	0.68	0.64
Mg	13.16	0.00	0.15
Na	7.14	7.05	0.03
K	12.59	1.39	0.32
Fe	4.14	19.08	4.73
Mn	7.65	11.99	0.04
Al	6.83	13.91	0.03
Elevation	1.94	5.73	35.49

Spatial and Temporal Scale of Effect

We conducted a multi-scale analysis to determine the optimal spatial extent (scale of effect) at which landscape structure (here the habitat coverage) best predicts our response variables (Brennan et al., 2002; Jackson and Fahrig, 2015). We considered four radii (500, 1000, 1500, and 2000 m, see **Figure 1**) for the landscape structure and estimated the scale of effect for each response variable by model comparison based on the small-sample Akaike Information Criteria (AICc). The scale of effect was the radius of the buffer (landscape) corresponding to the best model, chosen with the smallest AICc. Finally, to explore a legacy effect on current OTU diversity, we compared models built with either ancient or contemporary forest cover as habitat amount, at the relevant scale of effect for each response variable (**Figure 3**).

RESULTS

Bacterial and Fungal Diversity and Sampling Coverage

The sequencing run produced 3 014,848 and 2 137,868 sequences for fungi and bacteria, respectively (without singletons and low-quality sequences). After cleaning the sequences (using negative controls and taxonomic assignment), we detected 3 751 fungal OTUs from 1.31×10^6 sequences and 14 922 bacterial OTUs from 8.44×10^5 sequences. The number of sequences was lower for bacteria as sequences attributed to mitochondria and chloroplasts were removed. Rare OTUs, well identified to fungi and bacteria, represented a minor part of the dataset for fungi (10% of OTUs occurring once, 12% occurring twice), but a larger part of bacteria (22% occurring once, 22% occurring twice). For fungi, 36.5%, 27.4% and 9% of OTUs were unambiguously assigned to symbiotrophs, saprotrophs and pathotrophs, respectively. Other OTUs were either attributed to two trophic modes (e.g., pathotroph and saprotroph) or not attributed to a trophic mode given the low taxonomic resolution (for Dikarya sequences, i.e., 11.8% of OTUs). Ectomycorrhizal fungi represented 92.4% of symbiotroph fungi, while arbuscular mycorrhizal fungi represented 4.3% of OTUs. Other mycorrhizal fungi belonged to several guilds (e.g., ectomycorrhizal and orchid mycorrhizal or ericoid mycorrhizal), to endophytes (0.01%) or lichens (1.3%). Ectomycorrhizal OTUs were more diverse, with 1245 OTUs, mainly belonging to Russulaceae, Cortinariaceae and Atheliaceae (**Supplementary Figure S2**). Cortinariaceae were more abundant in sequences but Russulaceae were richer in OTUs (**Supplementary Figure S2**). Among bacteria, proteobacteria, acidobacteria and actinobacteria were dominant, with actinobacteria represented by more sequences and proteobacteria by more OTUs than other phyla (**Supplementary Figure S3**).

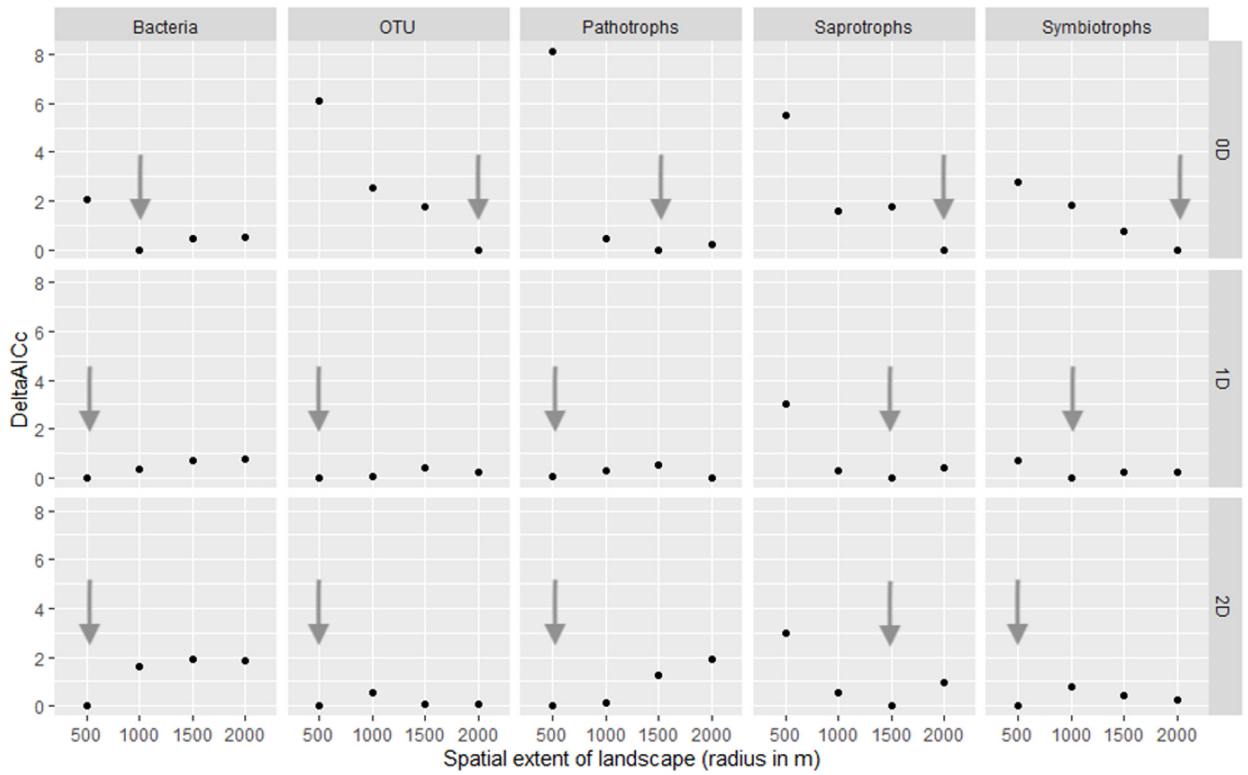


FIGURE 3 | Estimation of the scale of effect of the landscape variable (habitat amount) for each group and each Hill number (^{0}D: species richness, ^{1}D: Shannon index and ^{2}D: inverse of Simpson index). Arrows indicate the distance with the lower AIC and therefore the best model. OTU refer to all fungi OTUs.

With a mean sampling coverage of 94% and 99% for bacteria and fungi, respectively, our sampling is comprehensive at the site level (**Supplementary Figure S4**). Communities were relatively rich at each site, with at least 445 fungal and 2030 bacterial OTUs at each site (maximum of 1121 and 3948 OTUs, respectively). For fungi, when looking at the trophic group level, the sampling coverage was 99%, 98% and 95% for symbiotrophs, saprotrophs and pathotrophs, respectively (**Supplementary Figure S4**). Furthermore, the sampling coverage was not affected by the number of reads, which suggests that the sampling coverage was high even locally for composite sample. Therefore, we did not have to include the number of reads as a covariable in our models.

Habitat Amount Effect on Species Diversity

PCA was used to reduce the number of soil variables included in the GLMMs. The first three axis of the PCA on soil variables summarized 82.5% of total inertia: the first axis was mainly explained by Ca, Mg and K contents together with the CEC, the second axis was mainly explained by C/N and Fe content, and the third axis was mainly explained by P_2O_5 content and elevation (**Table 1**).

The use of Hill number allowed to compare the response of all fungi including rare species, more common species and the more abundant ones. In line with our predictions, habitat coverage was significantly and positively related to fungal OTU richness and with OTU richness of the three fungal trophic groups (**Table 2**) while patch area had no effect on OTU diversity. Such pattern did not emerge among fungi for common OTUs (^{1}D) and dominant OTUs (^{2}D). However, the patch area was significantly and positively related to the Simpson diversity (^{2}D) of saprotrophs (**Table 2**). Regarding bacteria, whatever the Hill number considered, we did not detect any significant relationship between diversity and habitat amount or patch area.

On the other hand, for all taxa and trophic groups, the first component of PCA was the main driver of OTU diversity (**Table 2**). This axis was mainly driven by soil CEC, other cations concentration (Ca, Mg, K). The second axis, driven by Fe concentration and C/N ratio, only correlated with bacterial Shannon diversity. The third axis, mainly driven by phosphate concentration and elevation, was significantly and negatively related with OTU richness of all fungi, and each trophic group, but not for bacteria (**Table 2**). Whereas this correlation was also observed for common and dominant symbiotrophic OTUs, no relationship emerged for pathotrophs and saprotrophs when decreasing the importance of rare species on diversity indices (**Table 2**). Finally, for all indices and taxonomic or functional groups, the effect of PCA axis was always more important and significant than the habitat amount effect, showing that both factors contribute to shape microorganism diversity but that soil factors are also extremely important locally.

TABLE 2 | Results from regression analyses.

		Mean	SE	Intercept		Habitat amount		Patch	area	PCA dim1		PCA dim2		PCA dim3	
				z	*p*	*z*	*p*	*z*	*p*	*z*	*p*	*z*	*p*	*z*	*p*
All fungi	0D (Richness)	803.37	28.7	**274.96**	**< 0.001**	**2.927**	**0.003**	−0.072	0.942	**5.215**	**< 0.001**	−1.035	0.301	**−2.504**	**0.012**
	^{1}D (Shannon diversity)	72.74	3.21	**129.543**	**< 0.001**	0.238	0.812	0.593	0.553	**3.987**	**< 0.001**	−0.359	0.72	−1.516	0.129
	^{2}D (Simpson diversity)	29.44	1.69	**71.518**	**< 0.001**	−0.656	0.512	0.849	0.396	**2.027**	**0.043**	−0.792	0.428	**−2.069**	**0.039**
Symbiotrophs	0D (Richness)	309.89	9.47	**246.549**	**< 0.001**	**2.039**	**0.041**	−0.591	0.554	**3.757**	**< 0.001**	−1.054	0.292	**−2.637**	**0.008**
	^{1}D (Shannon diversity)	38.38	1.53	**114.897**	**< 0.001**	0.485	0.628	−0.88	0.379	**3.656**	**< 0.001**	−0.283	0.777	**−2.302**	**0.021**
	^{2}D (Simpson diversity)	19.31	1.05	**64.887**	**< 0.001**	−0.608	0.543	0.914	0.361	**1.995**	**0.046**	−0.257	0.797	**−2.333**	**0.02**
Saprotrophs	0D (Richness)	227.74	9.41	**195.24**	**< 0.001**	**2.879**	**0.004**	0.261	0.794	**5.352**	**< 0.001**	−0.592	0.554	**−2.229**	**0.026**
	^{1}D (Shannon diversity)	40.33	3.09	**79.487**	**< 0.001**	−0.719	0.472	1.912	0.056	**6.336**	**< 0.001**	1.789	0.074	1.047	0.295
	^{2}D (Simpson diversity)	18.51	1.71	**43.553**	**< 0.001**	−1.458	0.145	**2**	**0.045**	**4.666**	**< 0.001**	1.512	0.13	1.26	0.208
Pathotrophs	0D (Richness)	75.3	2.91	**192.046**	**< 0.001**	**2.918**	**0.004**	0.855	0.393	**6.486**	**< 0.001**	−1.117	0.264	**−3.895**	**< 0.001**
	^{1}D (Shannon diversity)	26.8	1.27	**75.749**	**< 0.001**	0.718	0.473	0.733	0.463	**2.937**	**0.003**	−0.18	0.857	−0.945	0.345
	^{2}D (Simpson diversity)	14.23	1.02	**36.35**	**< 0.001**	−1.619	0.105	0.513	0.608	1.548	0.122	0.888	0.375	0.275	0.784
Bacteria	0D (Richness)	3281.11	78.66	**381.692**	**< 0.001**	0.735	0.462	−1.505	0.132	**2.764**	**0.006**	1.047	0.295	−1.671	0.095
	^{1}D (Shannon diversity)	405.74	15.54	**282.111**	**< 0.001**	−0.177	0.86	1.194	0.233	**6.091**	**< 0.001**	**2.295**	**0.022**	−1.785	0.074
	^{2}D (Simpson diversity)	97.99	5.11	**139.218**	**< 0.001**	0.637	0.524	1.36	0.174	**4.795**	**< 0.001**	1.653	0.098	−1.862	0.063

Values in boldfaces are significant ($P < 0.05$).

Habitat Amount Effect on Community Composition

The composition of communities was only affected by the three PCA axis but not by the habitat amount nor the patch area (**Table 3**). All fungal, symbiotrophic and saprotrophic communities were shaped by the three PCA axis, while bacterial communities were shaped by the two first axis, and pathotrophic fungi only by the first one.

Spatial Scale of Effect

The scale of effects (of the habitat amount) was variable according to Hill numbers and the group considered (**Figure 3**). As a general pattern, the scale of effect increased with increasing the weight of rare species in diversity indices (**Figure 3**, see arrows). Our landscapes were initially delineated in a radius of 500 m, which seems relevant to explain variations in ^{2}D Hill number (inverse of Simson index) of all micro-organisms but saprotrophic fungi. Variation in species number, on which HAH was based, are on the contrary better explained by landscape structure delineated with a radius greater than 500 m (at least for all fungal OTUs, saprotrophic and symbiotrophic ones).

Ancient vs Actual Forest Cover

For all models with a significant effect of habitat amount, the models based on ancient forest cover had lower AICc than those based on contemporary forest cover (**Table 4**). This pattern was observed for all taxonomic and functional groups, but the difference of AICc was more important for saprotrophs and pathotrophs.

DISCUSSION

While there are debates surrounding how fungi can be affected by fragmentation (Grilli et al., 2017), our study showed, for the first time, that habitat coverage in the landscape is a driver of the species richness of local communities in forest patches. We did not confirm this pattern for bacteria, nor for the more abundant species (according to Hill numbers), but showed that both ectomycorrhizal (the main symbiotrophic fungi in our analysis), pathotrophic and saprotrophic fungi responded to the amount of ancient forest in the landscape. This common trend for fungi probably contrasts with the differences in dispersal strategy among fungi (Douhan et al., 2011). However, all agree that most spores disperse locally (Adams et al., 2013). This result follows the observations of Peay et al. (2012), who measured the dispersal of ectomycorrhizal fungi from 1 m to 10 km, and showed a rapid decrease in colonization success with increasing distance to the source. Edman et al. (2004) also confirmed that local sources were particularly important for wood decaying fungi and recommended maintaining local sources of dead wood, paying attention to the vicinity of forest patches. Interestingly, this trend was not confirmed when decreasing the weight of rare species (see results for Shannon and Simpson indices), suggesting that rare species would be more sensitive to the habitat coverage and changes in landscape structure. For wood-inhabiting species, a similar pattern has been observed, and Nordén et al. (2013)

TABLE 3 | Results from distance-based redundancy analysis (db-RDA) using Bray-Curtis dissimilarity.

	Conditional inertia (%)	Constrained intertia (%)	Habitat amount		Patch area		PCA dim1		PCA dim2		PCA dim3	
			F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
All fungi	26	24	1.05	0.377	1.11	0.312	**1.57**	**0.014**	**1.72**	**0.009**	**1.52**	**0.016**
Symbiotrophs	25	25	1.09	0.357	1.37	0.083	**1.53**	**0.017**	**1.66**	**0.006**	**1.45**	**0.038**
Pathotrophs	28	23	1.14	0.25	0.86	0.688	1.37	0.097	**1.97**	**0.005**	1.43	0.064
Saprotrophs	26	24	0.98	0.459	0.78	0.769	**1.92**	**0.006**	**1.91**	**0.007**	**1.97**	**0.004**
Bacteria	24	25	0.95	0.549	0.94	0.572	**2.15**	**0.002**	**1.98**	**0.002**	1.34	0.088

Values in boldfaces are significant ($P < 0.05$).

TABLE 4 | Models comparisons, with AICc, when using either ancient or contemporary forest cover as habitat amount.

Response variables		AICc	
		Ancient Forest	Contemporary Forest
All fungi	^{0}D (Richness)	345.33	352.31
	^{1}D (Shannon diversity)	231.5	230.79
	^{2}D (Simpson diversity)	199.47	198.51
Symbiotrophs	^{0}D (Richness)	292.19	294.81
	^{1}D (Shannon diversity)	187.62	191.45
	^{2}D (Simpson diversity)	168.36	172.04
Saprotrophs	^{0}D (Richness)	284.65	292.06
	^{1}D (Shannon diversity)	215.62	217.38
	^{2}D (Simpson diversity)	189.59	190.67
Pathotrophs	^{0}D (Richness)	208.69	221.11
	^{1}D (Shannon diversity)	195.5	196.83
	^{2}D (Simpson diversity)	184.83	184.95
Bacteria	^{0}D (Richness)	414.34	414.85
	^{1}D (Shannon diversity)	301.01	301.79
	^{2}D (Simpson diversity)	246.1	247.3

showed that rare specialist fungi were more limited by their habitat loss than generalist fungi. As the importance of rare species may also be lowered with Bray-Curtis distances, this would also explain why we did not detect any relationship between habitat coverage and the composition of communities.

Conversely, the diversity of bacterial communities was not affected by the habitat coverage of ancient forests, but rather shaped by local environmental factors. While the habitat amount hypothesis has never been investigated *per se* on bacterial communities, early studies have already shown the importance of habitat fragmentation and patch size in experimental conditions (Burkey, 1997; Gilbert et al., 1998). Interestingly, a recent study of fragmented landscapes in Chile (Almasia et al., 2016) also highlighted the importance of local environmental factors but not historical factors, confirming that present day conditions would be more important than historical ones. This lack of response by bacteria to ancient forest quantity could also be explained by their high turnover and short life span. Some bacteria are able to be dormant (Kaprelyants et al., 1993), but this trait is not widespread, and might have been selected rather in extreme environments or at high elevations (Jones and Lennon, 2010; Pozzi et al., 2015). In the Cevennes national park, soil conditions are not extreme and may not select for dormant bacteria, which may reduce their sensitivity to past forest conditions. When considering bacterial dispersal, several studies agree that terrestrial bacteria are able to disperse over relatively long distances (Smith et al., 2013), greater than fungi (Chemidlin Prévost-Bouré et al., 2014; Vacher et al., 2016), but are still locally limited by environmental conditions (Horner-Devine et al., 2004). Our study corroborates the importance of local soil parameters in shaping bacterial diversity without definitively rejecting the HAH, which could be investigated in more contrasted soil landscapes (e.g., agricultural vs forest soils).

By revealing that past forest cover better explains variation of species diversity than recent forest cover does, our study raises questions on the persistence of past landscape structure and its effect. Numerous clues have accumulated for plant communities (Nordén et al., 2014), even in Amazonian forests where botanists have now revealed that plant diversity is higher close to pre-Colombian sites (Levis et al., 2017). Such legacy effects are therefore acknowledged for plants, especially for trees due to their long lifespan. For fungi, the persistence of effects raises questions, for example on fungal longevity in soil. Estimating the age of fungal genets is rarely done, and requires knowledge of growth rates, but Smith et al. (1992) revealed that a *Armillaria bulbosa* genet remained genetically stable over 1500 years. Interestingly, this fungus is also parasitic, illustrating that a fungus can survive longer than its host. In the case of ancient beech forests, fungal genets could be sampled to test if their age compared to recent forests, and therefore to determine if part of the diversity could be attributed to past conditions. A study of lichens and fungi associated with old oaks have revealed an in-between trend: their diversity was both shaped by past and recent conditions (Ranius et al., 2008a), but older oaks hosted the richer communities (Ranius et al., 2008a,b). Similarly, for plants, past fragmentation particularly affects the current distribution of only slow colonizing species (Vellend et al., 2006) causing a long-lasting extinction debt, which is possibly never paid (Dupouey et al., 2002). The same pattern may be true for fungi, demonstrating the need for further studies of fungal traits and especially their dispersal abilities in order to help understand differences of extinction debt observed between fungi (e.g., Berglund and Jonsson, 2005).

Beyond those distinct patterns for bacteria and fungi, our study has revealed new landscape effects on soil communities, and highlighted the interest of conserving large amount of

ancient forests in the vicinity of recent forests to favor fungal diversity in soil. Interestingly, the results of a study of English forests by Spake et al. (2016) already demonstrate the benefit in conserving patches of ancient forests close to younger forests, when they detected a high similarity of fungal diversity and community composition between closely located pairs of forests, both dominated by oaks. All these results raise the question as to whether ancient forests could act as sources for fungal dispersal. The study of Bidartondo et al. (2001) in and near ancient forests of bristlecone pine revealed a low diversity of ectomycorrhizal inoculum, contrasting with the idea that old-growth forests would host more species, and at least "indicator" species, specifically associated with old trees. While the literature on fungal indicators is rich for wood-decaying fungi, results for ectomycorrhizal fungi are more contrasted. For example, Dahlberg et al. (1997) and Richard et al. (2005) have revealed a high diversity of ectomycorrhizal species in boreal and Mediterranean old-growth forests, respectively. A large-scale inventory in Czechia recently highlighted the correlation between ancient forests plants and fungal diversity, and suggested that ancient forest plants could be used to predict fungal richness, especially for red-listed species (Hofmeister et al., 2014), but rarely for ectomycorrhizal fungi. These results may depend on dominant tree species, as Boeraeve et al. (2018a) revealed that only late successional tree species host more diverse ectomycorrhizal communities in the oldest forests.

Our results, based on soil metabarcoding, suggest that nearly all fungi could be favored by greater coverage of ancient forest, and in particular highlight the strong effect on ectomycorrhizal fungi, dominant in our samples and more generally in beech-forest soils (Guinberteau, 2011; Coince et al., 2013). Using such techniques could therefore revolutionize the debate surrounding indicator species for ecological continuity (Rolstad et al., 2002) and allow for the comparison of diverse types of ancient forests, under different hosts. On the other hand, indicator species will still be useful for an above-ground diagnostic, since most sequences cannot be identified at the species level with metabarcoding techniques. For example, while Russulaceae and Cortinariaceae were the more abundant families, typical beech-associated fungi (mentioned by Guinberteau, 2011), such as *Russula fageticola, R. faginea, R. rubroalba, R. mairei, Cortinarius montanus var. fageticola*, and *C. amoenolens* were not detected with our short markers. Moreover, the cost of metabarcoding can remain high for a single inventory, and decreases only if several samples are collected (e.g., Zinger et al., 2016). Finally, if wood-decaying fungi are considered as good indicators, wood samples could be used to detect their presence with metabarcoding (e.g., Ovaskainen et al., 2010), and therefore make more comparisons with the existing literature on fungal diversity in ancient or old-growth forests than our study based on soil sampling.

Our study focused on soil microorganism diversity patterns, and revealed that both soil conditions and habitat coverage in a landscape shaped fungal diversity in the forests of the Cevennes National Park, and that bacterial communities were only shaped by soil conditions. While results on fungi are sometimes contradictory in the literature (Grilli et al., 2017), we detected a common pattern for all fungal guilds, showing how strong the effect of habitat coverage can be. Our results provide a synthetic view and show the importance of conserving any ancient forest patch, whatever its size, for soil biodiversity conservation. These results are of interest for park management but also for urban areas, where isolated patches of forest can have persisted over time and host unique fungi communities (Vogt-Schilb et al., 2018). Environmental sequencing could now be applied to produce comprehensive soil inventories, and study isolated systems, for example in urban areas or dry ecosystems, where patches of forests are particularly threatened and fungi could contribute to their functioning and connectivity, and even resilience when facing climate change (Gilliam, 2016).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: DRYAD Repository (https://doi.org/10.5061/dryad.hmgqnk9c8).

AUTHOR CONTRIBUTIONS

SMe, FK, MR, and SMa handled the field work. SMe and SMa did the extractions and molecular biology. FK handled the soil analysis. SMe and SMa did the bioinformatic analyses. SMe and AB did the statistical analyses. All authors contributed to discussion of the results and the writing of the final document.

FUNDING

This research was financially supported by FEDER, FNADT, and EI-PURPAN. We also benefitted from the support of "Investissements d'Avenir" grants managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01; ANAEE-France: ANR-11-INBS-0001, TULIP: ANR-10-LABX-0041).

ACKNOWLEDGMENTS

This research was conducted at the Cevennes National Park as part of the national action plan to preserve ancient forests and financially supported by FEDER, FNADT, and EI-PURPAN. We thank Mathieu Baconnet and Sophie Giraud for their advice, Patricia Jargeat for this help with DNA extraction and PCR, Francois Calatayud for his assistance with GIS, Michel Goulard and Magali San-Cristobal for their help with statistics. We finally thank Alain Roy and Julian Donald for reading our manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.00118/full#supplementary-material

REFERENCES

Abrego, N., and Salcedo, I. (2014). Response of wood-inhabiting fungal community to fragmentation in a beech forest landscape. *Fungal Ecol.* 8, 18–27.

Adams, R. I., Miletto, M., Taylor, J. W., and Bruns, T. D. (2013). Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. *ISME J.* 7, 1262–1273. doi: 10.1038/ismej.2013.28

Alberdi, A., and Gilbert, M. T. P. (2019). hilldiv: an R package for the integral analysis of diversity based on Hill numbers. bioRxiv [Preprint]. doi: 10.1101/545665

Almasia, R., Carú, M., Handford, M., and Orlando, J. (2016). Environmental conditions shape soil bacterial community structure in a fragmented landscape. *Soil Biol. Biochem.* 103, 39–45.

Altieri, M. A. (1999). The ecological role of biodiversity in agroecosystems. *Agric. Ecosyst. Environ.* 74, 19–31.

Bani, A., Pioli, S., Ventura, M., Panzacchi, P., Borruso, L., Tognetti, R., et al. (2018). The role of microbial community in the decomposition of leaf litter and deadwood. *Appl. Soil Ecol.* 126, 75–84.

Bartoñ, K. (2019). *Package 'MuMIn'*. Available online at: https://CRAN.R-project.org/package=MuMIn (accessed April 15, 2020).

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Bergès, L., Avon, C., Arnaudet, L., Archaux, F., Chauchard, S., and Dupouey, J.-L. (2016). Past landscape explains forest periphery-to-core gradient of understorey plant communities in a reforestation context. *Divers. Distrib.* 22, 3–16. doi: 10.1111/ddi.12384

Bergès, L., and Dupouey, J.-L. (2017). Écologie historique et ancienneté de l'état boisé: concepts, avancées et perspectives de la recherche. *Revue Forestière Française* 4–5, 297–318. doi: 10.4267/2042/67863

Berglund, H., and Jonsson, B. G. (2005). Verifying an extinction debt among lichens and fungi in northern Swedish boreal forests. *Conserv. Biol.* 19, 338–348. doi: 10.1111/j.1523-1739.2005.00550.x

Bidartondo, M. I., Baar, J., and Bruns, T. D. (2001). Low ectomycorrhizal inoculum potential and diversity from soils in and near ancient forests of bristlecone pine (Pinus longaeva). *Can. J. Bot.* 79, 293–299. doi: 10.1139/b01-006

Birkhofer, K., Schöning, I., Alt, F., Herold, N., Klarner, B., Maraun, M., et al. (2012). General relationships between abiotic soil properties and soil biota across spatial scales and different land-use types. *PLoS One* 7:e43292. doi: 10.1371/journal.pone.0043292

Boeraeve, M., Honnay, O., and Jacquemyn, H. (2018a). Effects of host species, environmental filtering and forest age on community assembly of ectomycorrhizal fungi in fragmented forests. *Fungal Ecol.* 36, 89–98.

Boeraeve, M., Honnay, O., Mullens, N., Vandekerkhove, K., De Keersmaeker, L., Thomaes, A., et al. (2018b). The impact of spatial isolation and local habitat conditions on colonization of recent forest stands by ectomycorrhizal fungi. *For. Ecol. Manag.* 429, 84–92.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., and Coissac, E. (2016). obitools: a unix-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* 16, 176–182. doi: 10.1111/1755-0998.12428

Brennan, J., Darren, J., Contreras, T. A., and Fahrig, L. (2002). "Focal patch landscape studies for wildlife management: optimizing sampling effort across scales," in *Integrating Landscape Ecology into Natural Resource Management*, eds J. Liu and W. W. Taylor (New York, NY: Cambridge University Press), 68–91.

BRGM (2008). *Carte Lithologiquesimplifiée ® [WGS raster].1: 1 000 000*. Orléans: Bureau de Recherches Géologiques et Minières.

Burkey, T. V. (1997). Metapopulation extinction in fragmented landscapes: using bacteria and protozoa communities as model ecosystems. *Am. Nat.* 150, 568–591. doi: 10.1086/286082

Cateau, E., Larrieu, L., Vallauri, D., Savoie, J.-M., Touroult, J., and Brustel, H. (2015). Ancienneté et maturité: deux qualités complémentaires d'un écosystème forestier. *C. R. Biol.* 338, 58–73.

Chao, A., and Jost, L. (2012). Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology* 93, 2533–2547. doi: 10.1890/11-1952.1

Chemidlin Prévost-Bouré, N., Dequiedt, S., Thioulouse, J., Lelièvre, M., Saby, N. P. A., Jolivet, C., et al. (2014). Similar processes but different environmental filters for soil bacterial and fungal community composition turnover on a broad spatial scale. *PLoS One* 9:e111667. doi: 10.1371/journal.pone.0111667

Christensen, M., Heilmann-Clausen, J., Walleyn, R., and Adamcik, S. (2005). "Wood-inhabiting fungi as indicators of nature value in European beech forests," in *Proceeding of the Monitoring and Indicators of Forest Biodiversity in Europe–From Ideas to Operationality*, Florence, 229.

Coince, A., Caël, O., Bach, C., Lengellé, J., Cruaud, C., Gavory, F., et al. (2013). Below-ground fine-scale distribution and soil versus fine root detection of fungal and soil oomycete communities in a French beech forest. *Fungal Ecol.* 6, 223–235.

Culman, S. W., Young-Mathews, A., Hollander, A. D., Ferris, H., Sánchez-Moreno, S., O'Geen, A. T., et al. (2010). Biodiversity is associated with indicators of soil ecosystem functions over a landscape gradient of agricultural intensification. *Landsc. Ecol.* 25, 1333–1348.

Dahlberg, A., Jonsson, L., and Nylund, J.-E. (1997). Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. *Can. J. Bot.* 75, 1323–1335. doi: 10.1139/b97-844

de Vries, F. T., Manning, P., Tallowin, J. R., Mortimer, S. R., Pilgrim, E. S., Harrison, K. A., et al. (2012). Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecol. Lett.* 15, 1230–1239. doi: 10.1111/j.1461-0248.2012.01844.x

Diedhiou, A. G., Dupouey, J.-L., Buee, M., Dambrine, E., Laüt, L., and Garbaye, J. (2009). Response of ectomycorrhizal communities to past Roman occupation in an oak forest. *Soil Biol. Biochem.* 41, 2206–2213.

Diedhiou, A. G., Dupouey, J.-L., Buée, M., Dambrine, E., Laüt, L., and Garbaye, J. (2010). The functional structure of ectomycorrhizal communities in an oak forest in central France witnesses ancient Gallo-Roman farming practices. *Soil Biol. Biochem.* 42, 860–862.

Douhan, G. W., Vincenot, L., Gryta, H., and Selosse, M.-A. (2011). Population genetics of ectomycorrhizal fungi: from current knowledge to emerging directions. *Fungal Biol.* 115, 569–597. doi: 10.1016/j.funbio.2011.03.005

Dray, S., Legendre, P., and Peres-Neto, P. R. (2006). Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecol. Model.* 196, 483–493. doi: 10.1016/j.ecolmodel.2006.02.015

Dupouey, J. L., Dambrine, E., Laffite, J. D., and Moares, C. (2002). Irreversible impact of past land use on forest soils and biodiversity. *Ecology* 83, 2978–2984.

Dvořák, D., Vašutová, M., Hofmeister, J., Beran, M., Hošek, J., Běták, J., et al. (2017). Macrofungal diversity patterns in central European forests affirm the key importance of old-growth forests. *Fungal Ecol.* 27, 145–154. doi: 10.1016/j.funeco.2016.12.003

Edman, M., Kruys, N., and Jonsson, B. G. (2004). Local dispersal sources strongly affect colonization patterns of wood-decaying fungi on spruce logs. *Ecol. Appl.* 14, 893–901. doi: 10.1890/03-5103

Epp, L. S., Boessenkool, S., Bellemain, E. P., Haile, J., Esposito, A., Riaz, T., et al. (2012). New environmental metabarcodes for analysing soil DNA: potential for studying past and present ecosystems. *Mol. Ecol.* 21, 1821–1833. doi: 10.1111/j.1365-294X.2012.05537.x

Fahrig, L. (2013). Rethinking patch size and isolation effects: the habitat amount hypothesis. *J. Biogeogr.* 40, 1649–1663.

Fahrig, L. (2015). Just a hypothesis: a reply to Hanski. *J. Biogeogr.* 42, 993–994.

Ficetola, G., Coissac, E., Zundel, S., Riaz, T., Shehzad, W., Bessière, J., et al. (2010). An In silico approach for the evaluation of DNA barcodes. *BMC Genomics* 11:434. doi: 10.1186/1471-2164-11-434

Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., Jones, R., et al. (2007a). Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. *Appl. Environ. Microbiol.* 73, 7059–7066. doi: 10.1128/AEM.00358-07

Fierer, N., Morse, J. L., Berthrong, S. T., Bernhardt, E. S., and Jackson, R. B. (2007b). Environmental controls on the landscape-scale biogeography of stream bacterial communities. *Ecology* 88, 2162–2173. doi: 10.1890/06-1746.1

Fierer, N., and Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. U.S.A.* 103, 626–631. doi: 10.1073/pnas.0507535103

Fliegerova, K., Tapio, I., Bonin, A., Mrazek, J., Callegari, M. L., Bani, P., et al. (2014). Effect of DNA extraction and sample preservation method on rumen bacterial population. *Anaerobe* 29, 80–84. doi: 10.1016/j.anaerobe.2013.09.015

Gilbert, F., Gonzalez, A., and Evans-Freke, I. (1998). Corridors maintain species richness in the fragmented landscapes of a microecosystem. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 265, 577–582. doi: 10.1098/rspb.1998.0333

Gilliam, F. S. (2016). Forest ecosystems of temperate climatic regions: from ancient use to climate change. *New Phytol.* 212, 871–887. doi: 10.1111/nph.14255

Grilli, G., Longo, S., Huais, P. Y., Pereyra, M., Verga, E. G., Urcelay, C., et al. (2017). Fungal diversity at fragmented landscapes: synthesis and future perspectives. *Curr. Opin. Microbiol.* 37, 161–165. doi: 10.1016/j.mib.2017.09.003

Grilli, G., Urcelay, C., Galetto, L., Davison, J., Vasar, M., Saks, Ü, et al. (2015). The composition of arbuscular mycorrhizal fungal communities in the roots of a ruderal forb is not related to the forest fragmentation process. *Environ. Microbiol.* 17, 2709–2720. doi: 10.1111/1462-2920.12623

Guinberteau, J. (2011). Contribution to the knowledge of the mycoflora of the beech grove relic of Ciron under a landais climate. *Bull. Mycol. Botan Dauphiné Savoie* 51, 23–38.

Halme, P., Holec, J., and Heilmann-Clausen, J. (2017). The history and future of fungi as biodiversity surrogates in forests. *Fungal Ecol.* 27, 193–201. doi: 10.1016/j.funeco.2016.10.005

Haddad, N. M., Gonzalez, A., Brudvig, L. A., Burt, M. A., Levey, D. J., and Damschen, E. I. (2017). Experimental evidence does not support the habitat amount Hypothesis. *Ecography* 40, 48–55. doi: 10.1111/ecog.02535

Hanski, I. (2015). Habitat fragmentation and species richness. *J. Biogeogr.* 42, 989–993.

Harding, J. S., Benfield, E. F., Bolstad, P. V., Helfman, G. S., and Jones, E. B. D. (1998). Stream biodiversity: the ghost of land use past. *Proc. Natl. Acad. Sci. U.S.A.* 95, 14843–14847. doi: 10.1073/pnas.95.25.14843

Helander, M., Ahlholm, J., Sieber, T. N., Hinneri, S., and Saikkonen, K. (2007). Fragmented environment affects birch leaf endophytes. *New Phytol.* 175, 547–553. doi: 10.1111/j.1469-8137.2007.02110.x

Hermy, M., Honnay, O., Firbank, L., Grashof-Bokdam, C., and Lawesson, J. E. (1999). An ecological comparison between ancient and other forest plant species of Europe, and the implications for forest conservation. *Biol. Conserv.* 91, 9–22. doi: 10.1016/S0006-3207(99)00045-42

Hill, M. O. (1973). Diversity and evenness: a unifying notation and its consequences. *Ecology* 54, 427–432. doi: 10.2307/1934352

Hobara, S., Osono, T., Hirose, D., Noro, K., Hirota, M., and Benner, R. (2014). The roles of microorganisms in litter decomposition and soil formation. *Biogeochemistry* 118, 471–486.

Hofmeister, J., Hošek, J., Brabec, M., Dvořák, D., Beran, M., Deckerová, H., et al. (2014). Richness of ancient forest plant species indicates suitable habitats for macrofungi. *Biodivers. Conserv.* 23, 2015–2031.

Horner-Devine, M. C., Lage, M., Hughes, J. B., and Bohannan, B. J. (2004). A taxa–area relationship for bacteria. *Nature* 432:750.

Hsieh, T. C., Ma, K. H., and Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (H ill numbers). *Methods Ecol. Evol.* 7, 1451–1456. doi: 10.1111/2041-210X.12613

IGN (1996). *BD ALTI ® [MNT]. 1: 25 000*. Saint-Mandé: Institut National de l'information Géographique et forestière.

IGN (2011). *SCAN Etat-Major® [TIF6.0]. 1: 40 000*. Saint-Mandé: Institut National de l'information Géographique et forestière.

Jackson, H. B., and Fahrig, L. (2015). Are ecologists conducting research at the optimal scale: Is research conducted at optimal scales? *Glob. Ecol. Biogeogr.* 24, 52–63. doi: 10.1111/geb.12233

Jones, S. E., and Lennon, J. T. (2010). Dormancy contributes to the maintenance of microbial diversity. *Proc. Natl. Acad. Sci. U.S.A.* 107, 5881–5886. doi: 10.1073/pnas.0912765107

Kaprelyants, A., Gottschal, J. C., and Kell, D. B. (1993). Dormancy in non-sporulating bacteria. *FEMS Microbiol. Lett.* 104, 271–285. doi: 10.1016/0378-1097(93)90600-90607

Kuussaari, M., Bommarco, R., Heikkinen, R. K., Helm, A., Krauss, J., Lindborg, R., et al. (2009). Extinction debt: a challenge for biodiversity conservation. *Trends Ecol. Evol.* 24, 564–571. doi: 10.1016/j.tree.2009.04.011

Lauber, C. L., Hamady, M., Knight, R., and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl. Environ. Microbiol.* 75, 5111–5120. doi: 10.1128/AEM.00335-339

Levis, C., Costa, F. R., Bongers, F., Peña-Claros, M., Clement, C. R., Junqueira, A. B., et al. (2017). Persistent effects of pre-Columbian plant domestication on Amazonian forest composition. *Science* 355, 925–931. doi: 10.1126/science.aal0157

Lindgren, J. P., and Cousins, S. A. (2017). Island biogeography theory outweighs habitat amount hypothesis in predicting plant species richness in small grassland remnants. *Landsc. Ecol.* 32, 1895–1906.

Lucas, R., Groeneveld, J., Harms, H., Johst, K., Frank, K., and Kleinsteuber, S. (2017). A critical evaluation of ecological indices for the comparative analysis of microbial communities based on molecular datasets. *FEMS Microbiol. Ecol.* 93:fiw209. doi: 10.1093/femsec/fiw209

Martin, C. A. (2018). An early synthesis of the habitat amount hypothesis. *Landsc. Ecol.* 33, 1831–1835. doi: 10.1007/s10980-018-0716-y

Melo, G. L., Sponchiado, J., Cáceres, N. C., and Fahrig, L. (2017). Testing the habitat amount hypothesis for South American small mammals. *Biol. Conserv.* 209, 304–314.

Nagati, M., Roy, M., Manzi, S., Richard, F., Desrochers, A., Gardes, M., et al. (2018). Impact of local forest composition on soil fungal communities in a mixed boreal forest. *Plant Soil* 432, 345–357.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., et al. (2016). FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* 20, 241–248.

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. Bioinformatics* 4, 193–201. doi: 10.4137/ebo.s653

Nordén, B., Dahlberg, A., Brandrud, T. E., Fritz, Ö, Ejrnaes, R., and Ovaskainen, O. (2014). Effects of ecological continuity on species richness and composition in forests and woodlands: a review. *Écoscience* 21, 34–45. doi: 10.2980/21-1-3667

Nordén, J., Åström, J., Josefsson, T., Blumentrath, S., Ovaskainen, O., Sverdrup-Thygeson, A., et al. (2018). At which spatial and temporal scales can fungi indicate habitat connectivity? *Ecol. Indic.* 91, 138–148. doi: 10.1186/s13071-017-2482-0

Nordén, J., Penttilä, R., Siitonen, J., Tomppo, E., and Ovaskainen, O. (2013). Specialist species of wood-inhabiting fungi struggle while generalists thrive in fragmented boreal forests. *J. Ecol.* 101, 701–712.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., et al. (2019). *vegan: Community Ecology Package. R Package Version 2.5–6*. Available online at: http://CRAN.R-project.org/package=vegan (accessed September 1, 2019).

Ovaskainen, O., Nokso-Koivisto, J., Hottola, J., Rajala, T., Pennanen, T., Ali-Kovero, H., et al. (2010). Identifying wood-inhabiting fungi with 454 sequencing–what is the probability that BLAST gives the correct species? *Fungal Ecol.* 3, 274–283.

Parmasto, E. (2001). "Fungi as indicators of primeval and old-growth forests deserving protection," in *Fungal Conservation Issues and Solutions*, eds D. Moore, M. M. Nauta, S. E. Evans, and M. Rotheroe (Cambridge: Cambridge University Press), 81–88.

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. doi: 10.1111/j.1365-294X.2012.05666.x

Pozzi, A. C., Bautista-Guerrero, H. H., Nouioui, I., Cotin-Galvan, L., Pepin, R., Fournier, P., et al. (2015). In-planta sporulation phenotype: a major life history trait to understand the evolution of A lnus-infective F rankia strains. *Environ. Microbiol.* 17, 3125–3138. doi: 10.1111/1462-2920.12644

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2012). The SILVA ribosomal RNA genedatabaseproject: improved data processing and web-basedtools. *Nucleic Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219

R Core Team (2019). *A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Ranius, T., Eliasson, P., and Johansson, P. (2008a). Large-scale occurrence patterns of red-listed lichens and fungi on old oaks are influenced both by current and historical habitat density. *Biodivers. Conserv.* 17, 2371–2381.

Ranius, T., Johansson, P., Berg, N., and Niklasson, M. (2008b). The influence of tree age and microhabitat quality on the occurrence of crustose lichens associated with old oaks. *J. Veg. Sci.* 19, 653–662. doi: 10.3170/2008-8-18433

Richard, F., Millot, S., Gardes, M., and Selosse, M.-A. (2005). Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by Quercus ilex. *New Phytol.* 166, 1011–1023. doi: 10.1111/j.1469-8137.2005.01382.x

Rolstad, J., Gjerde, I., Gundersen, V. S., and Saetersdal, M. (2002). Use of indicator species to assess forest continuity: a critique. *Conserv. Biol.* 16, 253–257. doi: 10.1046/j.1523-1739.2002.00552.x

Schrott, G. R., With, K. A., and King, A. W. (2005). Demographic limitations of the ability of habitat restoration to rescue declining populations: demographic limits of habitat restoration. *Conserv. Biol.* 19, 1181–1193. doi: 10.1111/j.1523-1739.2005.00205.x

Seibold, S., Bässler, C., Brandl, R., Fahrig, L., Förster, B., Heurich, M., et al. (2017). An experimental test of the habitat-amount hypothesis for saproxylic beetles in a forested region. *Ecology* 98, 1613–1622. doi: 10.1002/ecy.1819

Smith, D. J., Timonen, H. J., Jaffe, D. A., Griffin, D. W., Birmele, M. N., Perry, K. D., et al. (2013). Intercontinental dispersal of bacteria and archaea by transpacific winds. *Appl. Environ. Microbiol.* 79, 1134–1139. doi: 10.1128/AEM.03029-12

Smith, M. L., Bruhn, J. N., and Anderson, J. B. (1992). The fungus Armillaria bulbosa is among the largest and oldest living organisms. *Nature* 356, 428–431. doi: 10.1038/356428a0

Spake, R., van der Linde, S., Newton, A. C., Suz, L. M., Bidartondo, M. I., and Doncaster, C. P. (2016). Similar biodiversity of ectomycorrhizal fungi in set-aside plantations and ancient old-growth broadleaved forests. *Biol. Conserv.* 194, 71–79. doi: 10.1016/j.biocon.2015.12.003

Taberlet, P., Bonin, A., Zinger, L., and Coissac, E. (2018). *Environmental DNA: For Biodiversity Research and Monitoring*. Oxford: Oxford University Press.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., and Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Mol. Ecol.* 21, 2045–2050. doi: 10.1111/j.1365-294X.2012.05470.x

Vacher, C., Cordier, T., and Vallance, J. (2016). Phyllosphere fungal communities differentiate more thoroughly than bacterial communities along an elevation gradient. *Microb. Ecol.* 72, 1–3. doi: 10.1007/s00248-016-0742-748

Vannette, R. L., Leopold, D. R., and Fukami, T. (2016). Forest area and connectivity influence root-associated fungal communities in a fragmented landscape. *Ecology* 97, 2374–2383. doi: 10.1002/ecy.1472

Vellend, M., Verheyen, K., Jacquemyn, H., Kolb, A., Van Calster, H., Peterken, G., et al. (2006). Extinction debt of forest plants persists for more than a century following habitat fragmentation. *Ecology* 87, 542–548. doi: 10.1890/05-1182

Vincenot, L., and Selosse, M.-A. (2017). "Population biology and ecology of ectomycorrhizal fungi," in *Biogeography of Mycorrhizal Symbiosis*, ed. L. Tedersoo (Cham: Springer International Publishing AG), 39–59.

Vogt-Schilb, H., Moreau, P.-A., Malaval, J.-C., Schatz, B., and Richard, F. (2018). Effects of long-term landscape dynamics and city growth on biodiversity in the Mediterranean: the case study of Montpellier, France. *Urban Ecosyst.* 21, 921–932. doi: 10.1007/s11252-018-0780-788

Zinger, L., Chave, J., Coissac, E., Iribar, A., Louisanna, E., Manzi, S., et al. (2016). Extracellular DNA extraction is a fast, cheap and reliable alternative for multi-taxa surveys based on soil DNA. *Soil Biol. Biochem.* 96, 16–19.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 01 June 2020
doi: 10.3389/fevo.2020.00152

Different Roles of Environmental Selection, Dispersal, and Drift in the Assembly of Intestinal Microbial Communities of Freshwater Fish With and Without a Stomach

Yinghua Zha[1,2], Eva S. Lindström[1], Alexander Eiler[1,3] and Richard Svanbäck[4]*

[1] Limnology, Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden, [2] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solna, Sweden, [3] Section for Aquatic Biology and Toxicology, Centre for BioGeoChemistry in the Anthropocene, Department of Biosciences, University of Oslo, Oslo, Norway, [4] Animal Ecology, Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden

The composition of intestinal microbiota commonly varies among animal hosts and may affect host health. However, we have limited knowledge about the different relative roles of assembly processes, such as drift, dispersal and environmental selection, for the composition of gut microbiota. Here, we conducted a field study analyzing intestinal microbial communities of two fish species that either have (perch) or lack (roach) a stomach. We used a suite of statistical tools to evaluate the role of different assembly processes for intestine microbiota, including null model analysis (Chase et al., 2011; Fine and Kembel, 2011; Stegen et al., 2013), SourceTracker analysis (Knights et al., 2011) and several multivariate analyses, such as pRDA and PLS analysis. Drift, dispersal (i.e., microbes associated with food sources) and environmental factors (i.e., diet, host habitats), appeared to be of equal importance for the assembly of intestinal microbial communities in roach, while drift appeared most important in perch, followed by dispersal and environmental selection. Furthermore, we found that microbes associated with macroinvertebrates had a positive association to fish body condition (weight/length^3) whereas microbes associated with zooplankton had a negative association to fish body condition. These results emphasize the important combined roles of drift, dispersal and environmental selection in shaping the host-associated microbial communities. We conclude that general conclusions about fish as a whole are not justified since different species differ in the relative roles of these important drivers of community assembly.

Keywords: intestinal microbial community, freshwater fish, metacommunity theory, environmental selection, dispersal, drift, fish body condition

OPEN ACCESS

Edited by:
Mathew A. Leibold, University of Florida, United States

Reviewed by:
James Skelton, United States Geological Survey, United States
Carly Rae Muletz-Wolz, National Zoological Park (SI), United States

****Correspondence:***
Yinghua Zha
yinghua.zha.goteman@ki.se

Specialty section:
This article was submitted to Biogeography and Macroecology, a section of the journal Frontiers in Ecology and Evolution

Received: *29 September 2019*
Accepted: *04 May 2020*
Published: *01 June 2020*

Citation:
Zha Y, Lindström ES, Eiler A and Svanbäck R (2020) Different Roles of Environmental Selection, Dispersal, and Drift in the Assembly of Intestinal Microbial Communities of Freshwater Fish With and Without a Stomach. Front. Ecol. Evol. 8:152. doi: 10.3389/fevo.2020.00152

INTRODUCTION

Microbes inhabiting animals have been shown to contribute to the health of their hosts, for instance by facilitating nutrient absorption from the diet, and by stimulating important processes such as the development of host immune systems (Costello et al., 2012; Dutton and Turnbaugh, 2012). The interaction between hosts and their microbes, as well as the interactions among microbes,

results in a rather stable ecosystem where host health can be seen as an ecosystem service (Costello et al., 2012). Therefore, how this microbial ecosystem works, and what internal and external factors influence its assembly is important for host ecology and evolution (Theis et al., 2016). Conceptually, intestinal microbial communities can be seen as metacommunities, i.e., multiple local communities that are connected by dispersal via interacting hosts (Leibold et al., 2004; Miller et al., 2018). Factors such as drift, selection by local environmental conditions and dispersal can differ in their importance affecting microbial communities depending on the circumstances (Vellend, 2010; Lindström and Langenheder, 2012).

Drift processes comprise of random recruitment from the regional species pool and stochastic community assembly, so the competitive interactions are less important in shaping the community composition (Chave, 2004; Schmidt et al., 2015). If a community is driven by ecological drift, there is a smaller role for environmental interactions, including species-interactions, in determining its community composition, and will show a less correlation between community assembly and environment (Nemergut et al., 2013). For instance, Dethlefsen et al. (2006) emphasized the importance of unpredictable events, such as colonization history, for gut microbiota assembly. In contrast Li and Ma (2016) suggested that in the human microbiome, the environmental conditions of the host generally dominate over ecological drift as a major assembly process.

Dispersal, the process and result of the spreading of organisms from one place to another, is an important regulator of microbial community assembly (Lindström and Langenheder, 2012; Veiga et al., 2014; Berga et al., 2015; Evan, 2016). In the intestine of vertebrates, microbes are dispersed from mother to offspring (Round and Mazmanian, 2009; Dominguez-Bello et al., 2010), from free-living microbes in the surrounding environment, or through ingestion of food particles (De Filippo et al., 2010; Koenig et al., 2011; Zhang et al., 2016). The dispersal ability of microbes from external sources to the intestine will likely vary, for instance because individual hosts may differ in their habitat and diet choice. This will affect which pools of environmental or food associated microorganisms can act as sources for intestinal microbial communities. When microbes successfully enter the host intestine they will have to compete with and integrate into the resident microbial communities (Derrien and van Hylckama Vlieg, 2015; Fukami, 2015; Zhang et al., 2016). Further they will also need to cope with the environment in the intestine (Benson et al., 2010; Campbell et al., 2012).

Environmental factors in the intestine could depend on host traits, such as host species, sex, and also characteristics of the host's habitat such as temperature and salinity (Stewart et al., 2005; Sullam et al., 2012; Nelson et al., 2013; Bolnick et al., 2014). Other environmental factors, for example, food availability in different habitats, lay more complexity to microbial community assembly (Skulason and Smith, 1995; Bolnick et al., 2003). One apparent environmental factor affecting gut microbiota is the host's diet, since it serves as a substrate for intestinal microbes (Spor et al., 2011; Wu et al., 2011; Dutton and Turnbaugh, 2012; Ravussin et al., 2012). The effect of food choice on gut microbiota is, thus, 2-fold: both as a source of dispersing microorganisms and as a factor affecting the local habitat conditions within the gut. Disentangling the complexity and quantifying the role of dispersal and environmental factors in the assembly of gut microbiota are important steps toward understanding the among individual variations in gut microbial composition.

Organisms that can use food more efficiently for growth will reduce their vulnerability to predation (Lundvall et al., 1999). The transfer of food into energy reserves is important for reproduction or for times when food is in short supply (Davis et al., 2011). Studies on gut microbiota suggest that microbes in the intestine are crucial for host energy gain and fat storage, where the composition of the microbiota matters for the efficiency of these processes (Bäckhed, 2011; Li et al., 2013; Shapira, 2016). For example bacteria can produce short chain fatty acids (SCFA) from the fermentation of carbohydrates, which then contribute to the energy maintenance of the host (Stevens and Hume, 1998). Furthermore, in wild animals, a higher body condition can be beneficial and result in both increased fecundity and increased survival when food is in short supply (Bender et al., 2008; Dutil and Lambert, 2000; Davis et al., 2011). Thus, detailed understanding of assembly processes in gut microbiota have direct implications for predicting host fitness and well-being (Nicholson et al., 2012).

In this study, we quantified how environmental factors, dispersal, and drift can influence the assembly of intestinal microbiota communities in two co-occurring fish species (Eurasian perch, *Perca fluviatilis*, and Roach, *Rutilus rutilus*). Perch and roach are two of the most dominant fish species in Swedish lakes (Persson et al., 1991; Svanbäck et al., 2008). These two species also have different digestive systems, with perch having and roach lacking a stomach. Both fish feed on zooplankton and macroinvertebrates. However, perch also include fish in their diet, and roach include plants and detritus in their diet (Svanbäck et al., 2008).

We analyzed fish intestinal microbial communities as well as microbes associated with their food sources and microbes in the surrounding environment. We hypothesized that environmental factors, such as fish diet and habitat choice will affect intestinal microbial communities (Ley et al., 2008; Spor et al., 2011; Wu et al., 2011). If microbes in the intestine are gained by dispersal via diet and are crucial for host energy gain and body condition (Bäckhed, 2011; Li et al., 2013; Shapira, 2016) we also expected that fish body condition would depend on bacterial dispersal sources.

MATERIALS AND METHODS

Field Sampling

Sampling was done in Lake Erken in Sweden between September 4 and 5, 2013. Perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) were caught from three sites in lake Erken, including littoral, pelagic and profundal zones using standard survey-link pelagic and benthic multi-mesh gill nets. Littoral nets (30 m long and 1.5 m deep) were set just outside the vegetation at 2 m depth.

Pelagic nets (27.5 m long and 6 m deep) were placed at the surface about 200 m from the shoreline and the littoral nets. Nets for the profundal zone (30 m long and 1.5 m deep) were set at 12-meter depth in the lake adjacent to the pelagic nets. We emptied nets immediately to get all fish after leaving the nets in the lake overnight. The fish were frozen immediately after removal from the nets at the Lake Erken field station. Fish were then kept frozen when transported to the lab at Uppsala University for analysis. From the pelagic nets, we divided the catch to represent fish from a depth of 0–3 meters and fish from a depth of 3–6 meters. We then choose maximum 50 individual fish of perch and roach from the littoral (perch: 50, roach: 46), pelagic (0–3 m) (perch: 44, roach: 32), pelagic (3–6 m) (perch: 50, roach: 6) and profundal (perch: 50, roach: 16) nets, respectively, summing up to 194 perch and 100 roach individuals. Perch and roach were chosen because they are the numerically dominant species in Lake Erken and were present in all nets. Water and microalgae were sampled with a Ruttner water sampler, meanwhile zooplankton was sampled with a plankton net (100 μm mesh size) from the littoral and pelagic sites. Sediment (N = 3 per site) and macro-invertebrate samples were taken from both littoral and profundal sites next to the gill-nets (see **Supplementary Table S3**).

Samples Processing

We determined the sex, measured the weight (W, to the nearest 0.1 g) and length (L, total length to the nearest mm) as well as the intestine length (to the nearest 0.1 mm) of each individual fish. Fish sex was categorized as male, female and YOY (young of the year). Fish body condition was calculated as W/L^3 and would indicate the fish nutritional status and fitness, which is similar to the body mass index (BMI) used in human studies. The whole intestine was thereafter stored at −20°C in Eppendorf tubes for later bacterial community analysis. Water and algae were filtered onto 0.2 μm membrane filter (Pall Corporation) and 0.7 μm glass microfiber filter (Whatman™) separately. Individuals from zooplankton and macro-invertebrate samples, 0.25 gram sediment and filters from water and algae were all stored into sterilized Eppendorf tubes at −20°C for later bacterial analysis.

Analysis of Short-Term Diet by Stomach Content and Long-Term Diet by Stable Isotopes

Stomach contents of each fish were examined under dissecting microscope and were identified to lowest possible taxonomic group, and lengths of ≤10 prey from each taxonomic group were measured to the nearest 0.1 mm. The lengths of all prey were then converted to biomass (dry weight) using our own length-mass relationship. The biomass-based diet was then grouped into macroinvertebrates, zooplankton and fish and represents measures of short-term diet.

Stable isotopes of carbon and nitrogen are widely used to study long-term feeding ecology in wild populations (Post, 2002, Newsome et al., 2007; Alves-Stanley and Worthy, 2009). We used standard formulas to calculate the proportion of littoral carbon in the diet of an individual fish and its trophic position (Post, 2002; Matthews et al., 2010) using the isotopes from mussels and snails as baselines (**Supplementary Table S3**). Part of the dorsal muscle was dissected from perch and roach and kept at −20°C. Each muscle sample was dried for 48h at 60°C and ground to fine powder. The powder (around 1 mg) was packed into 6 × 4 mm tin capsules for ^{13}C and ^{15}N analysis using a continuous-flow isotope ratio mass spectrometer at University of California at Davis Stable Isotope Facility. In order to get the baseline values of different carbon and nitrogen sources, we collected snails [*Theodoxus fluviatilis*, a grazing littoral primary consumer (Jacoby, 1985)] and mussels [Zebra mussels, a pelagic primary consumer filtering phytoplankton (Strayer et al., 1999)] mostly around the littoral zones in the lake while fish sampling (Svanbäck and Persson, 2009).

DNA Extraction and Bacterial 16S rRNA Genes Illumina Sequencing

Bacteria DNA from the whole fish intestine were extracted using PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, United States) including PCR grade water as negative extraction control (VWR). Bacteria from algae, zooplankton, macroinvertebrates, water and sediment were extracted in the same way. 16S rRNA bacterial genes were amplified by using two universal primers, and PCR grade water was used as PCR negative controls. Polymerase chain reaction was applied in two steps. The first step was amplified with the universal primers 515F (5′-GTGCCAGCMGCCGCGGTAA -3′) and 806R (5′- GGACTACHVGGGTWTCTAAT-3′). Triplicates of 20 μl reaction were carried out for each sample. Each reaction consisted of 10 μM of forward and reverse primers, 5 × reaction buffer, 2 mM of dNTPs and 2 U/μl Q5 HF DNA polymerase and 1 μl of DNA template. Reactions were started with initial denaturation at 98°C for 30 s, then followed with 30 cycles of denaturation at 98°C for 10 s, annealing at 58°C for 30 s and extension at 72°C for 30 s. A final extension was done at 72°C for 2 min. First step PCR products were purified and concentrated using Agencourt® AMPure® XP (Beckman Coulter). Purified products were then used as the template for the second step PCR. Forward and reverse barcode primers were used for the second step PCR. Triplicates were prepared for each sample. Each reaction consisted of 1.25 μM of primers, 5 × reaction buffer, 2 mM of dNTPs and 2 U/μl of Q5 HF DNA polymerase and 1 μl of template. Each reaction started with initial denaturation at 98°C for 30 s, followed by 20 cycles of denaturation at 98°C for 10 s, annealing at 68°C for 30 s and extension at 72°C for 30 s. Final extension was finished at 72°C for 2 min. The second step PCR products were also purified with Agencourt® AMPure® XP, then quantified with Quant-iT™ PicoGreen® dsDNAReagent Kit (Invitrogen) according to manual instructions. Equal amounts of PCR products were mixed with a final concentration of 2.68 ng/ul and sent to sequencing. Sequencing was performed using Illumina Miseq in SNP&SEQ technology platform in the national genomics infrastructure Sweden and science for life laboratory in Uppsala.

Sequencing Data Analysis

In total, 165 fish were included in the sequencing analysis (**Supplementary Table S1**) due to the failure of PCR for some fish intestine samples. The raw amplicon sequencing data was demultiplexed and sequence-pairs were assembled using pipeline developed by Sinclair et al. (2015). In short, every read-pair produced was parsed and checked for recognizable barcodes on both the forward and reverse sequences.

Next, sequences with missing primers and unassigned base pairs were removed and resulting quality filtered assembled reads were clustered into operational taxonomical units (OTUs) using UPARSE (cutoff of 3% sequence dissimilarity) (Edgar, 2013). Taxonomy was assigned using CREST (Lanzén et al., 2012) and the ribosomal sequence database SilvaMod. Raw sequences were deposited in the European Nucleotide Archive (ENA) under accession numbers ERS4181501–ERS4181737.

Statistical Analysis

Figure 1 gives an overview of the sample and statistical analysis of this study. More specifically, after sequences had been assigned into operational taxonomic units (OTUs) with a 97% sequence similarity, we removed non-bacterial OTUs (e.g., Archaea) prior to all downstream analyses. Each sample was rarified down to 15299 reads using package GUniFrac in R. The following statistical analyses were also run in R (version 3.2.2). Phylogenetic trees were constructed using MacQIIME with default settings (FastTree; Price et al., 2010). We used the Bray-Curtis (*vegan*, version 2.3-5) and weighted UniFrac distances (*phyloseq*, version 1.12.2) based on the OTU's relative abundance to calculate differences in community composition between samples (i.e., fish and environmental). Adding the phylogenetic perspective in distance matrix calculation takes into account the species phylogenetic relationship among communities, and this can help to capture of even small differences among communities (Cadotte et al., 2009). Non-metric multidimensional scaling (NMDS) of the distance matrices was done in package vegan. We used the function of metaMDS to test different *k* values (number of dimensions), and then used a Shepard plot to determine the value of *k*, then the first two axis were chosen to make the NMDS plot with ggplot2 package (version 2.1.0). PERMANOVA (*vegan*, version 2.3-5) was used to test the effects of all measured environmental factors on the intestinal microbial communities among individual fish.

Null Model Analysis

We applied a null model analysis to disentangle the contributions of environmental selection and other ecological processes for the assembly of fish intestinal microbial communities. First, we calculated phylogenetic beta diversity between pairs of fish using β-mean-nearest taxon distance (βMNTD) (Fine and Kembel, 2011; Stegen et al., 2013). Then in order to measure the degree to which community composition is determined by environmental selection, we calculated how much the observed βMNTD deviated from the mean of the null distribution based on random shuffling of the tip labels on the phylogenetic tree constructed by MacQIIME. We calculated βNTI (β-nearest taxon index) from 1000 random phylogenetic trees. βNTI indicates the number of standard deviations the observed βMNTD is from the mean of the distribution of the randomized phylogenetic trees (Stegen et al., 2012, 2013). We used the cut off $|\beta NTI| > 2$ to identify pairs of communities that were phylogenetically more similar than expected by chance, meaning that the observed difference between communities can be assumed to be determined by environmental selection (Stegen et al., 2013; Langenheder et al., 2017). Further we calculated Raup-Crick distances (RC_{bray}) between pairs of intestinal microbial communities (Raup and Crick, 1979) based on relative abundance data according to modification from Chase et al. (2011). Among the numerous metrices describing OTU turnover, Raup-Crick provides information on whether the OTU turnover in one community is different from a community that would be structured mainly by drift (Chase et al., 2011). In the following analyses, we combined the βNTI and RC_{bray} into β_{RCBray}, to further assess the contribution of ecological processes to fish intestinal microbial communities that were not determined by environmental selection ($|\beta NTI| < 2$) (Stegen et al., 2013; Yan et al., 2016; Langenheder et al., 2017). This metric provides some indication of the possible underlying mechanisms of community assembly, in particular the degree to which deterministic processes create communities that deviate from those based on stochastic (null) expectations. An assumption of the analysis is that the samples of each fish species' microbiome is representative for the regional species pool of the respective gut microbiome. When $\beta_{RCBray} > 0.95$ or < -0.95, it indicates that the community variation is influenced by dispersal limitation or mass effects, respectively, when β_{RCBray} is between -0.95 and $+0.95$, it suggests that community variation is determined by ecological drift (Stegen et al., 2013; Langenheder et al., 2017). The scripts of the null model analysis are attached as **Supplementary Material**.

Source Tracker Analysis

As fish diet can both be an environmental factor and a dispersal source for the fish intestinal communities, we treated it in two parts. Firstly, microorganisms attached associated with the diet and other external sources (i.e., water, sediment) were assigned as the dispersal sources for fish intestinal microbiota using section "Source Tracker Analysis". Secondly, stomach content (such as the proportion of zooplankton, macroinvertebrates as diet) were used as intestine environmental factors, together with fish traits such as fish species, sex, habitat, length, weight, intestine length, trophic position, and proportion of littoral carbon.

Source Tracker analysis was used to analyze the contribution and the relative importance of different dispersal sources (bacteria from fish diet including algae, zooplankton, macroinvertebrates, prey fish, and water and sediment as known from sequencing data) (defined as sources in section "Source Tracker Anaysis") to intestinal microbial communities of the individual fishes as known from sequencing data (defined as sinks in Source Tracker) (Knights et al., 2011). This analysis produces a table showing the proportion of each dispersal source explaining their contribution to the intestine microbiota of each individual fish. For roach of all sizes and perch that were shorter than 18 cm, bacteria attached to algae, zooplankton,

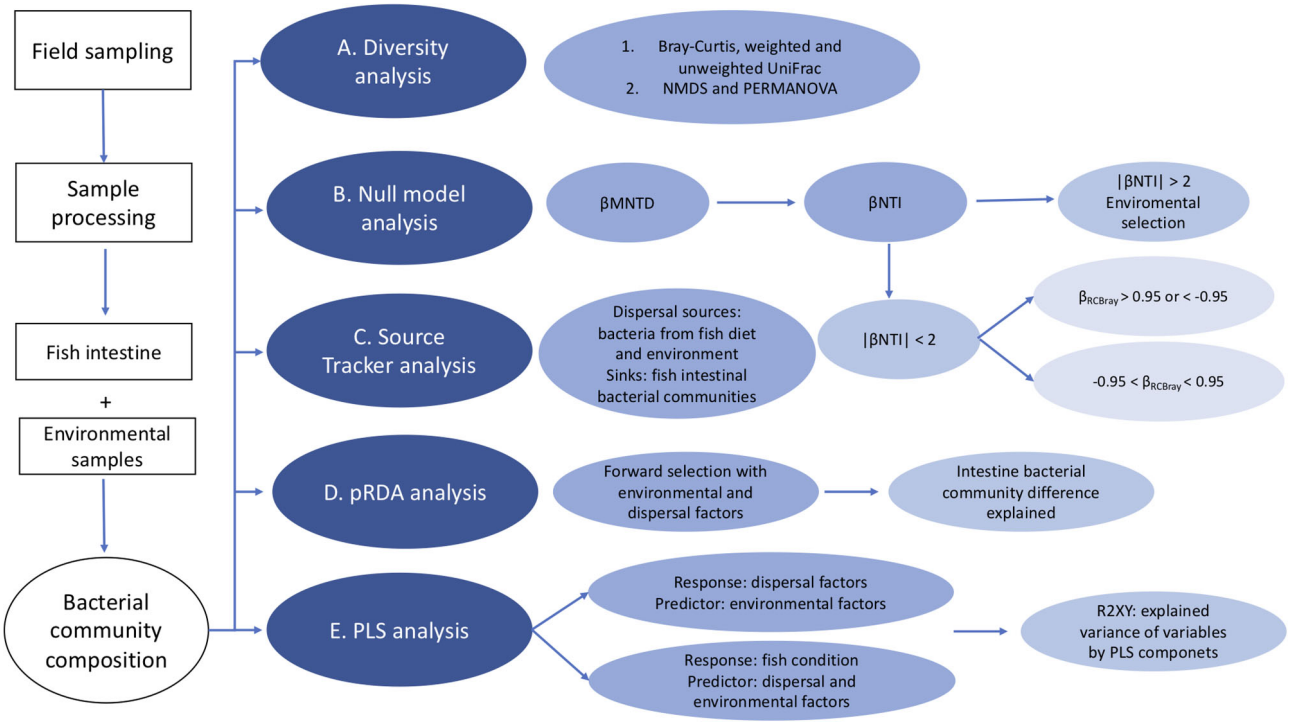


FIGURE 1 | Flow chart of the statistical analysis of this study. **(A)** Beta diversity analysis of the bacterial community composition using Bray-Curtis and weighted UniFrac distances based on the OTU's relative abundance. **(B)** We used null model analysis to determine the contributions of environmental selection and other ecological processes to the assembly of fish intestinal bacterial communities. In this analysis, βMNTD was used to measure the dissimilarities of bacterial communities between fish. Then βNTI is calculated as how much observed βMNTD deviated from the mean of the null distribution based on 1000 randomly shuffling of the tip labels on the phylogenetic tree, to check if the difference between microbial communities is determined by environmental selection when $|\beta NTI| > 2$ or not when $|\beta NTI| < 2$. For communities with $|\beta NTI| < 2$, Raup-Crick distances were calculated based on the relative abundance data. Then we combined βNTI and RC_{bray} into β_{RCBray}, to further assess the contribution of ecological processes. When $\beta_{RCBray} > 0.95$ or < -0.95, it indicates that the community variation is influenced by dispersal limitation or mass effects, respectively, when β_{RCBray} is between –0.95 and +0.95, it suggests that community variation is determined by ecological drift. **(C)** We used Source Tracker analysis, which is a Bayesian method to estimate the proportion of one community that originates from a set of source environments (Knights et al., 2011). In our study, we used it to determine the contribution and relative importance of different dispersal sources to intestinal bacterial communities. **(D)** pRDA analysis with forward selection is used to test how much the significant variables could explain the differences among fish intestinal bacterial communities. **(E)** PLS analysis is used to predict the relationship between dispersal factors and environmental factors on one hand, and fish body condition on the other with both environmental and dispersal factors.

macroinvertebrates, water and sediment were treated as external dispersal sources. As perch that are longer than 18 cm can be highly piscivorous and feeds on both smaller perch and roach (Svanbäck et al., 2015), we also included bacteria associated with the 5 shortest perch and 5 shortest roach as an extra source for perch longer than 18 cm. Furthermore, diet analyses of the perch showed that both smaller perch and roach was included in the diet of perch larger than 18 cm (data not shown). A limitation of the Source Tracker is that it computes a source to sink model, only considering unidirectional movement of bacteria into the gut.

pRDA Analysis

We used partial redundancy analysis (pRDA) to determine how much of the variation in intestinal microbial communities could be explained by dispersal and environmental factors (package vegan). According to the results from previous analyses (i.e., NMDS, and PERMANOVA) we found that intestinal microbial communities differed between perch and roach, thus pRDA analysis was implemented for perch and roach separately. Microbial community data of the fish intestines was Hellinger transformed to let us be able to implement ordination methods for species data containing many zeros and minimize the influence from rare species on the analysis (Legendre and Gallagher, 2001). Dispersal (i.e., the contribution of different dispersal sources as obtained from SourceTracker analysis) and environmental variables were log transformed [$\log(x + 1)$] to remove the zeros in the dataset and to make the Canonical coefficient comparable (Ferreira et al., 1999). The full list of dispersal sources includes algae, invertebrates, sediments, water, zooplankton and in some cases fish (see above). The full list of environmental factors includes habitat, sex, length, weight, proportion of littoral carbon, trophic level, macroinvertebrates as diet and zooplankton as diet. Forward selection of both environmental and dispersal variables was implemented in separate analyses prior to pRDA to select only significant variables ($p < 0.05$) to be included in the model. We used step selection to check which factors should

be added to the reduced RDA model with only the significant factors included. AIC value was used to estimate which was the best model, the lower the AIC value, the better the model. In the final reduced pRDA model for perch, the environmental variables were profundal habitat, fish length, trophic level, proportion of littoral carbon in the diet, and the dispersal variables for perch were the proportion of bacteria from zooplankton, macroinvertebrates and fish as obtained from the SourceTracker analysis. In the final reduced pRDA model for roach, the environmental variables were proportion of littoral carbon in the diet, macroinvertebrates as diet and zooplankton as diet; the dispersal variables for roach were the proportion of bacteria from algae, macroinvertebrates, water and zooplankton as obtained from the SourceTracker analysis (**Table 1**). The first pRDA model was run using environmental factors as constrained variables and dispersal factors as condition variables, while the second pRDA model used dispersal factors as constrained variable and environmental factors as a condition variable. In this way, we could analyze the effect of environmental and dispersal factors on the microbial community composition independent of each other. An ANOVA test of each pRDA model with 999 permutations was followed to test the significance of each variable in environmental and dispersal factors on the variance of intestinal microbial communities (vegan). Further we used variation partitioning based on RDA to quantify the amount of variation explained by environmental and dispersal factors, respectively. Projection of pRDA and variation partitioning was done by packages of ggplot2 and VennDiagram (version 1.6.17) separately.

PLS Analysis

We ran three separate partial least squares regression (PLS) models. In the first model we investigated if fish body condition (response variable) could be statistically explained by dispersal factors (predictor variables). In the second model, we investigated if fish body condition (response variable) could be explained by environmental factors (predictor variables). The factors included were the same as for the pRDA described above, i.e., dispersal factors were the contributions of the different dispersal sources as obtained from SourceTracker analysis.

In the third PLS model we wanted to explore how well bacteria from the different dispersal sources could establish in the intestines (based on SourceTracker data) considering how important that particular source was as a diet. In this analysis, therefore, the dispersal factors were used as response variables while the environmental factors were used as predictor variables.

For the PLS we used the package *plsdepot* version 0.1.17 and all analyses were made for perch and roach separately. The R2Xy value (explained variance of variables by PLS components) was used to evaluate how strong this relationship was with a cutoff at 0.8 (Eriksson et al., 2013). Variables in environmental and dispersal factors were log $(x + 1)$ transformed before analysis. The final PLS results were projected as principal component figures showing a circle of correlations with arrows indicating tested variables.

RESULTS

Fish intestinal bacteria were distinctively different from those from the dispersal sources (**Figure 2A** and **Supplementary Figure S1A**). Among intestinal microbial communities, fish species (perch and roach) seemed to be one of the major steering factors (**Figure 2B** and **Supplementary Figure S1B**). PERMANOVA showed that the microbial communities among individual fish were significantly dependent on the habitat (i.e., littoral, profundal or pelagic) and fish species (**Supplementary Table S2**).

The null model analysis showed that ecological drift could explain 60% of the assembly of the communities in perch, followed by dispersal limitation, environmental selection and mass effects (**Figure 3A**). In contrast, roach communities appeared to be assembled by environmental selection, dispersal and ecological drift to about an equal degree (approximately 30% each, **Figure 3B**).

Source Tracker analysis showed that dispersal sources contributed to microbiota to different degrees, and this was dependent on fish species. In perch, zooplankton appeared as the most important source of dispersal, followed by macroinvertebrates and fish (**Figure 4A**). In contrast, the most important dispersal source in roach appeared to be macroinvertebrates (**Figure 4B**), followed by zooplankton, and algae. Water and sediment seemed to be negligible as dispersal sources in both fish species. Still in both perch and roach, unknown dispersal sources contributed to 28–35% in our Source Tracker model ("unknown" in **Figure 4**). These unknown sources may be associated with zooplankton and macroinvertebrates that were not present during the time of sampling or not present at the place of sampling.

When analyzing the Source Tracker results grouped by habitat, we observed that fish as food sources could contribute to the intestinal bacteria in perch from littoral and profundal zones but not from pelagic zones (**Figure 4C**). Further, zooplankton contributed slightly more to microbes in pelagic fish than in fish from the littoral and profundal zones. In roach (**Figure 4D**), microbes attached to algae contributed to the microbes in pelagic fish but not to the gut microbiota of littoral and profundal fish.

Forward selection in RDA and subsequent pRDA analysis showed that several environmental and dispersal factors could significantly explain variation in microbial community composition among fishes, and that there were differences between perch and roach (**Table 1**). More specifically, profundal habitat, fish length and trophic position were significant environmental factors explaining variations in the gut microbiota of perch (**Figure 5A** and **Table 1**), while for roach the only significant environmental factor was macroinvertebrates as diet (**Figure 5B** and **Table 1**). There were also differences between the fish species when it came to the dispersal factors explaining variations in the microbiota. Bacteria from macroinvertebrates, zooplankton and fish could significantly explain the variation among perch gut microbiota (**Figure 5C** and **Table 1**), while for roach, bacteria from algae and water as well as from macroinvertebrates and zooplankton were significant dispersal variables (**Figure 5D** and **Table 1**).

TABLE 1 | ANOVA test the significance of each variable in the estimated environmental and dispersal factors explaining the variance of intestinal microbial communities from pRDA analysis in perch and roach.

	Perch				Roach		
	df	*F*	*p*		*df*	*F*	*p*
Environment				Environment			
Profundal	1	4.30	**0.001**	Littoral carbon use	1	0.76	0.74
Length	1	3.61	**0.001**	macroinvertebrates as diet	1	2.36	**0.008**
Littoral carbon use	1	1.80	0.075	Zooplankton as diet	1	0.83	0.67
Trophic position	1	2.71	**0.008**				
Dispersal				Dispersal			
Macroinvertebrates	1	3.24	**0.001**	Algae	1	3.80	**0.001**
Zooplankton	1	3.63	**0.001**	macroinvertebrates	1	7.88	**0.001**
Fish	1	2.62	**0.003**	Water	1	2.81	**0.001**
				Zooplankton	1	4.92	**0.001**

Significant variables are bold labeled.

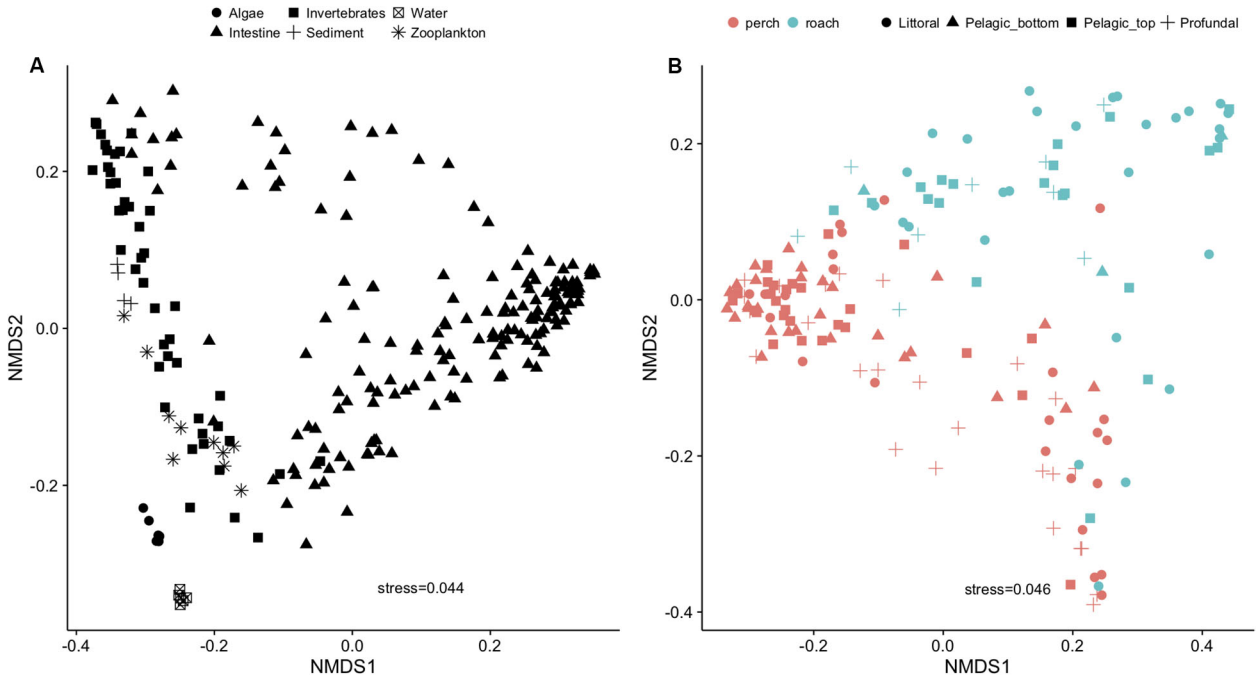


FIGURE 2 | NMDS plot using Bray-Curtis distance showing variations of microbial communities in fish intestine and external dispersal sources **(A)** and fish species as the main driver for the variation within gut microbial communities **(B)**.

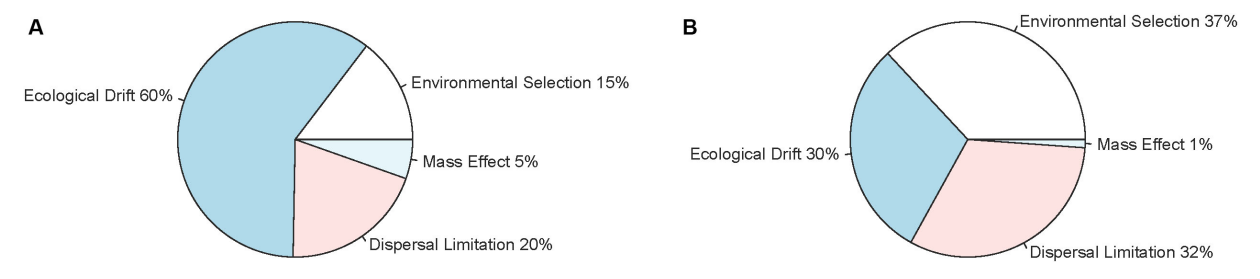


FIGURE 3 | Relative importance of environmental selection, mass effects, dispersal limitation and ecological drift for the variation of intestinal microbial communities among perch **(A)** and roach **(B)** as obtained from null model analysis.

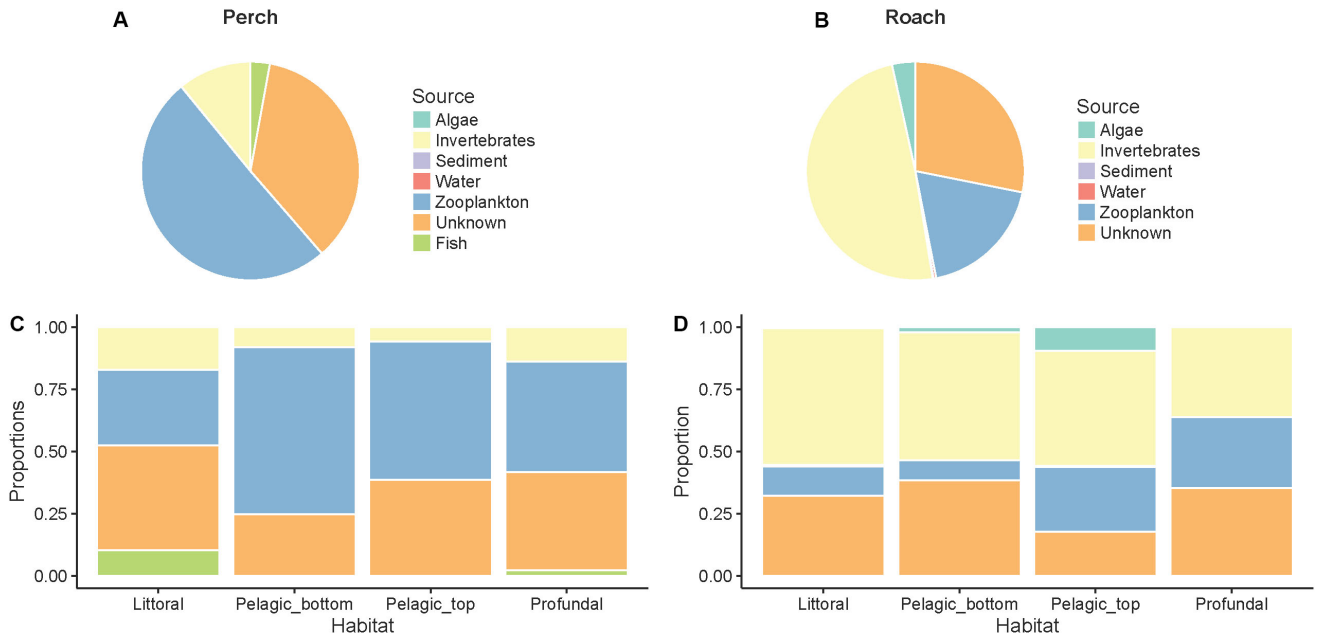


FIGURE 4 | Contributions of microbes from different dispersal sources to intestinal microbial communities as determined by SourceTracker analysis. In perch **(A)** and roach **(B)** across habitats in perch **(C)** and across habitats in roach **(D)**.

Variation partitioning analysis further revealed the differences between perch and roach in the percentage of environmental and dispersal factors that could explain the variation in microbiota (**Figures 5E,F**). While dispersal explained 21.63% of the variation in roach, only a small proportion of the variation could be explained by dispersal in perch (5.45%). The proportion that dispersal explained in perch was approximately equal to the proportions explained by environmental factors (7%) and their shared contribution (6.01%) (**Figure 5E**). In both fish species the largest part of the variation was unexplained by the RDA models.

Partial least squares analyses showed that different dispersal sources were related to fish body condition in perch and roach (**Figure 6** and **Table 2**), but only a relatively low proportion if the fish body condition variation could be explained (22.6% variance explained for perch, 4.88% variation explained for roach). More specifically, the effect of macroinvertebrates and zooplankton associated microbes appeared more pronounced compared with other dispersal factors, however, zooplankton as a dispersal source was negatively associated with fish body condition, while macroinvertebrates as a dispersal source was positively associated with fish body condition (**Figure 6**). When we also included environmental factors in the PLS analyses of fish body condition, more of the variation could be explained (39.03% for perch and 10.88% for roach, respectively), and different variables were related to fish body condition (**Tables 3**, **4**). In this analysis macroinvertebrates as diet was positively related to fish body condition in both perch and roach (**Figure 7**). Macroinvertebrates as diet was strongly related to roach condition, while this relation was weaker than that between microbes from macroinvertebrates and roach condition (**Figure 7**). PLS analysis between environmental and dispersal factors showed that, in both perch and roach, that the contribution of bacteria from zooplankton to microbiota in fish intestine was associated with the degree of zooplankton as food, while for macroinvertebrates this association was weaker (**Figure 8** and **Table 5**).

DISCUSSION

The composition and diversity of intestinal microbial communities can directly or indirectly affect hosts' health (Bäckhed, 2011; Li et al., 2013; Shapira, 2016). Therefore, the understanding of how intestinal microbes assemble in their hosts, and what factors contribute to the assembly is of great importance (Miller et al., 2018). In this field study, by sampling the gut microbiota of two different fish species as well as the multiple origins of the microbial species pool, we found an equal contribution of environmental selection, dispersal and ecological drift, contributed to the assembly of intestinal microbial communities in roach, whereas the dominant factor in perch was ecological drift. Berg et al. (2016) showed that in the free-living nematode *Caenorhabditis elegans* the assembly of gut microbiota communities is controlled by deterministic processes (host and interaction between microbiota members). Burns et al. (2016) on the other hand showed that neutral processes can explain a large part of the variation in microbiota composition in zebrafish. Possible reason for these differences in assembly processes between species can be hosts' habitat choice, which results in differences in environmental settings, food choice and anatomy (i.e., design of the digestive system).

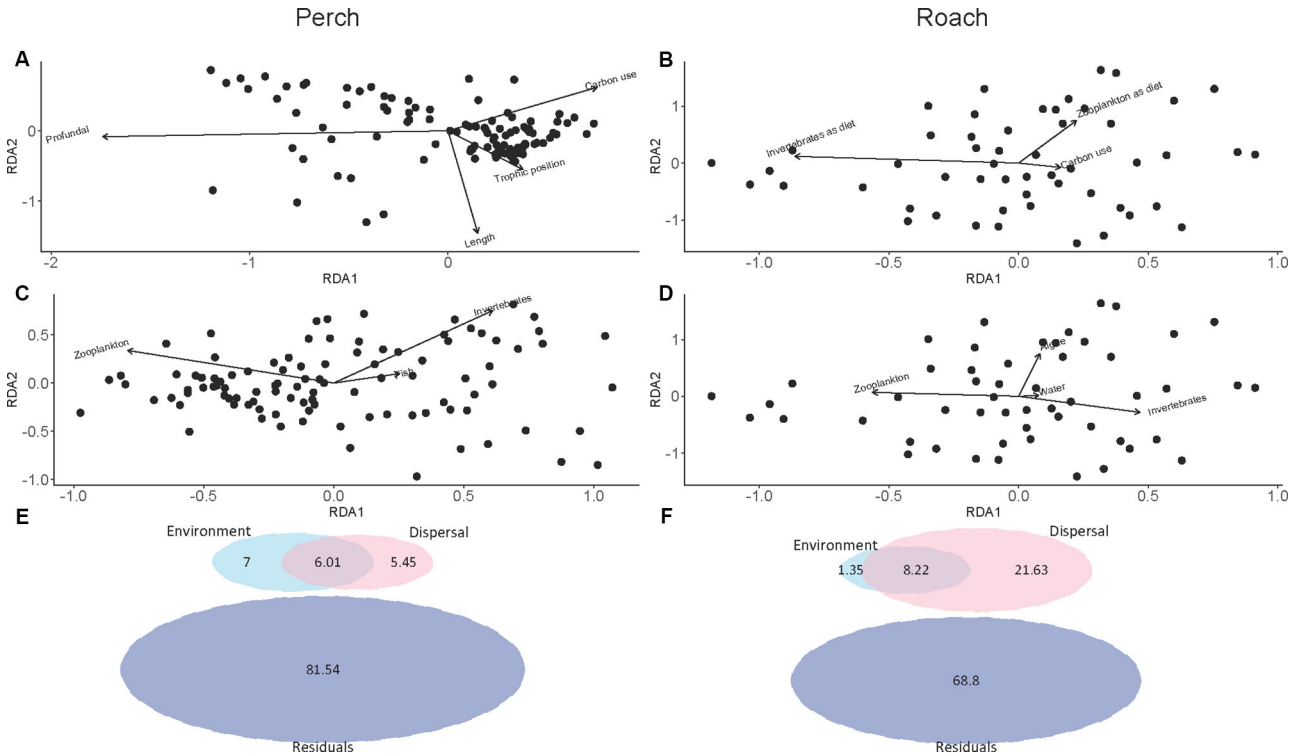


FIGURE 5 | pRDA plot showing the covariation between the composition of intestinal microbial communities and environmental factor in perch **(A)** and in roach **(B)**, shown as arrows. pRDA plot showing the covariation between the composition of intestinal microbial communities and dispersal factors in perch **(C)** and in roach **(D)**, shown as arrows. Proportion of variance in intestinal microbial communities explained by environmental and dispersal factors from variation partitioning analysis in perch **(E)** and in roach **(F)**. Residuals is variation in community composition unexplained by environment and dispersal factors.

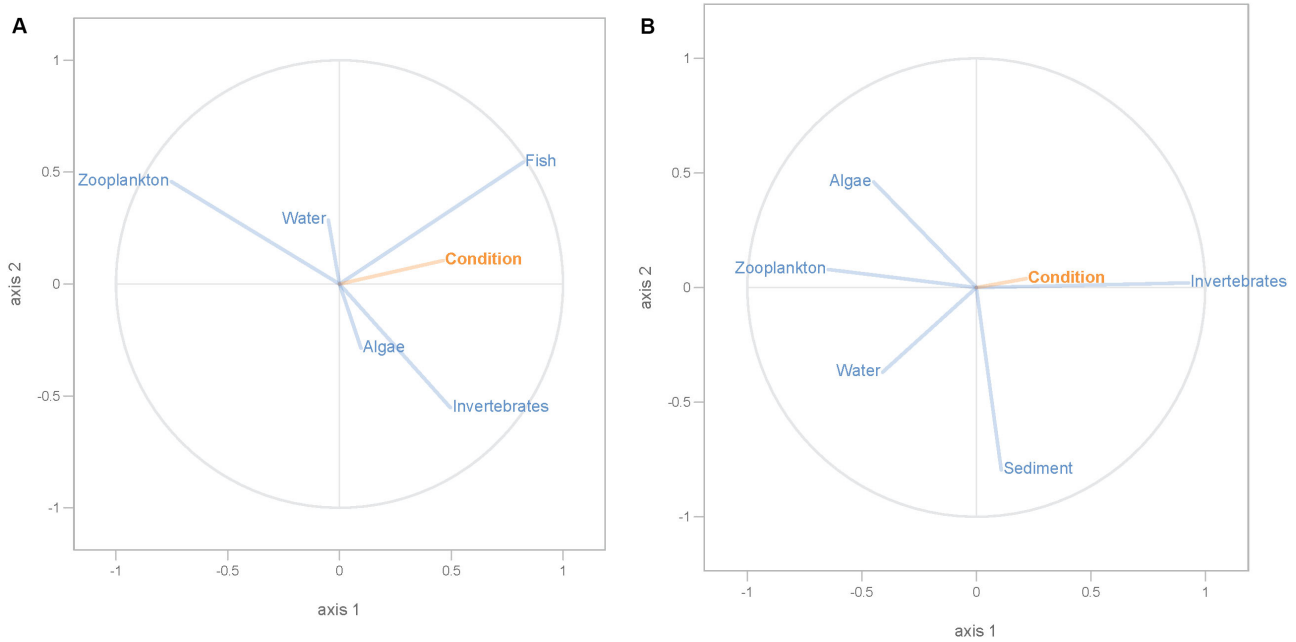


FIGURE 6 | PLS regression analysis between fish body condition and dispersal factors. Fish body condition was response variable (orange lines), and dispersal factors were predictor variables (blue lines) in perch **(A)** and roach **(B)**.

TABLE 2 | Results from PLS model 1 tested relationship between fish body condition and dispersal factors.

	t1	t2	t3	t4	t5	t6	t7
Algae	0.02	0.06	0.08	0.09	0.13	**0.98**	1.00
Macroinvertebrates	0.48	0.52	0.72	0.81	**0.93**	**0.93**	1.00
Sediment	0.01	0.15	0.49	0.50	0.57	0.62	1.00
Water	0.04	0.23	0.44	0.58	0.82	0.85	1.00
Zooplankton	0.52	**0.91**	**0.97**	**0.97**	**0.97**	**0.99**	1.00
Unknown	0.00	0.42	0.73	**0.88**	**0.98**	**0.98**	1.00
Fish	0.36	0.54	0.69	0.71	0.71	0.73	1.00
Condition	0.08	0.08	0.08	0.09	0.10	0.10	0.10
R^2	8E-2	3.65E-3	5.99E-4	8.88E-3	5.80E-3	1.20E-4	1.02E-7
Q^2	0.014	−0.034	−0.041	−0.045	−0.017	−0.011	−0.008

R2Xy value from PLS test shows the relationship between the response and predictor factors. R2Xy values are above 0.8 is bold labeled indicating a more pronounced relationship between fish body condition and dispersal factors. Q^2 and R^2 values from PLS also showed. Q^2 and R^2 values from PLS also showed. t1 to t7 is each of the PLS components.

TABLE 3 | Results from PLS model 2 tested relationship between perch body condition with both environmental and dispersal factors.

	t1	t2	t3	t4	t5	t6	t7	t8	t9	t10
HabitatPelagic_bottom	0.09	0.26	0.30	0.31	0.70	**0.83**	**0.83**	**0.84**	**0.88**	**0.97**
HabitatPelagic_top	0.15	0.29	0.60	0.60	0.72	0.76	**0.88**	**0.88**	**0.88**	**0.90**
HabitatProfundal	0.13	0.13	0.13	0.16	0.23	0.23	0.43	0.70	0.71	**0.80**
SexM	0.03	0.04	0.07	0.09	0.09	0.16	0.37	0.47	0.73	0.73
SexYO	0.17	0.18	0.18	0.20	0.21	0.21	0.30	0.36	0.39	**0.80**
Propor_Litt	0.31	0.41	0.62	0.70	**0.92**	**0.92**	**0.93**	**0.95**	**0.96**	**0.97**
Trophic_P	0.00	0.28	0.39	0.63	0.81	**0.92**	**0.92**	**0.93**	**0.95**	**0.96**
Algae	0.02	0.08	0.08	0.25	0.37	0.45	0.56	0.62	0.87	**0.90**
Macroinvertebrates	0.17	0.22	0.27	0.40	0.42	0.51	0.51	0.51	0.55	0.65
Water	0.01	0.07	0.07	0.09	0.09	0.10	0.11	0.11	0.14	0.33
Zooplankton	0.45	0.56	0.60	0.63	0.63	0.66	0.72	0.72	0.74	0.75
Fish	0.44	0.57	0.61	0.69	0.71	0.72	0.77	**0.86**	**0.92**	**0.92**
inverte_diet	0.06	0.06	0.06	0.08	0.08	0.09	0.22	0.23	0.44	0.48
zoop_diet	0.33	0.33	0.34	0.35	0.38	0.50	0.58	0.58	0.58	0.65
Condition	0.32	0.39	0.45	0.48	0.49	0.50	0.50	0.50	0.50	0.50
Q^2	0.22	−0.10	−0.18	−0.11	−0.14	−0.13	−0.11	−0.09	−0.08	−0.07
R^2	3.21E-1	6.8E-2	5.5E-2	3.5E-2	7.9E-3	6.6E-3	2.3E-3	1.5E-3	1.4E-4	1.8E-6

R2Xy value from PLS test shows the relationship between the response and predictor factors. R2Xy values are above 0.8 is bold labeled indicating a more pronounced relationship between both environmental and dispersal factors with perch body condition. Q^2 and R^2 values from PLS also showed. Q^2 and R^2 values from PLS also showed. t1 to t10 is each of the PLS components.

Here we caught both perch and roach at the same place, thus the differences in environmental conditions experienced by the two species should be minor. Genetic differences between perch and roach, which previously have been found to influence gut microbial communities (Benson et al., 2010; Koenig et al., 2011; Bolnick et al., 2014; Brooks et al., 2016), can therefore be one reason for differences in the assembly mechanisms. Another possible explanation could be the difference of the digestive system between perch and roach. In perch food passes through the stomach before it enters the intestine, while roach have no stomach, so food goes directly into the intestine. Studies on other vertebrates have shown that the stomach could work as an ecological barrier to filter microbial taxa before they enter into intestines due to the produced gastric acid (Martinsen et al., 2005; Beasley et al., 2015). Moreover, pH influence from the stomach could be an important factor to form gut microbial communities as it is for free-living microbes (Rousk et al., 2010). Thus, we would have expected environmental selection to be most important in perch, but our result was the opposite (**Figure 3**). The explanation for this could instead be that the stomach barrier leads to dispersal limitation, which would lead to more drift in perch than in roach. Still, further investigations are needed to confirm these speculations.

The results from the PLS analyses show that not all microbes associated with diet could establish themselves in the intestinal environment equally well. This is because the degree of macroinvertebrates as diet (obtained from gut analysis) was not related to the contribution of bacteria from macroinvertebrate to intestine microbiota (as obtained from SourceTracker analysis). This suggests that host's intestinal environment could select for

TABLE 4 | Results from PLS model 2 tested relationship between roach body condition with both environmental and dispersal factors.

	t1	t2	t3	t4	t5	t6	t7	t8	t9	t10
HabitatPelagic_top	0.31	0.35	0.43	0.59	0.72	0.75	0.77	0.78	**0.88**	**0.90**
HabitatProfundal	0.00	0.01	0.06	0.67	0.68	0.72	**0.86**	**0.88**	**0.88**	**0.89**
SexM	0.02	0.68	0.68	0.76	0.77	0.77	**0.83**	**0.84**	**0.84**	**0.88**
SexYO	0.07	0.18	0.19	0.21	0.28	**0.80**	**0.82**	**0.82**	**0.83**	**0.83**
Propor_Litt	0.20	0.59	0.75	0.77	0.77	0.79	0.79	**0.80**	**0.82**	**0.87**
Trophic_P	0.00	0.18	0.46	0.62	0.62	0.62	0.62	0.62	0.65	**0.87**
Algae	0.15	0.22	0.22	0.30	0.44	0.45	0.55	0.86	0.86	**0.88**
Macroinvertebrates	0.41	0.41	0.42	0.43	0.60	0.75	**0.81**	**0.90**	**0.90**	**0.91**
Sediment	0.02	0.03	0.15	0.20	0.27	0.32	0.34	0.49	**0.90**	**0.96**
Water	0.06	0.23	0.31	0.35	0.38	0.56	0.66	0.73	**0.90**	**0.95**
Zooplankton	0.46	0.59	0.59	0.73	0.75	0.77	**0.87**	**0.87**	**0.88**	**0.95**
inverte_diet	0.34	0.39	0.50	0.54	0.57	0.59	0.60	0.73	**0.83**	**0.95**
zoop_diet	0.21	0.23	0.24	0.37	0.45	0.45	0.46	0.65	0.70	**0.82**
Condition	0.09	0.11	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Q^2	−0.03	−0.07	−0.09	−0.07	−0.08	−0.07	−0.07	−0.05	−0.03	−0.03
R^2	9.1E-2	1.7E-2	8.3E-3	1.8E-3	1.7E-3	3.4E-4	1.8E-4	2.8E-6	3.3E-7	6.6E-8

R2Xy value from PLS test shows the relationship between the response and predictor factors. R2Xy values are above 0.8 is bold labeled indicating a more pronounced relationship between both environmental and dispersal factors with perch body condition. Q^2 and R^2 values from PLS also showed. Q^2 and R^2 values from PLS also showed. t1 to t10 is each of the PLS components.

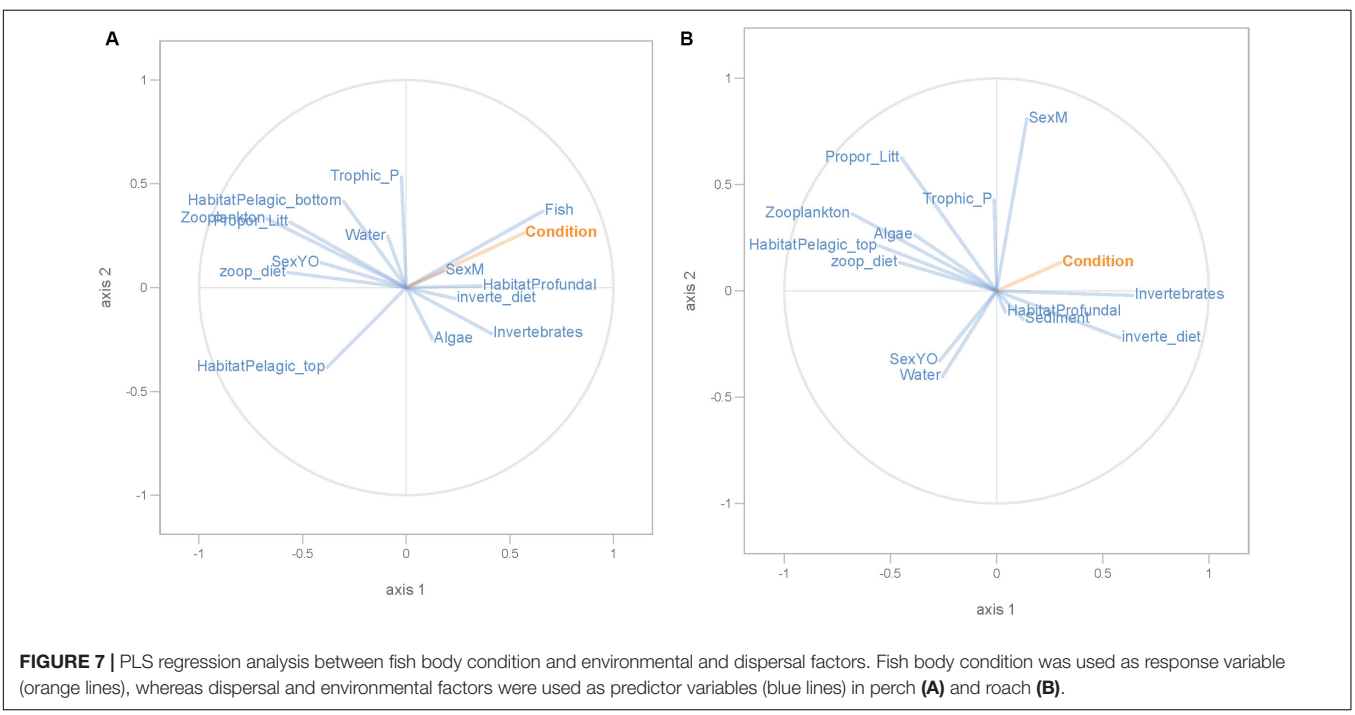


FIGURE 7 | PLS regression analysis between fish body condition and environmental and dispersal factors. Fish body condition was used as response variable (orange lines), whereas dispersal and environmental factors were used as predictor variables (blue lines) in perch **(A)** and roach **(B)**.

microbial communities that can locally adapt. One other reason for this pattern could be priority effects, which suggests that the colonization success of species is dependent on the order when they get into a site (Fukami, 2015). Juvenile perch usually feed on zooplankton, and when they get large enough they will switch to feed on macroinvertebrates and later on to fish (Svanbäck et al., 2015). Thus, when macroinvertebrate diet proportionally increases as perch grow, microbes attached to it will not increase as quickly in the intestine due to the pre-colonized microbes from zooplankton diet.

When foragers switch diets, as both perch and roach do with size (Svanbäck et al., 2008) this can both change the substrate diversity and amount of substrate for gut microbial communities. This could increase the competition between the bacteria attached to the new food intake and the original bacteria in the intestine if they have overlapping niche use. A change in diet use of the host might thus lead to invasion by bacterial species attached to the new food source into the niche of the original species (Mihaljevic, 2012), which will lead to variation in the local

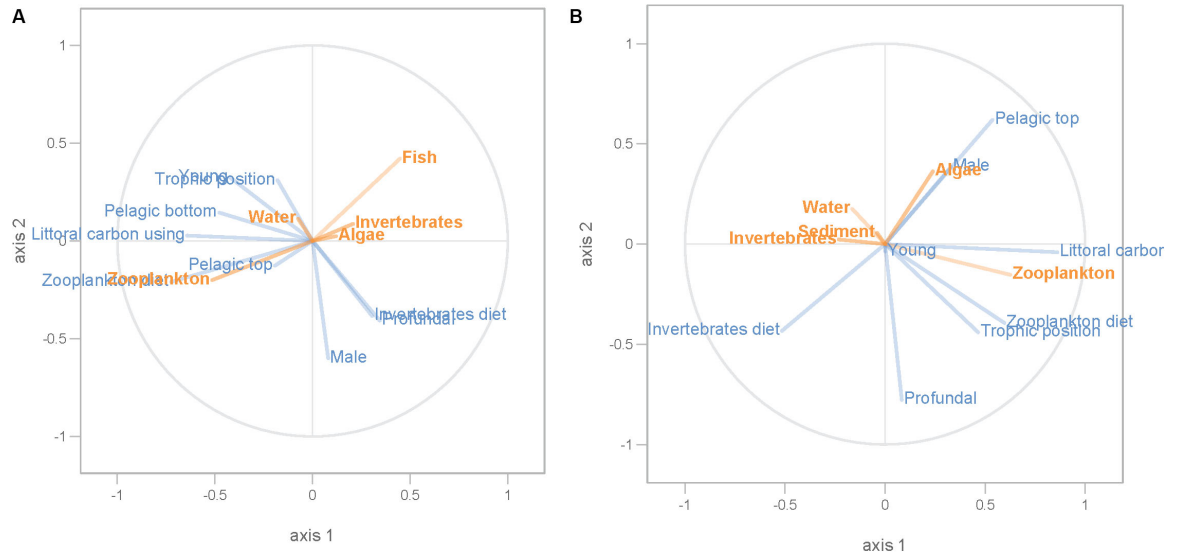


FIGURE 8 | PLS regression analysis between environmental and dispersal factors. Dispersal factors were used as response variable (orange lines), whereas environmental factors were used as predictor variables (blue lines) in perch **(A)** and roach **(B)**.

TABLE 5 | Results from PLS model 3 tested relationship between environmental and dispersal factors in perch.

	t1	t2	t3	t4	t5
Pelagic bottom	**1.1**	**0.9**	**0.8**	**0.8**	**0.9**
Pelagic top	0.7	0.7	**0.8**	**0.8**	**0.8**
Profundal	0.4	**0.8**	**0.8**	**0.9**	**0.9**
Male	0.2	0.7	**0.9**	**0.9**	**0.9**
Young	**0.9**	**0.9**	**0.9**	**0.8**	**0.8**
Littoral carbon using	**1.7**	**1.5**	**1.4**	**1.4**	**1.4**
Trophic position	0.3	**0.8**	**0.8**	0.7	**0.8**
Macroinvertebrates diet	0.08	**0.9**	**0.9**	**0.9**	**0.9**
Zooplankton diet	**1.7**	**1.5**	**1.5**	**1.4**	**1.4**

R2Xy value from PLS test shows the relationship between the response and predictor factors. R2Xy values are above 0.8 is bold labeled indicating a more pronounced relationship between environmental and dispersal factors. Q^2 and R^2 values from PLS also showed. t1 to t5 is each of the PLS components.

TABLE 6 | Results from PLS model 3 tested relationship between environmental and dispersal factors in roach.

	t1	t2	t3	t4	t5
Pelagic top	**1.1**	**1.3**	**1.2**	**1.1**	**1.1**
Profundal	0.4	0.7	0.7	0.7	**0.8**
Male	0.6	0.6	0.7	**0.9**	**0.9**
Young	0.2	0.4	0.3	0.6	0.7
Littoral carbon using	**1.7**	**1.4**	**1.4**	**1.3**	**1.3**
Trophic position	0.6	0.7	**0.9**	**0.8**	**0.8**
Macroinvertebrates diet	0.8	**0.9**	**0.8**	**0.9**	**0.9**
Zooplankton diet	**1.5**	**1.4**	**1.4**	**1.3**	**1.3**

R2Xy value from PLS test shows the relationship between the response and predictor factors. R2Xy values are above 0.8 is bold labeled indicating a more pronounced relationship between environmental and dispersal factors. Q^2 and R^2 values from PLS also showed. t1 to t5 is each of the PLS components.

diversity among individual hosts. These processes could either decrease or increase the microbial beta diversity among fish individuals depending on how competitive the bacteria species are and if fish individuals could diverge in their use of food sources (Costello et al., 2012). In other words, dispersal of bacterial species can be an effective way of altering gut microbiota in fish both at the local (in individual fish) and regional (in fish populations of a lake) level.

The metacommunity dynamics of gut microbiota and the surrounding environment is probably governed by bi-directional dispersal between hosts and the surrounding environment (Miller et al., 2018). For example, Escalas et al. (2017) suggests that fish may be a source for environmental bacteria. This may be true for our study as well. Source Tracker assumes that the local community is the sink only (Knights et al., 2011), and in our analysis, the gut microbial community is only receiving microbes from the environment and not contributing. Some studies highlighted that macro-organisms can disperse their microbial communities into the environment indicating that they are also a source of environmental microbes (e.g., De Filippo et al., 2010; Zhang et al., 2016), which suggesting a bi-directional influence of microbes from environment to hosts and from hosts to environment. Such bi-directional dispersal can probably influence persistence of bacteria in the metacommunities. However, the occurrence of bidirectional dispersal needs to be investigated in controlled experiments.

Gut microbiota composition has been shown to affect host energy uptake (Bäckhed, 2011; Li et al., 2013; Shapira, 2016). For example, studies on mice and humans have revealed strong links between the gut microbiome composition and both energy harvest and energy

storage in the host (Bäckhed, 2011; Li et al., 2013; Shapira, 2016). Previous findings, e.g., Bolnick et al. (2014) have shown that microbial diversity can be associated with fish fitness, though their results have not been consistent.

Similarly, our results revealed links between gut microbiome composition and fish body condition. Though, we went further by exploring the relationship between microbiome composition and fish body condition by differentiating the gut microbiome based on its sources. We show that while microbes from macroinvertebrates are less efficient at establishing in the intestinal environment, they still have a stronger association with fish body condition compared to zooplankton associated microbes. However, an increase in macroinvertebrate-associated microbiota could not be linked to the increased condition in perch and roach. Invertebrate diet compared to zooplankton diet, when given in the same amount, has been shown to lead to better growth in a previous study on perch (Borcherding et al., 2007). Furthermore, it has been shown that macroinvertebrates have access to better quality food during late summer and autumn in Lake Erken (our study lake) (Ahlgren et al., 1997), which potentially can lead to macroinvertebrates being of higher quality food for perch and roach. Thus, the association between macroinvertebrate and fish body condition can come from correlations between both invertebrate as diet and its associated microbiota. We found opposite associations of microbes attached to macroinvertebrates and zooplankton with fish body condition. This difference may be a result of the different metabolites produced by zooplankton and invertebrate associated microbes. If so, then this can be speculated to have consequences for the host, for example if poorer nutrients are produced by zooplankton associated microbes (Nicholson et al., 2012), which will lead to decreased fitness of their host.

In conclusion, by applying a metacommunity approach to investigate the assembly of intestinal microbiota communities, we show that both environmental selection and ecological drift have influences on community assembly of gut microbiota. Ecological drift was predicted to be more important in perch than roach leading to a smaller role for environmental interactions, including species-interactions in perch. Confirming previous studies, we infer using statistical means that environmental factors, such as fish diet and habitat choice can affect intestinal microbial communities. Both environment and dispersal factors contributed to the intestinal community composition but the relative contribution of these differed between perch and roach. This difference in assembly mechanisms may come from differences in the structure of the gut between perch and roach but also from genetic difference. Furthermore, we predict from our statistical analyses that fish body condition depends on bacterial dispersal sources, potentially affecting other traits such as immunity. While our understanding of how intestinal microbes assemble in their hosts, and what factors contribute to these processes has come a fair way, novel insights can be gained from comparative studies on natural animal populations as exemplified in our study.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the European Nucleotide Archive (ENA) under accession numbers ERS4181501 – ERS4181737.

ETHICS STATEMENT

The animal study was reviewed and approved by the ethical committee of Uppsala Djurförsöketiska Nämnd (permit number C80/13).

AUTHOR CONTRIBUTIONS

YZ, RS, and EL designed the study. YZ and RS did the field work. YZ did the laboratory sample process, data analysis, and wrote the first draft of the manuscript. AE processed the sequencing data. All authors have contributed to the writing and revising of all the previous versions of the manuscript.

FUNDING

This work was supported by the Swedish Research Council (VR) to RS and Malméns Stiftelse to YZ.

ACKNOWLEDGMENTS

We would like to thank Konrad Karlsson, Johnny Malmberg, and Dandan Shen for their great help in the sampling at Erken. We are also grateful to Silke Langenheder, William Jones, Mathew Leibold and three reviewers for constructive comments on earlier version of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.00152/full#supplementary-material

REFERENCES

Ahlgren, G., Goedkoop, W., Markensten, H., Sonesten, L., and Boberg, M. (1997). Seasonal variations in food quality for pelagic and benthic invertebrates in Lake Erken - the role of fatty acids. *Freshw. Biol.* 38, 555–570.

Alves-Stanley, C. D., and Worthy, G. A. J. (2009). Carbon and nitrogen stable isotope turnover rates and diet-tissue discrimination in Florida manatees (*Trichechus manatus latirostris*). *J. Exp. Biol.* 212, 2349–2355. doi: 10.1242/jeb.027565

Bäckhed, F. (2011). Programming of host metabolism by the gut microbiota. *Ann. Nutr. Metab.* 58, 44–52. doi: 10.1159/000328042

Beasley, D. E., Koltz, A. M., Lambert, J. E., Fierer, N., and Dunn, R. R. (2015). The evolution of stomach acidity and its relevance to the human microbiome. *PLoS One* 10:e0134116. doi: 10.1371/journal.pone.0134116

Bender, L. C., Cook, J. G., Cook, R. C., and Hall, P. B. (2008). Relations between nutritional condition and survival of North American elk *Cervus elaphus*. *Wildlife Biol.* 14, 70–80.

Benson, A. K., Kelly, S. A., Legge, R., Ma, F., Low, S. J., Kim, J., et al. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18933–18938. doi: 10.1073/pnas.1007028107

Berg, M., Stenuit, B., Ho, J., Wang, A., Parke, C., Knight, M., et al. (2016). Assembly of the *Caenorhabditis elegans* gut microbiota from diverse soil microbial environments. *ISME J.* 10, 1998–2009. doi: 10.1038/ismej.2015.253

Berga, M., Östman, Ö., Lindström, E. S., and Langenheder, S. (2015). Combined effects of zooplankton grazing and dispersal on the diversity and assembly mechanisms of bacterial metacommunities. *Environ. Microbiol.* 17, 2275–2287. doi: 10.1111/1462-2920.12688

Bolnick, D. I., Snowberg, L. K., Hirsch, P. E., Lauber, C. L., Knight, R., Caporaso, J. G., et al. (2014). Individuals' diet diversity influences gut microbial diversity in two freshwater fish (Threespine stickleback and Eurasian perch). *Ecol. Lett.* 17, 979–987. doi: 10.1111/ele.12301

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., et al. (2003). The ecology of individuals: incidence and implications of individual specialization. *Am. Nat.* 161, 1–28. doi: 10.1086/343878

Borcherding, J., Hermasch, B., and Murawski, P. (2007). Field observations and laboratory experiments on growth and lipid content of young-of-the-year perch. *Ecol. Freshw. Fish* 16, 198–209.

Brooks, A. W., Kohl, K. D., Brucker, R. M., van Opstal, E. J., and Bordenstein, S. R. (2016). Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history. *PLoS Biol.* 14:e2000225. doi: 10.1371/journal.pbio.2000225

Burns, A. R., Zac Stephens, W., Stagaman, K., Wong, S., Rawls, J. F., Guillemin, K., et al. (2016). Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. *ISME J.* 10, 655–664. doi: 10.1038/ismej.2015.142

Cadotte, M., Cavender-Bares, J., Tilman, D., and Oakley, T. (2009). Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PLoS One* 4:e5695. doi: 10.1371/journal.pone.0005695

Campbell, J. H., Foster, C. M., Vishnivetskaya, T., Campbell, A. G., Yang, Z. K., Wymore, A., et al. (2012). Host genetic and environmental effects on mouse intestinal microbiota. *ISME J.* 6, 2033–2044. doi: 10.1038/ismej.2012.54

Chase, J. M., Kraft, N. J. B., Smith, K. G., Vellend, M., and Inouye, B. D. (2011). Using null models to disentangle variation in community dissimilarity from variation in α-diversity. *Ecosphere* 2:art24.

Chave, J. (2004). Neutral theory and community ecology. *Ecol. Lett.* 7, 241–253.

Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J. M., and Relman, D. A. (2012). The application of ecological theory toward an understanding of the human microbiome. *Science* 336, 1255–1262. doi: 10.1126/science.1224203

Davis, B. E., Afton, A. D., and Cox, R. (2011). Factors affecting winter survival of female mallards in the lower mississippi alluvial valley. *Waterbirds* 34, 186–194.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., et al. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14691–14696. doi: 10.1073/pnas.1005963107

Derrien, M., and van Hylckama Vlieg, J. E. T. (2015). Fate, activity, and impact of ingested bacteria within the human gut microbiota. *Trends Microbiol.* 23, 354–366. doi: 10.1016/j.tim.2015.03.002

Dethlefsen, L., Eckburg, P. B., Bik, E. M., and Relman, D. A. (2006). Assembly of the human intestinal microbiota. *Trends Ecol. Evol.* 21, 517–523.

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., et al. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11971–11975. doi: 10.1073/pnas.1002601107

Dutil, J. D., and Lambert, Y. (2000). Natural mortality from poor condition in Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 57, 826–836.

Dutton, R. J., and Turnbaugh, P. J. (2012). Taking a metagenomic view of human nutrition. *Curr. Opin. Clin. Nutr. Metab. Care* 15, 448–454. doi: 10.1097/MCO.0b013e3283561133

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10, 996–998. doi: 10.1038/nmeth.2604

Eriksson, L., Byrne, T., Johansson, E., Trygg, J., and Vikström, C. (2013). *Multi- and Megavariate Data Analysis Basic Principles and Applications*, Vol. 1. Umeå: Umetrics Academy.

Escalas, A., Troussellier, M., Yuan, T., Bouvier, T., Bouvier, C., Mouchet, M. A., et al. (2017). Functional diversity and redundancy across fish gut, sediment and water bacterial communities. *Environ. Microbiol.* 19, 3268–3282. doi: 10.1111/1462-2920.13822

Evan, S. (2016). Effects of dispersal and selection on stochastic assembly in microbial communities. *Nat. Publ.* 1–10. doi: 10.1038/ismej.2016.96

Ferreira, M. T., Franco, A., Catarino, L., Moreira, I., and Sousa, P. (1999). Environmental factors related to the establishment of algal mats in concrete irrigation channels. *Hydrobiologia* 415, 163–168.

Fine, P. V. A., and Kembel, S. W. (2011). Phylogenetic community structure and phylogenetic turnover across space and edaphic gradients in western Amazonian tree communities. *Ecography* 34, 552–565.

Fukami, T. (2015). Historical contingency in community assembly: integrating niches, species pools, and priority effects. *Annu. Rev. Ecol. Evol. Syst.* 46, 1–23. doi: 10.1111/nph.13362

Jacoby, J. M. (1985). Grazing effects on periphyton by *Theodoxus fluviatilis* (Gastropoda) in a lowland stream. *J. Freshw. Ecol.* 3, 265–274.

Knights, D., Kuczynski, J., Charlson, E. S., Zaneveld, J., Mozer, M. C., Collman, R. G., et al. (2011). Bayesian community-wide culture-independent microbial source tracking. *Nat. Methods* 8, 761–763. doi: 10.1038/nmeth.1650

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., et al. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proc. Natl. Acad. Sci. U.S.A.* 108(Suppl.), 4578–4585. doi: 10.1073/pnas.1000081107

Langenheder, S., Wang, J., Karjalainen, S. M., Laamanen, T. M., Tolonen, K. T., Vilmi, A., et al. (2017). Bacterial metacommunity organization in a highly-connected aquatic system. *FEMS Micriobiol. Ecol.* 93:fiw225. doi: 10.1093/femsec/fiw225

Lanzén, A., Jørgensen, S. L., Huson, D. H., Gorfer, M., Grindhaug, S. H., Jonassen, I., et al. (2012). CREST - classification resources for environmental sequence tags. *PLoS One* 7:e49334. doi: 10.1371/journal.pone.0049334

Legendre, P., and Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia* 129, 271–280. doi: 10.1007/s004420100716

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.

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., and Gordon, J. I. (2008). Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* 6, 776–788. doi: 10.1038/nrmicro1978

Li, L., and Ma, Z. (2016). Testing the neutral theory of. (Biodiversity)with human microbiome datasets. *Sci. Rep.* 6:31448. doi: 10.1038/srep31448

Li, X., Yan, Q., Xie, S., Hu, W., Yu, Y., and Hu, Z. (2013). Gut microbiota contributes to the growth of fast-growing transgenic common carp (*Cyprinus carpio* L.). *PLoS One* 8:e64577. doi: 10.1371/journal.pone.0064577

Lindström, E. S., and Langenheder, S. (2012). Local and regional factors influencing bacterial community assembly. *Environ. Microbiol. Rep.* 4, 1–9. doi: 10.1111/j.1758-2229.2011.00257.x

Lundvall, D., Svanbäck, R., Persson, L., and Byström, P. (1999). Size-dependent predation in piscivores: interactions between predator foraging and prey avoidance abilities. *Can. J. Fish. Aquat. Sci.* 56, 1285–1292.

Martinsen, T. C., Bergh, K., and Waldum, H. L. (2005). Gastric juice: a barrier against infectious diseases. *Basic Clin. Pharmacol. Toxicol.* 96, 94–102. doi: 10.1111/j.1742-7843.2005.pto960202.x

Matthews, B., Marchinko, K. B., Bolnick, D. I., and Mazumder, A. (2010). Specialization of trophic position and habitat use by sticklebacks in an adaptive radiation. *Ecology* 91, 1025–1034. doi: 10.1890/09-0235.1

Mihaljevic, J. R. (2012). Linking metacommunity theory and symbiont evolutionary ecology. *Trends Ecol. Evol.* 27, 323–329. doi: 10.1016/j.tree.2012.01.011

Miller, E. T., Svanbäck, R., and Bohannan, B. J. M. (2018). Microbiomes as metacommunities: understanding host associated microbes through metacommunity ecology. *Trends Ecol. Evol.* 33, 926–935. doi: 10.1016/j.tree.2018.09.002

Nelson, T. M., Rogers, T. L., Carlini, A. R., and Brown, M. V. (2013). Diet and phylogeny shape the gut microbiota of Antarctic seals: a comparison of wild and

captive animals. *Environ. Microbiol.* 15, 1132–1145. doi: 10.1111/1462-2920.12022

Nemergut, D. R., Schmidt, S. K., Fukami, T., O'Neil, 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. doi: 10.1128/MMBR.00051-12

Newsome, S. D., del Rio, C. M., Bearhop, S., and Philips, D. L. (2007). A niche for isotopic ecology. *Front. Ecol. Environ.* 5, 429–436. doi: 10.1890/060150.1

Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., et al. (2012). Host-gut microbiota metabolic interactions. *Science* 108, 1262–1268.

Persson, L., Diehl, S., Johansson, L., Andersson, G., and Hamrin, S. F. (1991). Shifts in fish communities along the productivity gradient of temperate lakes - patterns and the importance of size-structure interactions. *J. Fish Biol.* 38, 281–293.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703–718. doi: 10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Price, M. N., Dehal, P. S., and Arkin, A. P. (2010). FastTree 2-approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490. doi: 10.1371/journal.pone.0009490

Raup, D. M., and Crick, R. E. (1979). Measurement of faunal similarity in paleontology. *J. Paleontol.* 53, 1213–1227.

Ravussin, Y., Koren, O., Spor, A., and LeDuc, C. (2012). Responses of gut microbiota to diet composition and weight loss in lean and obese mice. *Obesity* 20, 738–747. doi: 10.1038/oby.2011.111

Round, J. L., and Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 9, 313–324. doi: 10.1038/nri2515

Rousk, J., Bååth, 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. doi: 10.1038/ismej.2010.58

Schmidt, V. T., Smith, K. F., Melvin, D. W., and Amaral-Zettler, L. A. (2015). Community assembly of a euryhaline fish microbiome during salinity acclimation. *Mol. Ecol.* 24, 2537–2550. doi: 10.1111/mec.13177

Shapira, M. (2016). Gut microbiotas and host evolution: scaling up symbiosis. *Trends Ecol. Evol.* 31, 539–549. doi: 10.1016/j.tree.2016.03.006

Sinclair, L., Osman, O. A., Bertilsson, S., and Eiler, A. (2015). Microbial community composition and diversity via 16S rRNA gene amplicons: evaluating the illumina platform. *PLoS One* 10:e0116955. doi: 10.1371/journal.pone.0116955

Skulason, S., and Smith, T. B. (1995). Resource polymorphisms in vertebrates. *Trends Ecol. Evol.* 10, 366–370.

Spor, A., Koren, O., and Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat. Rev. Microbiol.* 9, 279–290. doi: 10.1038/nrmicro2540

Stegen, J. C., Lin, X., Fredrickson, J. K., Chen, X., Kennedy, D. W., Murray, C. J., et al. (2013). Quantifying community assembly processes and identifying features that impose them. *ISME J.* 7, 2069–2079. doi: 10.1038/ismej.2013.93

Stegen, J. C., Lin, X., Konopka, A. E., and Fredrickson, J. K. (2012). Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* 6, 1653–1664. doi: 10.1038/ismej.2012.22

Stevens, C. E., and Hume, I. D. (1998). Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiol. Rev.* 78, 393–427. doi: 10.1152/physrev.1998.78.2.393

Stewart, J. A., Chadwick, V. S., and Murray, A. (2005). Investigations into the influence of host genetics on the predominant eubacteria in the faecal microflora of children. *J. Med. Microbiol.* 54, 1239–1242. doi: 10.1099/jmm.0.46189-0

Strayer, D. L., Caraco, N. F., Cole, J. J., Findlay, S., and Pace, M. L. (1999). Transformation of freshwater ecosystems by bivalves – A case study of Zebra mussels in the Hudson River. *Bioscience* 49, 19–27.

Sullam, K. E., Essinger, S. D., Lozupone, C. A., O'Connor, M. P., Rosen, G. L., Knight, R., et al. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Mol. Ecol.* 21, 3363–3378. doi: 10.1111/j.1365-294X.2012.05552.x

Svanbäck, R., Eklöv, P., Fransson, R., and Holmgren, K. (2008). Intra-specific competition drives multiple species trophic polymorphism in fish communities. *Oikos* 117, 114–124.

Svanbäck, R., and Persson, L. (2009). Population density fluctuations change the selection gradient in Eurasian perch. *Am. Nat.* 173, 507–516. doi: 10.1086/597223

Svanbäck, R., Quevedo, M., Olsson, J., and Eklöv, P. (2015). Individuals in food webs: the relationships between trophic position, omnivory and among-individual diet variation. *Oecologia* 178, 103–114. doi: 10.1007/s00442-014-3203-4

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., John, F., Bosch, T. C. G., et al. (2016). Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes. *bioRxiv* [Preprint]. doi: 10.1101/038596

Veiga, P., Pons, N., Agrawal, A., Oozeer, R., Guyonnet, D., Faurie, J., et al. (2014). Changes of the human gut microbiome induced by a fermented milk product. *Sci. Rep.* 4:6328. doi: 10.1038/srep06328

Vellend, M. (2010). Conceptual synthesis in community ecology. *Q. Rev. Biol.* 85, 183–206. doi: 10.1086/652373

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334, 105–108. doi: 10.1126/science.1208344

Yan, Q., Li, J., Yu, Y., Wang, J., He, Z., Van Nostrand, J. D., et al. (2016). Environmental filtering decreases with fish development for the assembly of gut microbiota. *Environ. Microbiol.* 18, 4739–4754. doi: 10.1111/1462-2920.13365

Zhang, C., Derrien, M., Levenez, F., Brazeilles, R., Ballal, S. A., Kim, J., et al. (2016). Ecological robustness of the gut microbiota in response to ingestion of transient food-borne microbes. *ISME J.* 10, 2235–2245. doi: 10.1038/ismej.2016.13

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 25 September 2020
doi: 10.3389/fevo.2020.521668

Evaluating Alternative Metacommunity Hypotheses for Diatoms in the McMurdo Dry Valleys Using Simulations and Remote Sensing Data

Eric R. Sokol[1,2], J. E. Barrett[3], Tyler J. Kohler[4], Diane M. McKnight[2], Mark R. Salvatore[5] and Lee F. Stanish[1]*

[1] National Ecological Observatory Network (NEON), Battelle, Boulder, CO, United States, [2] Institute of Arctic and Alpine Research (NSTAAR), University of Colorado Boulder, Boulder, CO, United States, [3] Department of Biological Sciences, Virginia Tech, Blacksburg, VA, United States, [4] Stream Biofilm and Ecosystem Research Laboratory, School of Architecture, Civil and Environmental Engineering, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [5] Department of Astronomy and Planetary Science, Northern Arizona University, Flagstaff, AZ, United States

OPEN ACCESS

Edited by:
Mathew A. Leibold, University of Florida, United States

Reviewed by:
Janne Soininen, University of Helsinki, Finland
Sophia I. Passy, The University of Texas at Arlington, United States

***Correspondence:**
Eric R. Sokol
sokole@gmail.com

Specialty section:
This article was submitted to Biogeography and Macroecology, a section of the journal Frontiers in Ecology and Evolution

Received: *19 December 2019*
Accepted: *17 August 2020*
Published: *25 September 2020*

Citation:
Sokol ER, Barrett JE, Kohler TJ, McKnight DM, Salvatore MR and Stanish LF (2020) Evaluating Alternative Metacommunity Hypotheses for Diatoms in the McMurdo Dry Valleys Using Simulations and Remote Sensing Data. Front. Ecol. Evol. 8:521668. doi: 10.3389/fevo.2020.521668

Diatoms are diverse and widespread freshwater Eukaryotes that make excellent microbial subjects for addressing questions in metacommunity ecology. In the McMurdo Dry Valleys of Antarctica, the simple trophic structure of glacier-fed streams provides an ideal outdoor laboratory where well-described diatom assemblages are found within two cyanobacterial mat types, which occupy different habitats and vary in coverage within and among streams. Specifically, black mats of *Nostoc* spp. occur in marginal wetted habitats, and orange mats (*Oscillatoria* spp. and *Phormidium* spp.) occur in areas of consistent stream flow. Despite their importance as bioindicators for changing environmental conditions, the role of dispersal in structuring dry valley diatom metacommunities remains unclear. Here, we use MCSim, a spatially explicit metacommunity simulation package for R, to test alternative hypotheses about the roles of dispersal and species sorting in maintaining the biodiversity of diatom assemblages residing in black and orange mats. The spatial distribution and patchiness of cyanobacterial mat habitats was characterized by remote imagery of the Lake Fryxell sub-catchment in Taylor Valley. The available species pool for diatom metacommunity simulation scenarios was informed by the Antarctic Freshwater Diatoms Database, maintained by the McMurdo Dry Valleys Long Term Ecological Research program. We used simulation outcomes to test the plausibility of alternative community assembly hypotheses to explain empirically observed patterns of freshwater diatom biodiversity in the long-term record. The most plausible simulation scenarios suggest species sorting by environmental filters, alone, was not sufficient to maintain biodiversity in the Fryxell Basin diatom metacommunity. The most plausible scenarios included either (1) neutral models with different immigration rates for diatoms in orange and black mats or (2) species sorting by a relatively weak environmental filter, such that dispersal dynamics also influenced diatom community assembly, but there was not such a strong disparity

in immigration rates between mat types. The results point to the importance of dispersal for understanding current and future biodiversity patterns for diatoms in this ecosystem, and more generally, provide further evidence that metacommunity theory is a useful framework for testing hypotheses about microbial community assembly.

Keywords: Antarctica, Bacillariophyceae, *Nostoc*, dispersal, stream ecology

INTRODUCTION

Metacommunity theory provides a framework to integrate dispersal dynamics with local community assembly processes, such as environmental filtering, over a patchy and heterogeneous landscape (Leibold et al., 2004; Leibold and Chase, 2017). In general, different levels of dispersal, habitat heterogeneity, and species sorting are predicted to result in different biodiversity outcomes (Leibold et al., 2004; Sokol et al., 2017). However, dispersal can be difficult to assess, especially for microorganisms, which have been long-assumed to have nearly unlimited, widespread dispersal (Baas Becking, 1934). The dogma that all microbes are ubiquitous has been challenged over the past two decades, and evidence is mounting that metacommunity theory applies to microbial life (Martiny et al., 2006; Nemergut et al., 2013; Langenheder and Lindström, 2019). Simulations can be used to overcome some of the challenges of understanding microbial community assembly by characterizing the likely roles of environmental filters (e.g., species sorting) and dispersal (e.g., source-sink dynamics) in structuring empirically observed metacommunities (Sokol et al., 2015) to compliment field-based experimental observations.

The ice-free desert oases of Antarctica represent ideal locations to test the application of metacommunity theory to microbial communities because these ecosystems are characterized by geographical isolation, relatively low anthropogenic impacts, and low trophic complexity. Here, freshwater habitats often harbor dense cyanobacterial mats, which are the major primary producers in the landscape (McKnight et al., 1998). Because these ecosystems lack vascular plants, data products derived from satellite imagery (e.g., NDVI maps) can be used to map the distribution of such mats throughout the landscape (Salvatore et al., 2020). In the McMurdo Dry Valleys (MDV) of East Antarctica, "orange" and "black" mat types are common in and around glacial meltwater streams. These mats differ based on their appearance, dominant taxa, and location in the stream channel (Alger, 1997; McKnight and Tate, 1997; McKnight et al., 1998; Kohler et al., 2015; Van Horn et al., 2016). Orange mats are dominated by the filamentous cyanobacterial genera *Oscillatoria* and *Phormidium*, and typically occur in areas with consistent stream flow. In contrast, black mats are dominated by *Nostoc* spp. and occur in intermittently wet habitats, such as seeps and along stream margins. Furthermore, the coverage of mat growth varies dramatically within and among streams, where surveys of stream reaches have reported cyanobacterial mat percent coverages ranging from near 0 to near 100% (Alger, 1997; Kohler et al., 2015).

These cyanobacterial mats provide the primary habitat for diatom (Bacillariophyceae) assemblages in the ice-free landscape of the MDV (Esposito et al., 2006, 2008), where diatoms typically account for up to 10% of total mat biomass (Alger, 1997; McKnight et al., 1998; Kohler et al., 2015). Different diatom taxa are often associated with specific mat types (Darling et al., 2017; Andriuzzi et al., 2018; Kopalová et al., 2019). Thus, the heterogeneous distribution of mat types within and across MDV streams provides a patchy mosaic in which spatial habitat complexity can mediate the dispersal dynamics of these benthic diatoms, integrating local community assembly processes across the landscape, and creating a unique opportunity for study.

Here we focus on the resident diatom assemblages in the cyanobacterial mats because diatoms are excellent organisms for ecological monitoring and examination of biogeography due to their species-specific morphology, excellent preservation of their siliceous cell walls, and individual environmental tolerances (Smol and Stoermer, 2010; Spaulding et al., 2010). In the MDV, diatoms are an important sentinel taxonomic group because they are the most diverse and broadly distributed Eukaryote in the region (Adams et al., 2006; Spaulding et al., 2010; Kociolek et al., 2017). Thus, it is particularly important to understand the dynamics that control diatom biodiversity in the MDV.

Past work suggests that metacommunity theory can provide insight into the processes organizing MDV microbial biodiversity in general (Sokol et al., 2013) and diatom biodiversity in particular (Sakaeva et al., 2016). In MDV streams, several aspects of the flow regime appear to be dominant environmental filters for diatom communities. Specifically, the occurrence of flood pulses and periods without flow during the austral summer, as well as the interannual frequency of flow, have been linked to diatom assemblage composition (Esposito et al., 2006; Stanish et al., 2011, 2012). Laboratory and field experiments indicate that optimal growth temperatures (Darling et al., 2017) and nutrient concentrations (Kohler et al., 2016) may also influence species composition, but these factors are also strongly influenced by stream hydrology (Wlostowski et al., 2018). Thus, there is strong support that hydrology is a master environmental variable for understanding diatom assemblage structure in the MDV (Esposito et al., 2006; Stanish et al., 2012). In addition, dispersal dynamics can also influence benthic diatom community composition (Soininen et al., 2004; Verleyen et al., 2009; Heino et al., 2010; Sakaeva et al., 2016; Chen et al., 2019). Recently, Sakaeva et al. (2016) provided evidence that diatom assemblages residing in cyanobacterial mats at pond margins showed trends in composition across both spatial and environmental gradients in the MDV, indicating that dispersal dynamics may modify how environmental filters structure diatom assemblages across the landscape, aligning with other similar studies from the region (Michaud et al., 2012; Sokol et al., 2013).

Both wind and water are potential vectors for diatom dispersal in the MDV. Wind-driven transport has been documented for organic matter and biota in Taylor Valley (Šabacká et al., 2012; Diaz et al., 2018), where the most extreme, and likely most consequential, foehn wind events occur during the austral winters (Nylen et al., 2004). Many MDV diatom genera are considered to be aerophilic (e.g., *Luticola*, *Hantzschia*, *Humidophila*, and *Muelleria*), and thus adapted to survive the challenges associated with both aeolian transport (Diaz et al., 2018) and the desiccation-rehydration and freeze-thaw cycles associated with life in MDV cyanobacterial mat habitats (McKnight et al., 1999). Streams have also been shown to be an important vector for the movement of organic matter, including diatoms, downstream toward the terminal lakes (Cullis et al., 2014; Kohler et al., 2018). Black mats are probably more easily mobilized than orange mats (e.g., by high wind or high flow events) and appear to be more sensitive to changes in the hydrologic regime (Kohler et al., 2015, 2018; Gooseff et al., 2017). Thus, there is the potential for differences in the dispersal of diatoms among patches of cyanobacterial mats in the MDV landscape to mediate how local (i.e., "alpha-scale" or "patch-scale") community assembly processes scale to maintain the biodiversity of the diatom metacommunity (i.e., "gamma-scale" or "regional-scale").

It is to be expected that diatom biodiversity in the MDV will be influenced by physical controls over the hydrologic habitat template (Poff and Ward, 1990), largely because hydrology controls the distribution of the cyanobacterial mats that provide habitat for diatom assemblages. Indeed, the past three decades of research in the MDV provide strong support for this general hypothesis. However, the ways in which an altered landscape translates to a shift in biodiversity can vary dramatically given different metacommunity dynamics (e.g., Sokol et al., 2015, 2017). Thus, if we assume incorrect underlying community assembly mechanisms, our predictions about how future climatic conditions will affect MDV diatom biodiversity will be incorrect. Furthermore, as warming is expected in the coming decades (Shindell and Schmidt, 2004), a mechanistic understanding of the underlying diatom community assembly dynamics will be crucial for interpreting trends in the composition and diversity of these indicator taxa in a non-stationary world (Milly et al., 2008; Fitzpatrick et al., 2018).

Given that cyanobacterial mats provide a patchy mosaic of habitats for the MDV diatom metacommunity, our alternative hypotheses are:

H1: Neutral model (NM) hypothesis: all taxa are functionally equivalent and respond similarly to environmental filters (sensu Hubbell, 2001), but orange and black mats represent patches characterized by different dispersal dynamics (i.e., one habitat type allows for a high colonization success rate for immigrants).

H2: Moderate species sorting (MSS) hypothesis: different mat types represent patches with both different dispersal dynamics and different local environmental filters that moderately affect species sorting.

H3: Strong species sorting (SSS) hypothesis: differences in environmental filters between mat types exert a very strong influence over species sorting, largely overwhelming any spatial patterns that would otherwise emerge from dispersal dynamics.

All three of these hypotheses provide different mechanisms for the maintenance of diatom biodiversity in the MDV, and different processes by which an altered landscape might drive changes in diatom biodiversity in future climate scenarios. H1 provides a mechanism based on NM metacommunity dynamics where the stochastic processes that determine emergent diatom biodiversity patterns are governed by the spatial arrangement of habitable patches in the MDV landscape and the movement of diatoms among these patches (i.e., cyanobacterial mats). Alternatively, H3 provides a deterministic mechanism based on SSS dynamics where environmental filters sort diatom assemblages by functional traits at the patch (i.e., a cyanobacterial mat) scale. Following H3, emergent biodiversity patterns are simply predicted by the relative dominance of different mat types, and thus different types of environmental filters, in the MDV landscape. H2 (MSS) represents a hybrid hypothesis allowing for stochastic dispersal and colonization dynamics (H1) to play out in a non-neutral landscape (H3). Given existing evidence, we expect both species sorting and dispersal dynamics to play roles in organizing the diatom metacommunities residing in cyanobacterial mats in and around MDV streams. Modeling provides an opportunity to characterize what those stochastic (dispersal) and deterministic (species sorting) dynamics might look like.

Here, our objectives are (1) to test the plausibility of the three alternative metacommunity hypotheses described above to explain observed patterns in diatom biodiversity in cyanobacterial mats in stream channels in the Fryxell Basin of Taylor Valley, MDV and (2) to characterize the stochastic (dispersal) and deterministic (environmental filtering) dynamics of the most plausible scenarios. To evaluate the plausibility of the alternative hypotheses, biodiversity must be characterized at both the patch (alpha-diversity) and metacommunity (gamma-diversity) scales in order to understand its drivers. Among-patch variation in community composition (beta-diversity) provides a measure of scaling, i.e., the contribution of each local patch to the regional species pool. We apply a spatially explicit numerical model, MCSim (Sokol et al., 2015, 2017; Sokol, 2019), to predict diatom biodiversity outcomes under different metacommunity scenarios, using satellite imagery (i.e., a derived map of cyanobacterial mats) and ground-based observations of diatom assemblages to constrain simulation initial conditions (**Figure 1**). We use simulation outcomes to evaluate the plausibility of each of the three hypotheses outlined above.

MATERIALS AND METHODS

Site Description

The MDV are approximately 4,500 km^2 in area, making them the largest ice-free area on the continent (Levy, 2013). The MDV

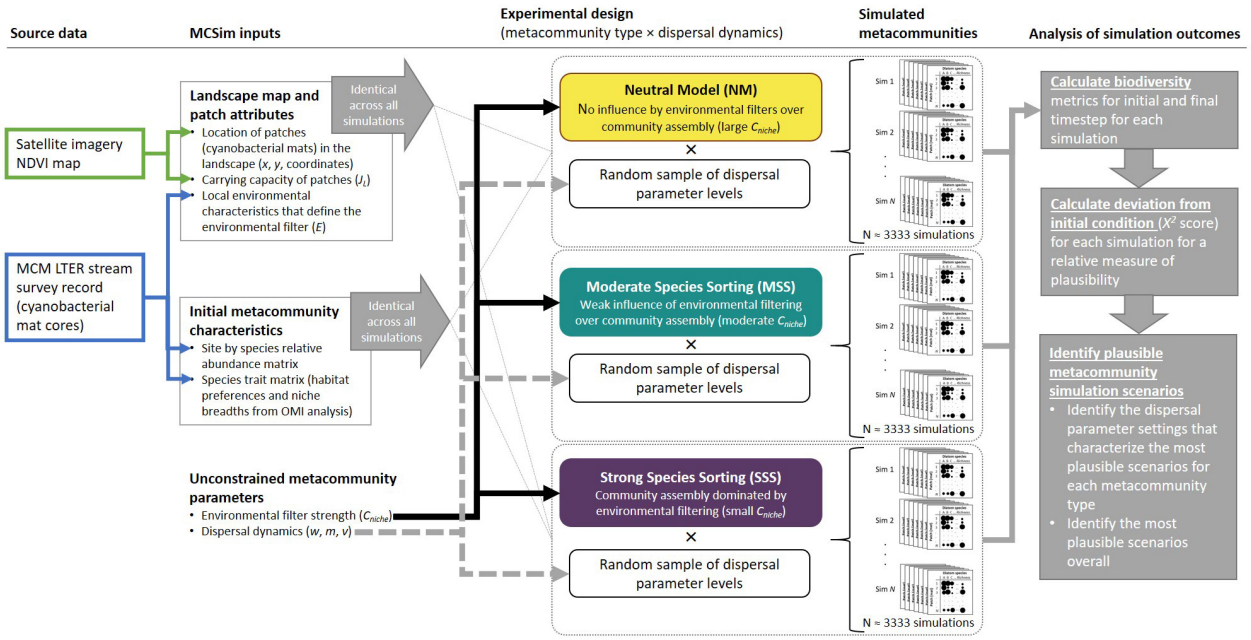


FIGURE 1 | Workflow to incorporate source data from the McMurdo Long Term Ecological Research (MCM LTER) program and remotely sensed imagery to constrain spatially explicit metacommunity simulations based on the MCSim framework to assess the plausibility of different metacommunity scenarios. Simulations that best maintained initial biodiversity levels were considered the most plausible.

provide an excellent model ecosystem for integrating community assembly theory and models with patterns of diatom distribution observed in the field because the diatom metacommunity presents a tractable number (∼50) of taxa (Esposito et al., 2008) whose distribution and ecological preferences have been rigorously described in streams since 1994 by the McMurdo Dry Valleys Long Term Ecological Research (MCM LTER) program (Esposito et al., 2006; Stanish et al., 2011, 2012; Kohler et al., 2016; Darling et al., 2017).

During the austral summer, the MDV streams are fed by glacial meltwater and are underlain by permafrost, receiving no groundwater inflow, and flow into perennially ice-covered lakes (Conovitz et al., 1998). The active layer thaws to a depth of about 60 cm and water in the stream channel exchanges with water in saturated sediments underlying and adjacent to the streambed, referred to as the hyporheic zone (Conovitz et al., 2006). This melt period typically lasts for 4–10 weeks, and provides a short growing season when cyanobacterial mats become biologically active (McKnight and Tate, 1997). Due to the influence of the adiabatic lapse rate, the elevation of the source glacier or snowfield exerts a strong influence on the flow regime for a given stream. During cold cloudy summers meltwater generation for the higher elevation glaciers is limited and storage of meltwater in the hyporheic zone further limits flow in the downstream reaches of the longer streams (Conovitz et al., 1998). During warm sunny summers, high flows can occur in most streams and flood pulses can act to scour the mats (Cullis et al., 2014). Overall, these differences in flow regime among streams influence the abundance and coverage of the different mat types (Kohler et al., 2015).

The water chemistry in MDV streams has been monitored by the MCM LTER program for over three decades and is generally dilute compared to temperate streams. Higher concentrations of major ions and silica occur in the longer streams driven by the greater extent of weathering reactions occurring in the hyporheic zone as the water flows from the glacier to the lake (Gooseff et al., 2002). The primary sources of nutrients for the cyanobacterial mats are nitrate deposited on the glacier surfaces associated with auroral activity, weathering of apatite releasing phosphorus in hyporheic sediments, and recycling of nutrients from mat biomass that has become entrained in hyporheic sediments (McKnight et al., 2004; Kohler et al., 2018). In streams with abundant mats, nutrient concentrations are low (**Table 1**) compared with streams where geomorphic conditions, such as unstable sandy substrates, do not support mat growth (McKnight et al., 2004).

In this study, our aim was to model the diatom metacommunity dynamics among patches of cyanobacterial mats located in the sub-catchments of eight streams that drain meltwater from alpine glaciers into Lake Fryxell, which is one of three terminal lakes located in Taylor Valley, in the MDV (**Table 1** and **Figure 2**). The Lake Fryxell drainage basin was selected as our focal system because the hydrological and biological characteristics of its streams have been well documented by the MCM LTER (e.g., Kohler et al., 2015; Wlostowski et al., 2016; Gooseff et al., 2017). Four of the streams are relatively short and have less variable hydrology (Kohler et al., 2015; Wlostowski et al., 2016). These include Canada Stream and Huey, which flow into Lake Fryxell from the north, and Bowles and Green Creek from the west. Canada Glacier and Lake Fryxell provide

TABLE 1 | Characteristics of streams where source data were collected.

Stream group	Stream	Length (km)	Source glacier	NO_3-N (μM)	SRP (μM)
SG1	Canada stream	1.5[b]	Canada glacier	1.10[d]	0.168[d]
	Huey[a]	2.1[b]	Asgard range snowfield	7.70[d]	0.780[d]
SG2	Bowles	0.9[c]	Canada glacier	0.710[e]	0.165[e]
	Green creek	0.8[b]	Canada glacier	0.756[d]	0.193[d]
SG3	Delta	8.0[b]	Howard glacier	2.28[d]	0.250[d]
	Harnish	5.8[b]	Kukri hills glaciers	2.16[d]	0.621[d]
	Crescent	5.9[b]	Crescent glacier	1.56[d]	0.424[d]
	Von Guerard	5.2[b]	Von Guerard glacier	2.00[d]	0.614[d]

Nutrient concentrations are mean nitrate nitrogen (NO_3-N) and soluble reactive phosphorus (SRP) concentrations reported by the MCM LTER program (Welch et al., 2010). [a]Sparse mat coverage in this stream reported by Koch et al. (2010), all other streams harbor patches of abundant mats (McKnight et al., 2004); [b]Wlostowski et al. (2016); [c]Kohler et al. (2015); [d]Welch et al. (2010); [e]K. Welch, personal communication, July 14, 2020.

geographic barriers between the two pairs of streams. The other four streams (Delta, Harnish, Crescent, and Von Guerard) have much longer flow paths, which result in more variable hydrographs, and drain meltwater from alpine glaciers from the south into Lake Fryxell (Kohler et al., 2015; Wlostowski et al., 2016). Streams were grouped such that cyanobacterial mat habitats located in the same stream group would be located in catchments with similar hydrologic characteristics and are not separated by any significant geographic barriers (i.e., Lake Fryxell or Canada Glacier). Stream group 1 (SG1) includes Canada Stream and Huey; stream group 2 (SG2) includes Bowles and Green; and stream group 3 (SG3) includes Delta, Harnish, Crescent, and Von Guerard. These groupings were used in our data sampling procedure (detailed below) to create a more realistic initial metacommunity to serve as a common simulation starting point.

MCSim Metacommunity Modeling

We used v0.4.9 of the MCSim metacommunity simulation package for R (Sokol et al., 2015, 2017; Sokol, 2019) to model scenarios that represent H1, H2, and H3. The simulations required three general components to run (**Figure 1**, MCSim inputs): (1) a landscape map, (2) an initial metacommunity, and (3) parameters that determine the dispersal and recruitment dynamics that occur with each time step of the simulation. We used remotely sensed imagery from the WorldView-2 satellite (**Figure 2**) (Salvatore et al., 2020) and data from the MCM LTER monitoring record (**Figure 3** and **Supplementary Data Sheet 1**) to create a simulation map (**Figure 4A** and **Supplementary Data Sheet 2**) and an initial contrived diatom metacommunity (**Supplementary Data Sheet 3**) as a common starting point for all simulation scenarios. We created 10,000 independent simulations to explore how well different dispersal dynamics crossed with NM, MSS, and SSS metacommunity dynamics could maintain initial metacommunity composition.

The Simulation Landscape: Based on Remote Imagery

Satellite imagery (**Figure 2**) was used to derive a two-dimensional map (**Figure 4A**) highlighting the distribution of photosynthetic mat communities throughout the watershed of Lake Fryxell (i.e., Fryxell Basin). Recent studies have demonstrated that satellite data and the use of common spectral parameters like the Normalized Difference Vegetation Index (NDVI) can effectively identify the distribution of these cyanobacterial mats in the MDV (Salvatore, 2015; Salvatore et al., 2020). NDVI is a commonly used spectral parameter in remote sensing that is used to identify the distribution and relative photosynthetic capacity of vegetation. Most photosynthetic vegetation contains dark pigments that absorb solar radiation at visible wavelengths, while the lack of absorption in the near-infrared results in a significant increase in reflectance at wavelengths just beyond the visible. These contrasting spectral signatures are unique to photosynthetic species relative to nearly all other natural materials. More specifically, NDVI is a ratio between the difference in reflectance at near-infrared and visible wavelengths normalized to the sum of the reflectance at these wavelengths ([NIR-Red]/[NIR + Red], where red and NIR wavelength bands are centered at 0.659 and 0.831 μm, respectively). The product of this ratio is a value between −1 and +1, with higher values representing greater mat density, photosynthetic activity, or the presence of otherwise spectrally dominant vegetation. While NDVI values are non-unique in most terrestrial ecosystems due to the heterogeneity in photosynthetic species, higher NDVI values are far more closely linked to the presence and areal coverage of cyanobacterial mats in the MDV due to the relative simplicity of these ecosystems (Salvatore et al., 2020). Here, we specifically use NDVI to identify likely patches of cyanobacterial mat communities in the Fryxell Basin to provide a proxy for the spatial distribution of diatom habitats.

High-resolution WorldView-2 data were processed in accordance with the methods described in Salvatore (2015) and Salvatore et al. (2020). Data were calibrated to surface reflectance using the Dark Object Subtraction and Regression (DOS-R) methods before NDVI was derived. Surface reflectance represents a fundamental property of the surface itself and, in theory, is independent of other external influences. These calibration steps ensured that differences in the surface composition and coverage were the only properties being measured and not, for example, relative changes in atmospheric properties or illumination conditions.

The derived NDVI raster image (**Figure 2C**) was then imported into R and processed to only include pixels located in Fryxell Basin itself, minimizing any influences due to bedrock

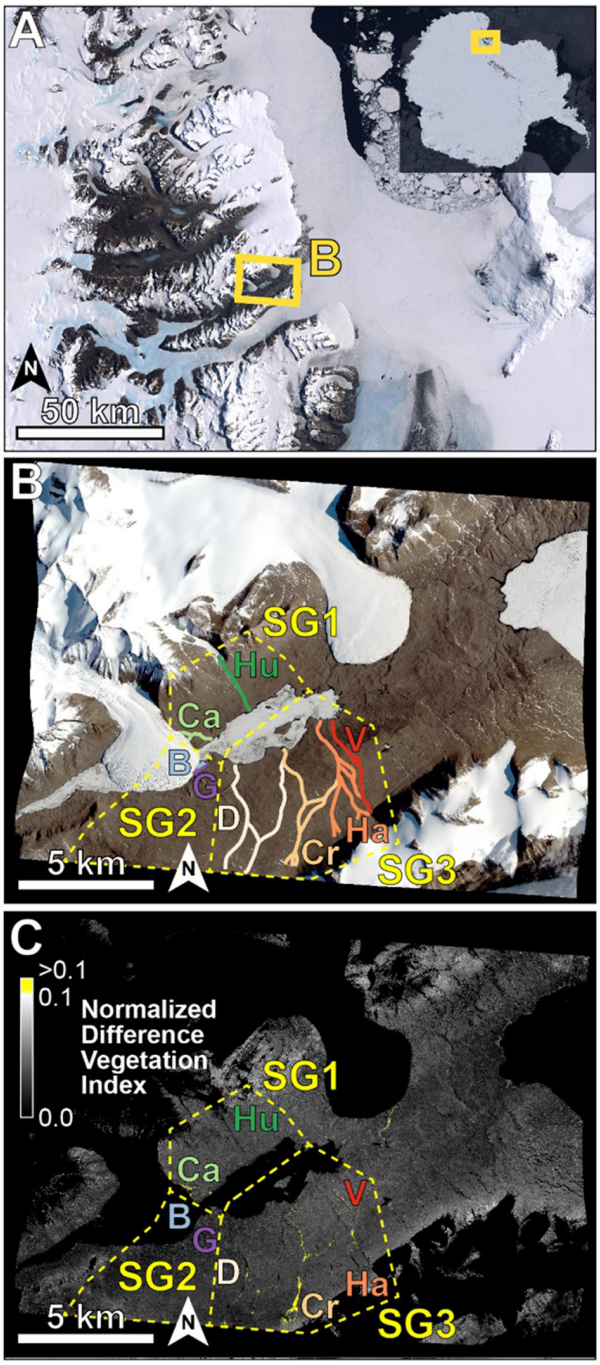


FIGURE 2 | Maps of **(A)** the location of the study area (Fryxell Basin) in the McMurdo Dry Valleys in Antarctica, **(B)** a visible remote image of Fryxell Basin (acquired 21 January 2015 GMT) with stream channels highlighted (labeled clockwise, Ca, Canada Stream; Hu, Huey; V, Von Guerard; Ha, Harnish; Cr, Crescent; D, Delta; G, Green Creek; B, Bowles), and **(C)** the NDVI metric calculated for habitats other than ice and water. Yellow patches represent areas of high NDVI values (>0.1) present within the Fryxell basin. These patches highlight regions of high photosynthetic mat coverage associated with perennial stream channels. Regions of shadow, ice, snow, and high elevation and steep slopes have been excluded from this thresholding to highlight regions of interest. Approximate outlines of stream group (**Table 1**) areas are indicated by dotted lines in **(B,C)**. Imagery © 2015 DigitalGlobe, Inc.

composition, illumination geometry, or topographic effects (R code available in Sokol et al., 2020). We then selected pixels with the 500 greatest NDVI values (summarized in **Table 2**) to represent 500 patches in the simulated metacommunity landscape (**Figure 4A**). Thus, patch locations in the simulated landscape were based on the pixel coordinates in the source NDVI raster image. Further, we assumed that cyanobacterial mat areal coverage within a pixel was positively correlated with the pixel's NDVI score (Salvatore et al., 2020), and that the size of a diatom assemblage within a pixel (J_L) scaled monotonically with the available habitat (i.e., mat coverage) in the pixel. Following these assumptions, total diatom assemblage size (J_L, i.e., the count of extant individuals in an assemblage at a location) in each of the 500 patches included in a simulated metacommunity were a function of observed NDVI score, such that values for J_L followed a log-normal distribution with minimum value of 50 and maximum value of 10,000. These bounds for J_L were set based on a prior sensitivity analysis of how simulated biodiversity metrics were affected by both J_L and overall metacommunity size (J_M) (Sokol et al., 2017), and were chosen to allow for reasonable compute times, but still produce metacommunities that were sufficiently large such that simulation behavior was consistent with previously published studies (Sokol et al., 2015, 2017).

While the metacommunity map, including patch location and patch carrying capacity (J_L), were determined from remote imagery, we were not able to derive other patch attributes in the same way. Specifically, we do not currently have a workflow to derive mat type (orange or black) or local environmental characteristics from available remote imagery. Thus, to create simulation scenarios in which patch attributes reflected realistic distributions of mat type and local environmental characteristics, we used a stratified sampling procedure to assign attributes from the MCM LTER stream monitoring records (described below) to patches in the simulated metacommunities.

Initial Conditions: Based on Empirical Observations

Simulation initial conditions were derived from the long-term stream monitoring data maintained by the MCM LTER program (see Stanish et al., 2011, 2012). The source dataset, assembled from data available on the Antarctic Freshwater Diatoms Database[1] and the MCM LTER data portal[2], spans 1994–2013 and includes 189 records of data from samples (mat cores) collected from orange and black cyanobacterial mats in and near stream channels in Fryxell Basin (summarized in **Table 3**, raw data available in **Supplementary Data Sheet 1**). Each record includes mat type (orange or black), a measurement of cyanobacterial mat chlorophyll-a standing biomass, and estimates of the relative abundances of observed diatom taxa.

To construct the patch attributes (see **Supplementary Data Sheet 2**) and diatom assemblages for the initial contrived metacommunity (see **Supplementary Data Sheet 3**) to serve as a starting point for the simulations, we randomly sampled, with replacement, 500 records from the source dataset derived from the MCM LTER monitoring program (see **Supplementary Data**

[1]http://huey.colorado.edu/diatoms/about/index.php
[2]http://mcm.lternet.edu/

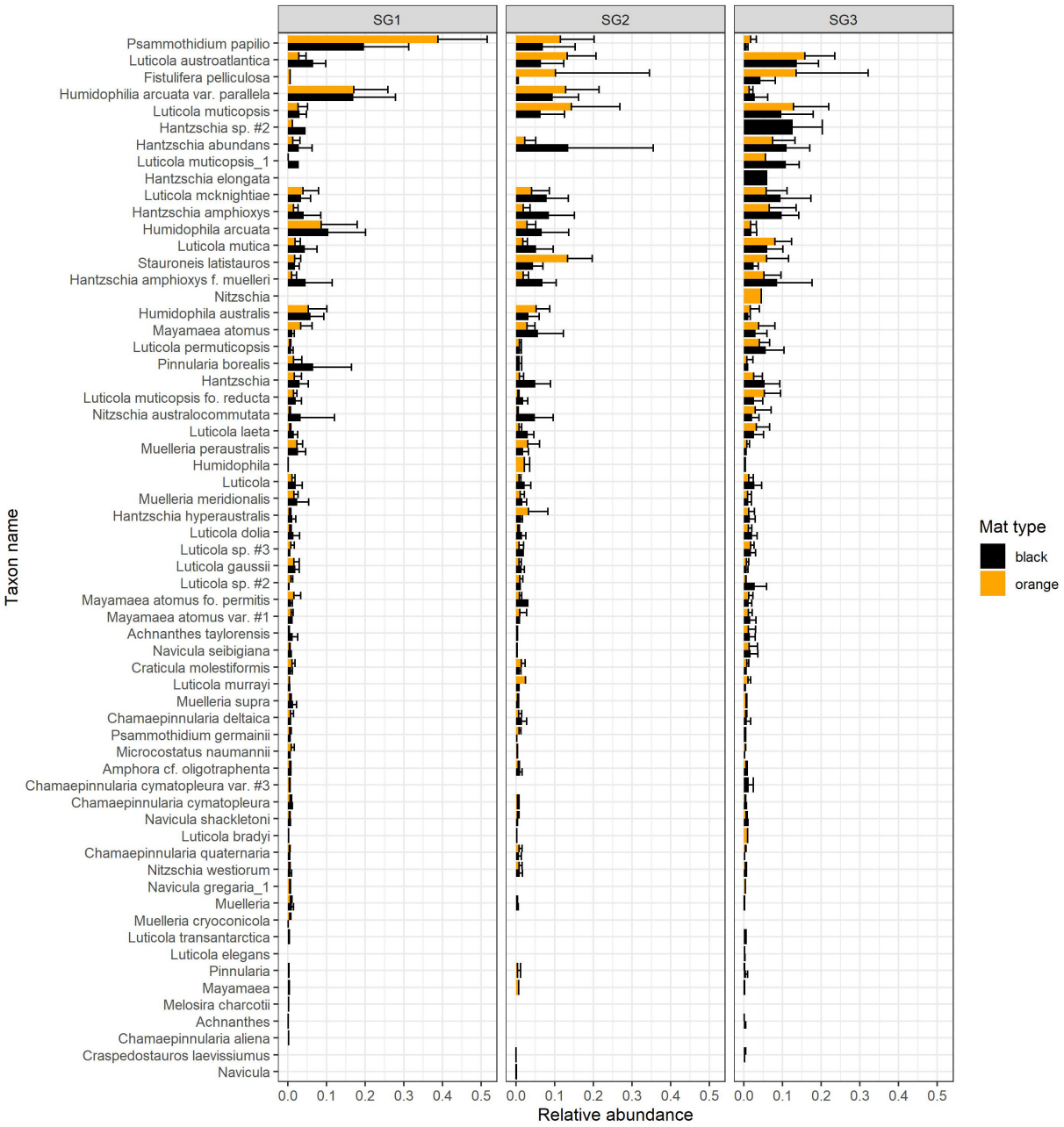


FIGURE 3 | Summary of relative abundances (mean ± sd) of diatom taxa in the MCM LTER record for samples collected from orange and black cyanobacterial mats for each "stream group". Morphospecies designations in the source database were preserved.

Sheet 1), described above. The data from each random sample were assigned to a patch in the simulation map (**Figure 4A**), thus, defining mat type, local environmental characteristics, and the relative abundances of the starting diatom assemblage for each patch in the simulated metacommunity. The sampling procedure was stratified by stream group, such that only empirically observed records from SG1 streams were assigned to the patches in SG1 on the simulation map, only records from SG2 streams were assigned to the SG2 patches on the simulation map, and only records from SG3 streams were assigned to the SG3 patches on the simulation map. This sampling procedure maintained a relative dominance by mat type, a distribution of chlorophyll-a standing biomass values, and diatom community compositions similar to what was observed to occur in each group of sub-catchments (**Figure 3**).

Using the ade4 package for R (Chessel and Dufour, 2006), we conducted an Outlying Mean Index (OMI) analysis (Dolédec et al., 2000) using the 189 records from the MCM LTER

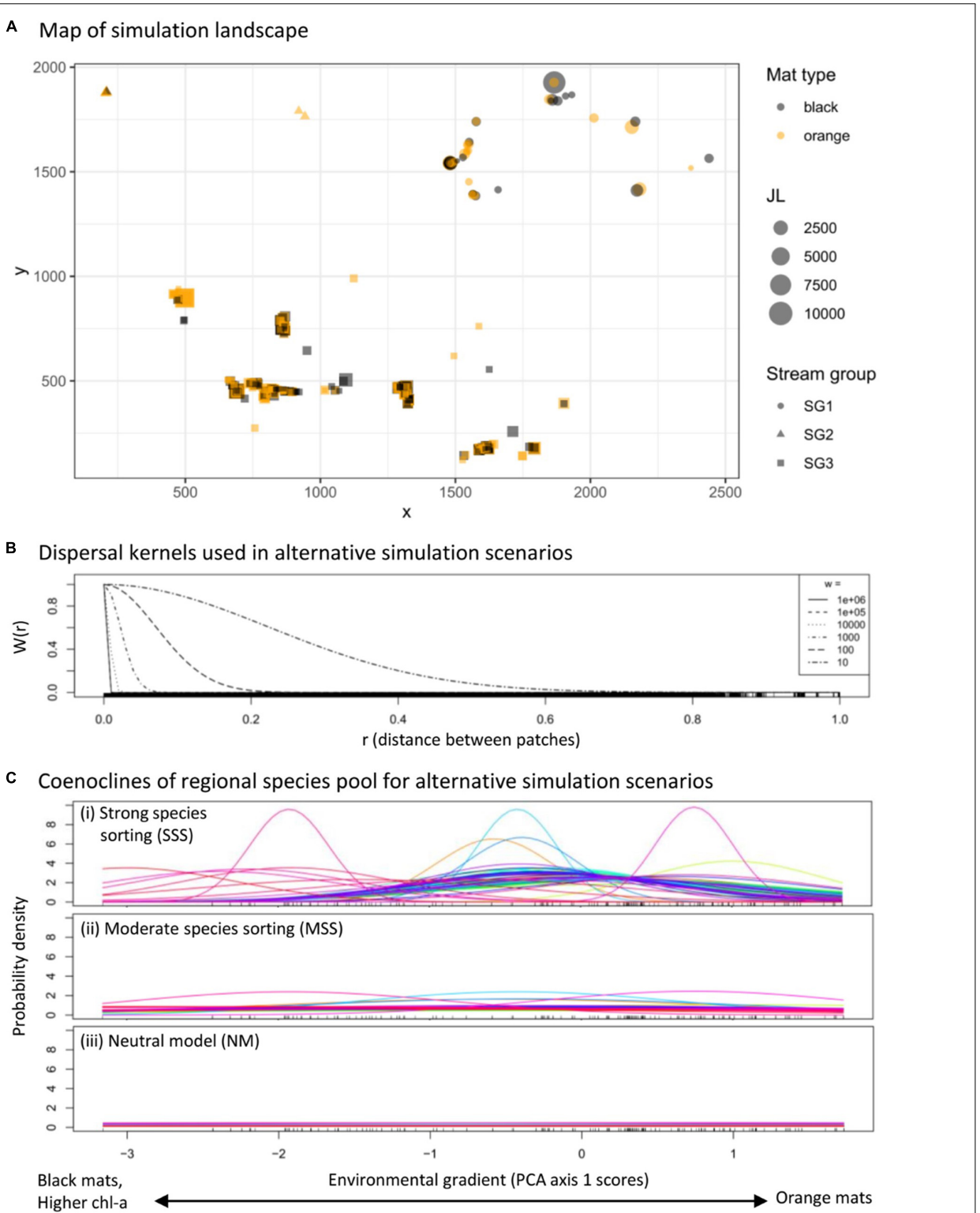


FIGURE 4 | All metacommunity simulations used the same map, where J_L indicates community size at each patch, *x*- and *y*- axes indicate patch coordinates in pixels, which are the spatial units used in the simulation and correspond to a ~2 m × 2 m area in the original raster map **(A)**, each simulation scenario was randomly assigned one of the dispersal kernels (see Eq. 2) plotted in **(B)**, and included species habitat preferences and niche breadths resulting in different types of metacommunity dynamics: **(C-i)**, where C_{niche} = 1, strong species sorting (SSS); **(C-ii)**, where C_{niche} = 4, moderate species sorting (MSS); or **(C-iii)**, where C_{niche} = 20, neutral model (NM). The "rug" (upward ticks along the *x*-axis) in **(B)** indicates all the pairwise distances between patches in the simulation landscape, and the "rug" in **(C)** is the distribution of patches along the environmental gradient used in the simulation.

TABLE 2 | Summary of NDVI source data used in metacommunity simulations.

Stream group	N pixels (patches in simulation)	NDVI		
		Mean	Minimum	Maximum
SG1	65	0.266	0.218	0.675
SG2	7	0.232	0.221	0.246
SG3	428	0.245	0.218	0.530

TABLE 3 | Summary of MCM LTER source data records used in metacommunity simulations.

Stream group	Mat type	N obs.	Chlorophyll-a ($\mu g\ cm^{-2}$)		
			Mean	Minimum	Maximum
SG1	Black	29	14.70	0.054	146.2
	Orange	32	6.33	0.062	20.3
SG2	Black	10	7.96	0.055	27.2
	Orange	26	7.72	0.649	25.9
SG3	Black	35	16.73	0.024	52.9
	Orange	57	7.50	0.29	31.2

source data to construct a univariate environmental gradient (E) and diatom trait matrix. Specifically, black mat occurrence and $\log(x + 1)$ transformed chlorophyll-a standing biomass measurements were scaled and used in a PCA to create a composite variable (E) to serve as an environmental state variable. We used these measures of chlorophyll-a standing biomass in cyanobacterial mat cores from the MCM LTER record as a proxy for local hydrologic characteristics (Kohler et al., 2015). We included mat type (i.e., a binary dummy variable indicating if a mat was "black" or "orange") to provide a mechanism by which mat color could act as an environmental filter on species composition (i.e., an alternative to mat type affecting immigration rate, m). We chose not to include water chemistry data, as previous studies have demonstrated such data are not major predictors of diatom community composition in this system (Esposito et al., 2006; Stanish et al., 2012). The E scores calculated for each MCM LTER record were mapped onto their respective patches in the simulation (see **Supplementary Data Sheet 2**). E scores were then used in an OMI analysis along with Hellinger transformed (Legendre and Gallagher, 2001) diatom relative abundances to estimate habitat preferences (μ) and niche widths (σ) for each taxon (see Sokol et al., 2020 for R code).

A detailed description of simulation initial conditions derived from empirical observations is available in **Supplementary Table 1**.

Metacommunity Model Details

The MCSim metacommunity model is explained in detail elsewhere (Sokol et al., 2015, 2017), but briefly, it is a spatially explicit, zero-sum lottery model based on Hubbell's neutral model (Hubbell, 2001), modified following Gravel et al. (2006) to be spatially explicit, include a dispersal kernel term, $W(r)$, and the influence of an environmental filter, $\lambda(E)$. Similar to the neutral model, MCSim parameters include m and ν, where m represents a relativized immigration rate at a patch in the metacommunity and ν is the probability a non-extant taxon will be recruited in a particular lottery event. The value of m for a patch with J_L individuals is related to the number of immigrants, I, such that

$$m = \frac{I}{I + J_L - 1} \tag{1}$$

The dispersal kernel is defined as:

$$W(r) = \exp\left(-wr^2\right) \tag{2}$$

where $W(r)$ is the probability of a successful dispersal event, r is the distance between the source and sink patch in the landscape, and w is the slope of the dispersal kernel. Larger values for w create steeper dispersal kernels and increase the effect of dispersal limitation (e.g., **Figure 4B**). MCSim uses $W(r)$ to weight the contributions of the diatom communities from each of the neighboring patches to the immigrant pool for any location in the landscape. Then m and ν (each take a value between 0 and 1) represent the proportion of individuals that are recruited from neighboring patches within the metacommunity, and from a pool of potential invaders from outside the modeled metacommunity (e.g., propagules transported by wind from other lake basins located up-valley), respectively.

Given the relative contributions of the novel invader pool (defined by ν), the immigrant pool (defined by m), and the extant resident community to the recruitment pool, the environmental filter, $\lambda(E)$, further weights the recruitment probabilities of each taxon, following

$$\lambda(E) = \exp\left(\frac{-(E-\mu)^2}{2\,(C_{\text{niche}}\sigma)^2}\right) \tag{3}$$

where $\lambda(E)$ is a taxon's re-weighted effective relative abundance in the recruitment pool, E represents the value of a local environmental state variable, μ is the taxon's optimal value for E, σ is the taxon's niche-breadth, and C_{niche} is a constant that can modify the strength of an environmental filter. When C_{niche} is increased for all taxa in a metacommunity, it weakens the influence of the environmental filter uniformly across all taxa, while preserving cross-taxa heterogeneity in niche breaths (e.g., **Figure 4C**).

Given a set of coordinates describing the location and attributes of patches in a landscape (e.g., **Figure 4A** and **Supplementary Data Sheet 2**) and a table of initial taxon abundances at each location (see **Supplementary Data Sheet 3**), MCSim allows metacommunity dynamics to play out over a series of generations. For a metacommunity with J_M total individuals, J_M lottery recruitment events occur with each time step. Each recruitment event is stochastic, but the probability of each taxon being recruited is weighted by its relative abundance in the effective recruitment pool as determined by the interaction of all the terms described above (also see Sokol et al., 2017).

Simulation Experiment

We simulated 10,000 independent metacommunities using random combinations of the parameter values outlined in **Table 4** (see **Supplementary Data Sheet 4** for a complete

TABLE 4 | Model parameters used in metacommunity simulations.

Model parameter	Parameter values tested
Niche breadth coefficient (C_{niche})*, which affects environmental filter [$\lambda(E)$] strength, see Eq. 3.	1, strong species sorting (SSS), n = 3272; 4, moderate species sorting (MSS), n = 3372; 20, neutral models (NM), n = 3356
Dispersal kernel slope (w), larger values indicate shorter dispersal distance, see Eq. 2	10, 10^2, 10^3, 10^4, 10^5, 10^6
Immigration rate for diatoms in black mats (m_{black}), defined in Eq. 1	0.001, 0.005, 0.009, 0.01, 0.05, 0.09, 0.1, 0.5, 0.9
Immigration rate for diatoms in orange mats (m_{orange}), defined in Eq. 1	0.001, 0.005, 0.009, 0.01, 0.05, 0.09, 0.1, 0.5, 0.9
Invasion rate (ν), probability of novel taxa invading the metacommunity	10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}

*See **Supplementary Data Sheet 4** for a complete design matrix for how parameter values were applied across the 10,000 simulations used in this study. *see **Figure 3C** for more information on how different niche breadth coefficients were implemented.*

design matrix, Sokol et al., 2020 for R code to run the simulations). Each simulation used the same map (**Figure 4A** and **Supplementary Data Sheet 2**) and initial metacommunity composition (**Figure 3** and **Supplementary Data Sheet 3**) and ran for a duration of 100 time-steps. This approach provided a relatively balanced exploration of how simulation outcomes vary across the metacommunity parameter space represented in **Table 4**. Choices for simulation duration and parameter ranges were based off of previous work (Sokol et al., 2015, 2017), where we found simulations with a duration of >30–50 time steps usually reached a steady state with respect to measures of alpha, beta, and gamma diversity. Preliminary model runs were used to determine that the values chosen for C_{niche} would create SSS, MSS, and NM metacommunity dynamics (**Figure 4C**).

An important modification to how MCSim was applied in this study is that immigration rate (m) was based on mat type. Within each simulation all orange mats had immigration rates of m_{orange} and all black mats had immigration rates of m_{black}. The values of m_{orange} and m_{black} were varied across simulation scenarios (**Table 4** and **Supplementary Data Sheet 4**), and immigration disparity between mat types in each simulation was summarized as $\log(m_{black}/m_{orange})$. Thus, log-ratio immigration disparity provides a measure of complexity in dispersal dynamics that can be compared across simulations, where negative values indicate higher immigration rates in orange mats, positive values indicate higher immigration rates in black mats, and values near zero indicate uniform immigration (less complex dispersal dynamics) across all mat types.

Evaluating Simulation Plausibility

The MDV ecosystem is considered "metastable" (Gooseff et al., 2017) and sediment cores from Lake Fryxell indicate the regional diatom species pool has been relatively stable throughout the Holocene (Whittaker et al., 2008; Konfirst et al., 2011), but diatom assemblage composition has been shown to vary through time at the patch scale (Esposito et al., 2006; Stanish et al., 2011). Thus, we considered a suitable criterion for a "plausible" simulation to be a scenario that maintains richness and evenness at both the patch (alpha) and metacommunity (gamma) scales but can allow species composition to turnover as time progresses. To this end, we used multiplicative diversity partitioning (Jost, 2007) to calculate mean alpha-scale diversity (D_α), gamma-scale diversity (D_γ, calculated using all patches in a simulated metacommunity), and compositional turnover among patches (D_β) in each simulated metacommunity, where $D_\beta = D_\gamma/D_\alpha$. We used order $q = 1$ species equivalents because this version of the metric incorporates both richness and evenness. Diversity partitions were calculated for the first and last time steps of each simulation with the vegetarian package for R (Charney and Record, 2012), using equal weights for all local communities because original source data available in the MCM LTER database were effectively relative abundance data. To quantify deviation from the reference condition for each of the simulations, we used a modification of a X^2 statistic (Sokol et al., 2015):

$$\chi^2_{\text{sim}} = \sum_{scale \in \{\alpha,\beta,\gamma\}} \frac{\left(D_{\text{scale,sim,final}} - D_{\text{scale,initial}}\right)^2}{\sigma_{D_{\text{scale,final}}}} \quad (4)$$

where χ^2_{sim} provides a multiscale measure of change in biodiversity. Specifically, this statistic provides a measure of how much D_α, D_β, and D_γ at the final time step (t = 100) deviate from initial values for a given simulation, *sim*. Squared differences are each standardized by the standard deviation of diversity measured at that scale at the final time step across all simulations, so that deviation at each scale contributes equally to the χ^2_{sim} metric. Thus, simulations with the smallest values represent those in which D_α, D_β, and D_γ changed the least during the course of the simulation.

Among the ∼3333 simulations for each metacommunity type, we considered those with χ^2_{sim} scores in the 5th percentile (i.e., the scenarios that best maintained initial diversity) to represent the most plausible metacommunity simulations. We used this approach to identify the simulations with the optimal dispersal kernel slope (w), immigration rates (m_{black} and m_{orange}), and invasion rate (ν) for each metacommunity type. Thus, comparing the top 5% most plausible simulation outcomes (those with χ^2_{sim} scores in the 5th percentile) for each metacommunity type provides a more apples-to-apples comparison between metacommunity types because the dispersal and immigration parameters have been optimized independently for each metacommunity type.

To quantify the change in taxonomic composition during the course of each simulation, we calculated patch-scale temporal beta-diversity. For each patch in a simulated metacommunity, we calculated the Bray–Curtis dissimilarity between the extant diatom assemblages at the first (t = 1) and last (t = 100) time steps using Hellinger transformed abundances (Legendre and Gallagher, 2001). We took the median Bray–Curtis dissimilarity as a representative measurement of patch-scale temporal beta-diversity for each simulation. We used the decostand and vegdist functions in the vegan package for R (Oksanen et al., 2017) to transform the data and calculate dissimilarity metrics, respectively.

RESULTS

The dataset that was simulated for this analysis includes 10,000 independent metacommunities, each with a duration of 100 time-steps (i.e., generations). The simulation output provides the information necessary to explore how local and metacommunity-scale trends in biodiversity vary over the region of metacommunity parameter space described in **Table 4**. Simulations were roughly evenly split across the neutral model (NM), moderate species sorting (MSS), and strong species sorting (SSS) metacommunity types (**Table 4**). Simulations with both NM and MSS metacommunity dynamics produced outcomes with lower χ^2_{sim} scores than SSS simulations (**Figure 5**), indicating that NM and MSS type metacommunity dynamics were better able to maintain D_α, D_β, and D_γ than SSS metacommunity dynamics within the metacommunity simulation framework that was used for this study.

In comparing the top 5% scenarios across metacommunity types, it is clear that NM and MSS metacommunity types, both with χ^2_{sim} scores < 1, are each capable of providing much more plausible dynamics than SSS scenarios (min $\chi^2_{sim} = 1.99$) for diatoms in Fryxell Basin (**Figure 5**).

Overall, all three metacommunity types produced scenarios that maintained gamma diversity ($D_\gamma \sim 22$ species equivalents, **Figure 6**). Additionally, simulations with both MSS and NM metacommunity dynamics were capable of maintaining values of D_α and D_β similar to initial conditions, which were 10.6 and 2.07 species equivalents, respectively. However, none of the scenarios with SSS dynamics were able to maintain D_α, and therefore produced metacommunities with inflated values of D_β by the final time step.

Additionally, NM and MSS scenarios better maintained initial community composition than SSS scenarios (**Figure 7**). Patch-scale temporal beta-diversity is a metric that is bounded between 0 and 1, and represents the median change in diatom assemblage taxonomic composition during the course of a simulation. Patch-scale temporal beta-diversity was much greater for SSS scenarios (>0.60) than NM and MSS scenarios (<0.50). However, even the most plausible scenarios experienced some taxonomic

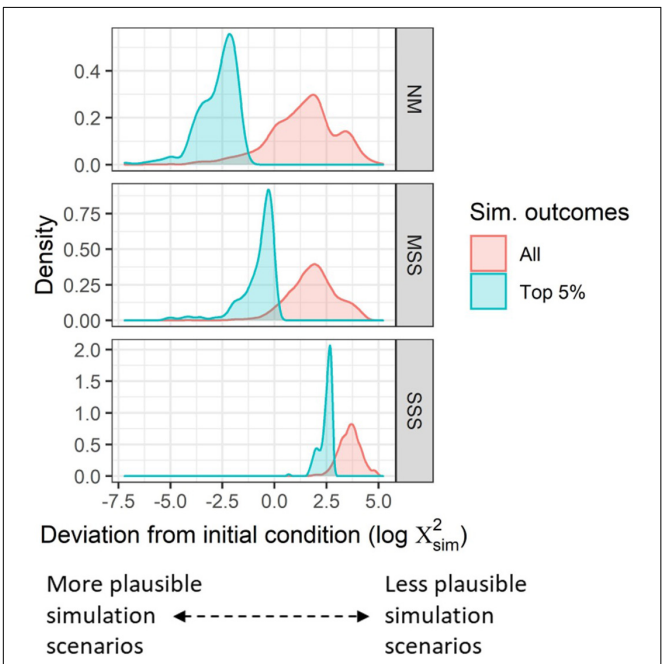


FIGURE 5 | Distribution of χ^2_{sim} scores (measurements of simulation deviation from initial condition) (*x*-axis) for all simulations (red) and the top 5% most plausible simulations (blue) for each metacommunity type: neutral model (NM, **top**), moderate species sorting (MSS, **middle**), and strong species sorting (SSS, **bottom**).

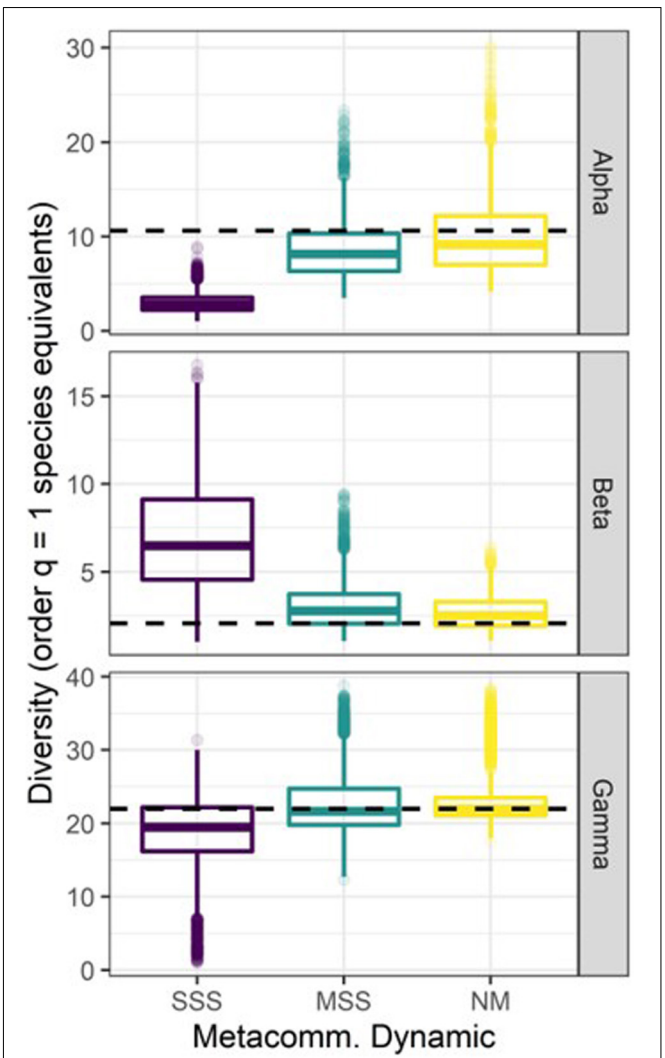


FIGURE 6 | Diversity outcomes for the final time step ($t = 100$) of the simulated metacommunities. Diversity partitions follow the Jost (2007) order $q = 1$ multiplicative method to calculate diversity at alpha (patch), beta (turnover among patches), and gamma (metacommunity) scales. Outcomes are displayed separately for scenarios with strong species sorting (SSS), moderate species sorting (MSS), and neutral model (NM) metacommunity dynamics. Dashed lines indicate initial diversity partitions at $t = 1$, which were the same for all 10,000 simulations.

turnover, as the lowest observed patch-scale temporal beta-diversity was still >0.30.

A comparison of the distribution of dispersal parameter values for the "most plausible" simulated metacommunities (i.e., with 5th percentile χ^2_{sim} scores) against the full population of ∼3333 simulations provided insight into the dispersal dynamics necessary to maintain diversity for each metacommunity type (**Figure 8**). For NM metacommunities, the most plausible scenarios had higher immigration rates in orange mats than black mats (i.e., $m_{orange} > m_{black}$) and low rates of invasion of non-extant taxa (low values of ν), relative to the full population of all ∼3333 NM simulations. On the other hand, there was no difference in the distribution of dispersal kernel slopes (w) between the top 5% and the full population of NM simulations. For MSS simulations, the top 5% simulations had a bias toward higher immigration rates in black mats than orange mats (i.e., $m_{black} > m_{orange}$), more moderate invasion rates (ν), and lower dispersal kernel slopes (w) than the full population of all ∼3333 MSS simulations. As described above, no SSS metacommunity simulations were capable of maintaining initial biodiversity, however, the top 5% SSS simulations that deviated the least from initial conditions had high values of ν and dispersal kernels with shallow slopes (small values for w) relative to the full population of ∼3333 SSS simulations.

DISCUSSION

Here we tested the plausibility of alternative metacommunity hypotheses to explain controls over diatom biodiversity in cyanobacterial mats residing in Fryxell Basin, MDV, using spatially explicit, process-based simulations (**Figure 1**). By using the NDVI metric derived from a remotely sensed image of Fryxell Basin to create the simulation map, our simulated landscape provides a realistic model of the patchy distribution of cyanobacterial mats in which the diatom assemblages reside. Because the results are based on simulations with known differences in the underlying species sorting and dispersal parameters (**Table 4** and **Supplementary Data Sheet 4**), outcomes can explicitly link spatial biodiversity patterns to specific metacommunity dynamics. Similar to inferences made from empirical observations by Sokol et al. (2013) and Sakaeva et al. (2016), the most plausible simulation outcomes in this study suggest species sorting by environmental filters, alone, does not provide a suitable mechanism to maintain microbial biodiversity in the MDV. Rather, simulations that included mechanisms for dispersal to influence the composition of the recruitment pool at each patch in the landscape provided the best candidate models for the dynamics that govern the diatom metacommunity in Fryxell Basin.

In this study, a metacommunity simulation was considered "plausible" if it was capable of maintaining both patch-scale (alpha) and metacommunity-scale (gamma) diversity (thus, also beta-diversity) similar to what was observed in the MCM LTER record for Fryxell Basin. NM and MSS metacommunity simulations both offered plausible candidate models that maintained diversity over time, but the dispersal characteristics in the most plausible NM simulations were different than those in the most plausible MSS simulations. It is important to note that the composition of diatom assemblages can still change over time in simulations that maintain diversity. Even the most plausible NM simulations generally showed moderate change in composition over time (patch-scale temporal beta-diversity was >0.30) because NM dynamics allow for drift in species composition (Chase, 2007). We did not use temporal trends in composition to evaluate the plausibility of alternative scenarios because we know the assumption of invariant community composition over time would be incorrect (Esposito et al., 2006; Stanish et al., 2011). The best simulation scenarios identified in this study (summarized in **Figures 5–8**) provide plausible and testable explanations about the types of metacommunity dynamics that might explain how spatial variation in stream hydrology and cyanobacterial mat coverage are linked to emergent patterns in diatom biodiversity in the MDV.

Ecological Interpretation of Plausible NM Simulations

The most plausible NM simulations suggest both regional diversity (gamma-scale) and patch level (alpha-scale) diversity can be maintained in the Fryxell Basin diatom metacommunity (**Figure 6**) if (1) there is a disparity in immigration rates between different mat types (higher immigration in orange mats) and (2) if the probability of invasion of novel taxa from outside the metacommunity (e.g., from other terminal lake basins located up-valley) is low (**Figure 8**). This outcome suggests that adding complexity to dispersal dynamics in the diatom

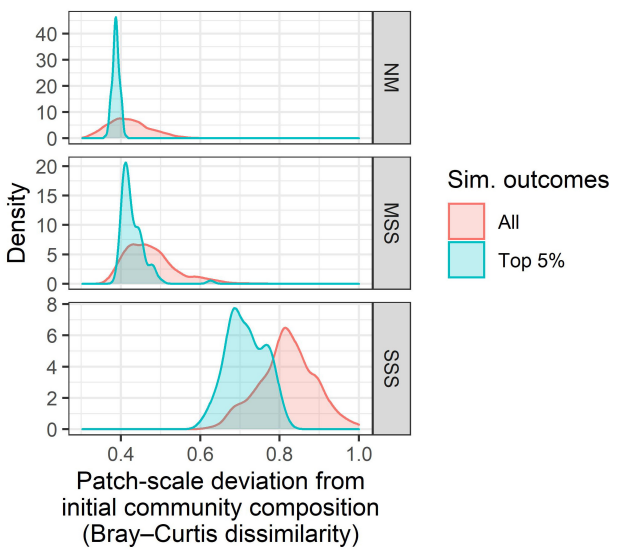


FIGURE 7 | The distribution of patch-scale temporal beta-diversity (*x*-axis) for all simulations (**red**) and the top 5% most plausible simulations (blue) for each metacommunity type: neutral model (NM, **top**), moderate species sorting (MSS, **middle**), and strong species sorting (SSS, **bottom**). Patch-scale temporal beta-diversity was quantified for each simulation as the median of the Bray–Curtis dissimilarities between the initial and final (t = 100) time steps at each patch, using Hellinger transformed abundances.

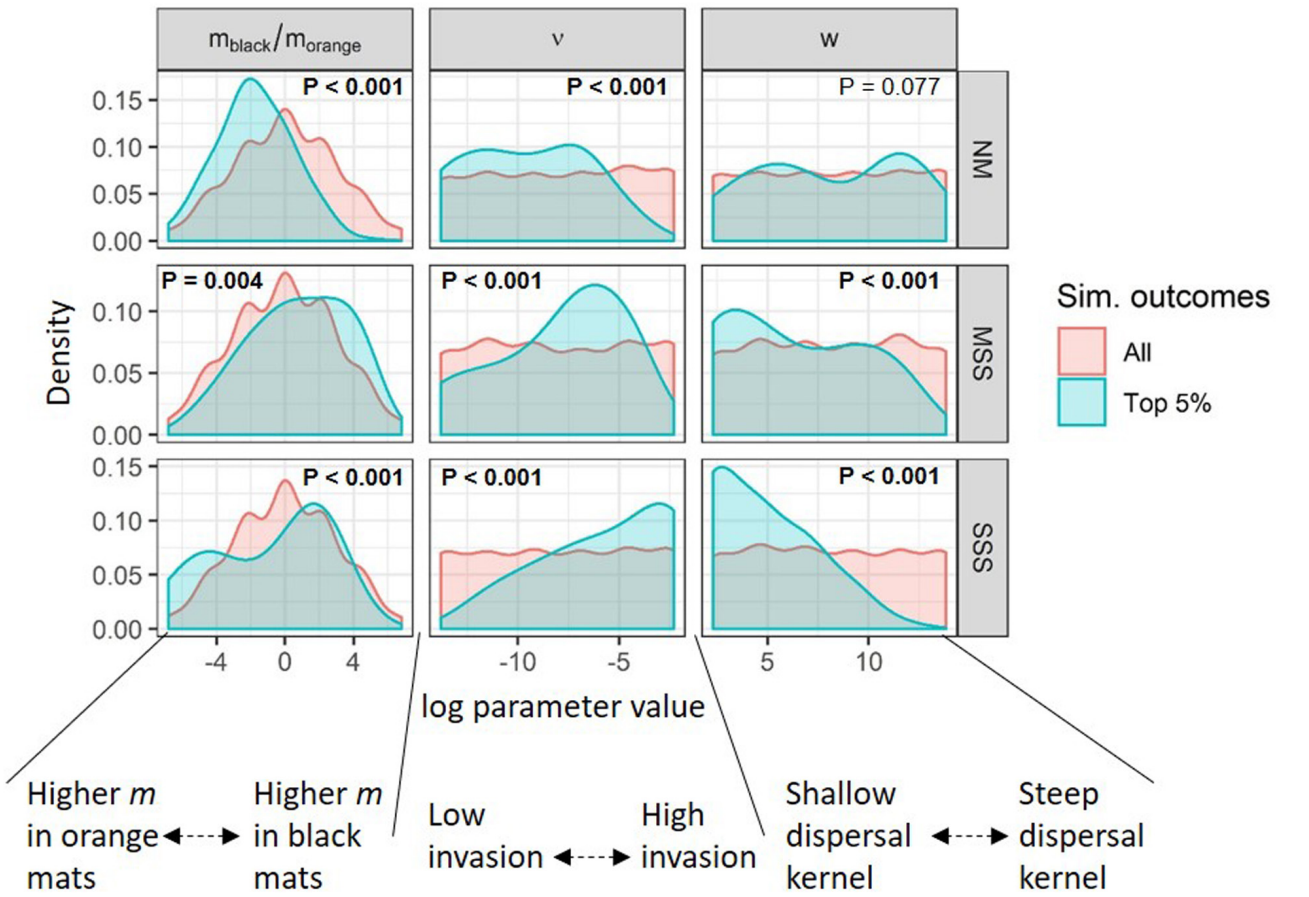


FIGURE 8 | Distribution of metacommunity parameter values (log transformed, *x*-axis) used in All (red) and the top 5% most plausible (blue) simulated metacommunities for neutral model (NM, **top** row), moderate species sorting (MSS, **middle** row), and strong species sorting (SSS, **bottom** row) simulation scenarios. Metacommunity parameters represent disparity in immigration between black and orange mats (m_{black}/m_{orange}, left column), invasion rate by novel taxa (ν, middle column), and dispersal kernel slope (*w*, right column). *P*-values are from a two-sample Kolmogorov–Smirnov test, used to compare the distribution of parameter values in the top 5% simulations against the total population of parameter values across all simulations represented in each panel.

metacommunity (i.e., different immigration rates in different mat types) can maintain biodiversity, and the functional complexity required for species sorting dynamics is not necessary for the maintenance of biodiversity. However, complexity in dispersal dynamics alone was not sufficient because NM simulations in which the relative magnitude of immigration rates were reversed (i.e., black mats had higher immigration rates than orange mats) were not equally plausible. This outcome suggests that both the spatial arrangement and relative abundance of patch types with different immigrant fluxes (i.e., values of *m* in the model) are important characteristics that govern biodiversity at both local and regional scales.

This result provides a potential mechanism by which hydrologic controls over mats in Antarctic streams can control diatom biodiversity at both local and regional scales by modifying bulk diatom dispersal and colonization dynamics. Indeed, stream harshness, characterized by increased flow intermittency in addition to diel and seasonal flow variability, has been linked to the characteristics of both the MDV stream cyanobacterial mats (e.g., orange vs. black) (Kohler et al., 2015) and their resident diatom communities (Esposito et al., 2006; Stanish et al., 2011, 2012). Because the most plausible NM simulations had larger values of *m* for orange mats than black mats, immigrants had a higher success rate for establishment in orange mats than in black mats in these scenarios. We interpret this property of the most plausible NM scenarios to indicate there is likely some characteristic of orange mat habitats that make them more suitable for colonization by immigrant diatoms dispersing from neighboring patches. Alternatively, simulation outcomes suggest recruitment from one generation to the next in black mats is more likely to be biased toward resident taxa that are already established in their respective mats (i.e., priority effects) (Chase, 2003). Past empirical work has shown that orange and black mats have different physical characteristics that might relate to diatom dispersal and colonization dynamics in this system. Orange mats are typically located in stream channels and have greater standing biomass and coverage (McKnight and Tate, 1997; McKnight et al., 1998), whereas black mats are located along wetted margins and more loosely attached to the substrate, and thus, more likely to be dislodged during high wind and/or high flow events

(Kohler et al., 2015). Therefore, during high wind and/or flow events, black mats may be more likely to be sources of emigration and orange mats may be more likely to be immigrant sinks. Further, simulation outcomes suggest relative dominance of black mat communities in the landscape may determine the importance of priority effects in the metacommunity.

Future empirical and modeling studies should test if and how these mat characteristics predict colonist success versus resident taxon success. For example, do orange mats indeed experience higher immigrant fluxes? If so, is it because orange mats are often located in thalweg where they are bathed in a stream of diatoms dispersing via drift? Alternatively, do orange mats just provide a more suitable habitat for a wider variety of diatoms to successfully colonize, irrespective of whether they are wind-dispersed or drifting in the water column? Would repeated observations reveal that priority effects are indeed stronger in black mat diatom assemblages? Regardless, the NM simulation outcomes suggest that by controlling the distribution of different mat types, physical processes (e.g., hydrology) can effectively control the dispersal dynamics of the diatom metacommunity by way of controlling the areal extent and spatial arrangement of patch types with different immigrant fluxes. If this mechanism provides a major control over the structure of the diatom metacommunity, then it also suggests local and regional diatom biodiversity will be sensitive to hydrologically driven shifts in the presence of and coverage of different mat types predicted to occur as the MDV climate changes (Gooseff et al., 2017).

Ecological Interpretation of Most Plausible MSS Simulations

Importantly, NM scenarios did not provide the only plausible metacommunity dynamics for this study system. Simulations with MSS dynamics were nearly as successful as NM scenarios at maintaining diatom diversity at both the patch (alpha) and metacommunity (gamma) scales (**Figure 6**). However, in contrast with the most plausible NM simulations, the most plausible MSS simulations (1) had a small bias toward higher immigration rates in black mats, (2) had higher rates of invasion by novel taxa from outside the metacommunity, and (3) relied on a broader dispersal kernel. The most plausible MSS simulations demonstrate that interactions between a moderately strong environmental filter and dispersal dynamics in a complex landscape can create similar emergent biodiversity patterns to NM simulations and provide a plausible alternative hypothesis about the metacommunity dynamics that maintain diatom biodiversity in the MDV.

A major difference between NM and MSS simulations was how mat identity (orange or black, **Figure 4A**) was linked to immigration and recruitment dynamics at each patch. In both NM and MSS simulations, the magnitude of m was defined by mat identity. However, in MSS simulations, mat type also influenced the environmental filter [$\lambda(E)$, Eq. 3] because the patch specific values for E were a function of both mat identity and chlorophyll-a standing biomass (x-axis in **Figure 4C**). While $\lambda(E)$ had no effect on the magnitude of the immigrant flux, it did control which taxa were more likely to be successfully recruited at a given patch based on the local environment (E) and the habitat preferences (μ) and niche breadths (σ) of the resident and immigrant taxa in the recruitment pool.

With the addition of an environmental filter affecting species sorting, MSS simulations changed the nature of dispersal dynamics required to maintain biodiversity at both local and regional levels relative to NM simulations (**Figure 8**). In contrast with the NM simulations, scenarios with moderate environmental filtering do not support the predictions that black mats are more insular (e.g., more likely to show priority effects) or that orange mats are more likely to be sink habitats for dispersing propagules. Further, simulation outcomes demonstrated that metacommunities with moderate environmental filtering required flatter dispersal kernels (e.g., curves for small values of w in **Figure 4B**) to maintain biodiversity. Thus, the most plausible MSS scenarios require that propagules in the immigrant pool are well dispersed among the patches in the landscape.

MSS metacommunities could include dynamics that resemble the mass effects (ME) metacommunity paradigm. Under ME dynamics, environmental filters determine the composition of source communities in productive habitats, but emigration from source communities into nearby sink communities can overwhelm the influence of environmental filtering at those less productive habitats (Leibold et al., 2004). Thus, dispersal can moderate the spatial extent of the influence of environmental filters under ME dynamics. While both MSS and ME dynamics involve an interaction between dispersal and species sorting to determine community assembly outcomes, the ME paradigm represents a specific case of MSS dynamics, and not all MSS metacommunity scenarios would necessarily conform to the ME paradigm.

Testable Predictions Derived From Simulation Outcomes

The most plausible NM and MSS simulations identified in this study predict quite different dispersal dynamics for diatoms in the MDV. The NM simulations predict diatom assemblages in orange mats act as sinks in the metacommunity, and priority effects are stronger in black mats. These predictions could be tested by comparing field observations of diatom assemblages in the different mat types with observations from potential sources of immigrants. Two likely vectors for diatom dispersal in this system are aeolian transport (Šabacká et al., 2012; Diaz et al., 2018) and drift in the water column in streams (Cullis et al., 2014; Kohler et al., 2018). If NM dynamics govern the structure of the diatom metacommunity, and wind/saltation is the primary driver of diatom dispersal, then these modeling outcomes suggest the composition of the pool of dispersing diatoms in both aeolian and drift samples will more closely represent the extant community in nearby, downwind or downstream orange mats than black mats.

Alternatively, if local environmental filters are influencing species sorting, the most plausible MSS simulation outcomes suggest that some specific dispersal dynamics are required to maintain the observed levels of alpha, beta, and gamma diversity. Specifically, **Figure 8** shows that as filter strength increases (from NM to MSS to SSS), the dispersal kernel slope (w) must

decrease (i.e., mean dispersal distances increase). Immigration rates (m) can still be low, but propagules must be capable of dispersing broadly for species sorting dynamics to maintain the observed biodiversity patterns. However, species sorting alone cannot maintain local diversity when environmental filters are too strong (as the SSS scenarios demonstrate). MSS model dynamics predict that the diatom assemblages in either aeolian samplers or drift nets distributed across Fryxell Basin would have very low levels of beta diversity compared to samples collected for orange and black mats. However, the flat dispersal kernel and larger values for ν identified in the most plausible MSS simulations suggest aeolian transport is a more likely vector than drift. Because of the influence of environmental filtering in MSS simulations, the MSS hypothesis predicts orange and black mat beta diversity should correlate somewhat with environmental variables, whereas beta diversity from samples of the dispersing pool should not correlate with environmental variables (or that correlation should be much weaker than currently observed in the extant communities). Lastly, MSS simulations suggest orange and black mat communities are equally insular, or orange mats are slightly more insular than black mats. Thus, we would not expect dispersing diatoms in either upstream drift or aeolian samples to be more similar to nearby orange mats than nearby black mats.

Moving Beyond the Model Assumptions

It is important to note the limitations on how these simulation outcomes can be interpreted. While the most plausible metacommunity models were able to maintain alpha, beta, and gamma diversity, we did not assess how simulated trends in community composition compared to the long-term record maintained by the MCM LTER. Past empirical studies have provided quite strong evidence that variation in stream hydrologic regimes serves as an environmental filter (Poff and Ward, 1990) to control diatom community composition in Fryxell Basin (Stanish et al., 2011, 2012). Thus, a next step is to refine our modeling approach to test predictions about community composition. However, identifying metacommunity dynamics that can maintain biodiversity was an important first step. Importantly, our simulation outcomes indicate that species sorting alone does not maintain biodiversity in this ecosystem that has been shown to be relatively stable.

Our results may enhance previous findings (Stanish et al., 2011, 2012) by pointing to specific mechanisms by which hydrology might exert control over diatom community composition in MDV streams. We implemented filtering in MCSim using chlorophyll-a concentrations as a proxy for local stream harshness, based on the empirical relationships described in Stanish et al. (2011, 2012), Kohler et al. (2015), and used empirically observed diatom occurrence data in the OMI analysis (**Figure 4C**) to determine the habitat preferences for each taxon in the environmental filtering process (Eq. 3). However, a large proportion, but not all of the taxa in the regional species pool are aerophilic (Esposito et al., 2008; Spaulding et al., 2010; Stanish et al., 2011). In the current study, we did not link functional traits to dispersal—taxa were neutral with respect to dispersal ability—though the simulation outcomes already suggest dispersal dynamics are important. A crucial next step in modeling metacommunity dynamics in this system is to use diatom functional traits to provide variation in dispersal abilities in the next generation of metacommunity models. It is likely that simulations that incorporate dispersal traits will better predict composition than the models used in the current study, but we expect that such simulations will involve dispersal dynamics similar to those identified in the present study.

Lastly, the simulation scenarios used in this study did not include temporal variability in the landscape. We attempted to indirectly include the influence of spatial variation in hydrologic regimes in the landscape by using spatial variation in chlorophyll-a standing biomass as a proxy, but there is obviously an important temporal component to this that interacts with the recruitment process that was not captured in the metacommunity dynamics that were modeled in these simulation scenarios. We suspect that adding realistic temporal variation to the distribution of mat types in addition to diatom dispersal traits will be key to more accurately modeling diatom metacommunity dynamics.

CONCLUSION

Overall, the results presented in this study point to the importance of dispersal dynamics for maintaining both patch-scale and metacommunity-scale measures of biodiversity in the diatom metacommunity in Fryxell Basin in the MDV. Both remote imagery and the MCM LTER record provided crucial information for characterizing the patchy mosaic of cyanobacterial mats in which the diatom assemblages reside. Results from this study suggest that shifts in the distribution of different mat types in Fryxell Basin, which can potentially be tracked using remote imagery (Salvatore, 2015; Salvatore et al., 2020), can be consequential for both local and metacommunity scale biodiversity. Importantly, this study highlights the necessity of an accurate understanding of how dispersal affects the scaling of community assembly processes, which will be important for predicting how diatom biodiversity will change in future climate scenarios (Shindell and Schmidt, 2004; Gooseff et al., 2017). Such an approach may also prove useful for understanding how dispersal affects other microbial metacommunities (Martiny et al., 2006; Heino et al., 2010; Soininen, 2012), both in the MDV, and beyond.

DATA AVAILABILITY STATEMENT

Code to run the simulations used in this study, input data used to initialize the simulations, and simulation results are available as an archived data package hosted by the Environmental Data Initiative (EDI) at: https://portal.edirepository.org/nis/mapbrowse?scope=edi&identifier=597 (doi: 10.6073/pasta/928879746d04e847c1b5acfa31f1cbfa). The code and input data are also available on GitHub at: https://github.com/sokole/diatom_metacommunity_simulations.

AUTHOR CONTRIBUTIONS

ES designed the study, created the MCSim modeling platform, conducted the analyses, and wrote the manuscript. ES, JB, MS, and LS secured funding to support the analysis of remote imagery used in the study. MS analyzed the remote imagery and created the visible and NDVI maps used in this study. DM and JB are PIs with the MCM LTER program, which provided the algal mat and diatom observational data used in the study. DM, TK, and LS designed and executed the collection of the MCM LTER data used in this study. All authors contributed to interpretation of data and writing the manuscript.

FUNDING

This work was funded by United States National Science Foundation OPP (Antarctic Organisms and Ecosystems) awards 1744785, 1744849, and 1745053 and NSF OPP award 1637708 supporting the MCM LTER Project.

ACKNOWLEDGMENTS

We thank the Algae Ops team who contributed to the field, lab, and data management work required to provide the MCM LTER record and the Antarctic Freshwater Diatoms Database. We also thank the contractors who have supported field research through the United States Antarctic Program: Antarctic Support Associates, Raytheon Polar. We thank the Polar Geospatial Center for geospatial and satellite image support, DigitalGlobe, Inc., and the National Science Foundation for access to these orbital data.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.521668/full#supplementary-material

Supplementary Material Table 1 | Description of simulation initial conditions derived from empirical observations.

Supplementary Material Data Sheet 1 | Algae Ops source data from MCM LTER, available as a .csv file.

Supplementary Material Data Sheet 2 | A patch attribute table for the simulation map, available as a .csv file.

Supplementary Material Data Sheet 3 | Initial contrived metacommunity composition in long format, available as a .csv file.

Supplementary Material Data Sheet 4 | The design matrix, generated by code in Sokol et al. (2020), available as a .csv file.

REFERENCES

Adams, B. J., Bardgett, R. D., Ayres, E., Wall, D. H., Aislabie, J., Bamforth, S., et al. (2006). Diversity and distribution of victoria land biota. *Soil Biol. Biochem.* 38, 3003–3018. doi: 10.1016/j.soilbio.2006.04.030

Alger, A. S. (1997). *Ecological Processes in a Cold Desert Ecosystem: the Abundance and Species Distribution of Algal Mats in Glacial Meltwater Streams in Taylor Valley, Antarctica. Occas. Pap. Colo*. Available online at: http://agris.fao.org/agris-search/search.do?recordID=US19970146317 (accessed May 16, 2016).

Andriuzzi, W. S., Stanish, L. F., Simmons, B. L., Jaros, C., Adams, B. J., Wall, D. H., et al. (2018). Spatial and temporal patterns of microbial mats and associated invertebrates along an Antarctic stream. *Polar Biol.* 41, 1911–1921. doi: 10.1007/s00300-018-2331-4

Baas Becking, L. G. M. (1934). *Geobiologie of inleiding tot de milieukunde*. The Hague: W.P. Van Stockum & Zoon.

Charney, N., and Record, S. (2012). *vegetarian: Jost Diversity Measures for Community Data. R Package Version 1.2*. Available online at: http://CRAN.R-project.org/package=vegetarian (accessed October 29, 2012).

Chase, J. M. (2003). Community assembly: when should history matter? *Oecologia* 136, 489–498. doi: 10.1007/s00442-003-1311-7

Chase, J. M. (2007). Drought mediates the importance of stochastic community assembly. *Proc. Natl. Acad. Sci. U.S.A.* 104:17430. doi: 10.1073/pnas.0704350104

Chen, S., Zhang, W., Zhang, J., Jeppesen, E., Liu, Z., Kociolek, J. P., et al. (2019). Local habitat heterogeneity determines the differences in benthic diatom metacommunities between different urban river types. *Sci. Total Environ.* 669, 711–720. doi: 10.1016/j.scitotenv.2019.03.030

Chessel, D., and Dufour, A. B. (2006). *Analysis of Environmental Data: Exploratory and Euclidean methods in Environmental sciences*.

Conovitz, P. A., MacDonald, L. H., and McKnight, D. M. (2006). Spatial and temporal active layer dynamics along three glacial meltwater streams in the McMurdo Dry valleys, Antarctica. *Arct. Antarct. Alp. Res.* 38, 42–53. doi: 10.1657/1523-0430(2006)038[0042:satald]2.0.co;2

Conovitz, P. A., McKnight, D. M., MacDonald, L. H., and Fountain, A. G. (1998). "Fryxell Basin, Antarctica," in *Ecosystem Dynamics in a Polar Desert: The McMurdo Dry Valleys, Antarctica*, ed. J. C. Priscu (Washington, DC: American Geophysical Union), 93. doi: 10.1029/ar072p0093

Cullis, J. D. S., Stanish, L. F., and McKnight, D. M. (2014). Diel flow pulses drive particulate organic matter transport from microbial mats in a glacial meltwater stream in the McMurdo Dry valleys: pom transport dynamics in MDV stream. *Water Resour. Res.* 50, 86–97. doi: 10.1002/2013WR014061

Darling, J. P., Garland, D. D., Stanish, L. F., Esposito, R. M. M., Sokol, E. R., and McKnight, D. M. (2017). Thermal autecology describes the occurrence patterns of four benthic diatoms in McMurdo Dry valley streams. *Polar Biol.* 40, 2381–2396. doi: 10.1007/s00300-017-2151-y

Diaz, M. A., Adams, B. J., Welch, K. A., Welch, S. A., Opiyo, S. O., Khan, A. L., et al. (2018). Aeolian material transport and its role in landscape connectivity in the McMurdo Dry valleys. Antarctica. *J. Geophys. Res. Earth Surf.* 123, 3323–3337. doi: 10.1029/2017JF004589

Dolédec, S., Chessel, D., and Gimaret-Carpentier, C. (2000). Niche separation in community analysis: a new method. *Ecology* 81, 2914–2927. doi: 10.1111/j.0022-3646.1995.00002.x

Esposito, R. M. M., Horn, S. L., McKnight, D. M., Cox, M. J., Grant, M. C., Spaulding, S. A., et al. (2006). Antarctic climate cooling and response of diatoms in glacial meltwater streams. *Geophys. Res. Lett.* 33:4.

Esposito, R. M. M., Spaulding, S. A., McKnight, D. M., Van de Vijver, B., Kopalová, K., Lubinski, D., et al. (2008). Inland diatoms from the McMurdo Dry Valleys and james ross Island. Antarctica. *Botany* 86, 1378–1392. doi: 10.1139/B08-100

Fitzpatrick, M. C., Blois, J. L., Williams, J. W., Nieto-Lugilde, D., Maguire, K. C., and Lorenz, D. J. (2018). How will climate novelty influence ecological forecasts? Using the Quaternary to assess future reliability. *Glob. Change Biol.* 24, 3575–3586. doi: 10.1111/gcb.14138

Gooseff, M. N., Barrett, J. E., Adams, B. J., Doran, P. T., Fountain, A. G., Lyons, W. B., et al. (2017). Decadal ecosystem response to an anomalous melt season in a polar desert in Antarctica. *Nat. Ecol. Evol.* 1, 1334–1338. doi: 10.1038/s41559-017-0253-0

Gooseff, M. N., McKnight, D. M., Lyons, W. B., and Blum, A. E. (2002). Weathering reactions and hyporheic exchange controls on stream water chemistry in a glacial meltwater stream in the McMurdo Dry valleys. *Water Resour. Res.* 38:279.

Gravel, D., Canham, C. D., Beaudet, M., and Messier, C. (2006). Reconciling niche and neutrality: the continuum hypothesis. *Ecol. Lett.* 9, 399–409. doi: 10.1111/j.1461-0248.2006.00884.x

Heino, J., Bini, L. M., Karjalainen, S. M., Mykrä, H., Soininen, J., Vieira, L. C. G., et al. (2010). Geographical patterns of micro-organismal community structure: are diatoms ubiquitously distributed across boreal streams? *Oikos* 119, 129–137. doi: 10.1111/j.1600-0706.2009.17778.x

Hubbell, S. P. (2001). *A Unified Theory of Biodiversity and Biogeography*. Princeton, NJ: Princeton University Press.

Jost, L. (2007). Partitioning diversity into independent alpha and beta components. *Ecology* 88, 2427–2439. doi: 10.1890/06-1736.1

Koch, J. C., McKnight, D. M., and Baeseman, J. L. (2010). Effect of unsteady flow on nitrate loss in an oligotrophic, glacial meltwater stream. *J. Geophys. Res. Biogeosciences* 115:G01001. doi: 10.1029/2009JG001030

Kociolek, J. P., Kopalová, K., Hamsher, S. E., Kohler, T. J., Van de Vijver, B., Convey, P., et al. (2017). Freshwater diatom biogeography and the genus Luticola: an extreme case of endemism in Antarctica. *Polar Biol.* 40, 1185–1196. doi: 10.1007/s00300-017-2090-7

Kohler, T. J., Stanish, L. F., Crisp, S. W., Koch, J. C., Liptzin, D., Baeseman, J. L., et al. (2015). Life in the main channel: long-term hydrologic control of microbial mat abundance in McMurdo Dry valley streams. Antarctica. *Ecosystems* 18, 310–327. doi: 10.1007/s10021-014-9829-6

Kohler, T. J., Stanish, L. F., Liptzin, D., Barrett, J. E., and McKnight, D. M. (2018). Catch and release: Hyporheic retention and mineralization of N-fixing Nostoc sustains downstream microbial mat biomass in two polar desert streams. *Limnol. Oceanogr. Lett.* 3, 357–364. doi: 10.1002/lol2.10087

Kohler, T. J., Van Horn, D. J., Darling, J. P., Takacs-Vesbach, C. D., and McKnight, D. M. (2016). Nutrient treatments alter microbial mat colonization in two glacial meltwater streams from the McMurdo Dry valleys, Antarctica. *FEMS Microbiol. Ecol.* 92:fiw049. doi: 10.1093/femsec/fiw049

Konfirst, M. A., Sjunneskog, C., Scherer, R. P., and Doran, P. T. (2011). A diatom record of environmental change in fryxell basin, taylor valley, Antarctica, late pleistocene to present. *J. Paleolimnol.* 46, 257–272. doi: 10.1007/s10933-011-9537-6

Kopalová, K., Soukup, J., Kohler, T. J., Roman, M., Coria, S. H., Vignoni, P. A., et al. (2019). Habitat controls on limno-terrestrial diatom communities of Clearwater Mesa. James Ross Island, Maritime Antarctica. *Polar Biol.* 42, 1595–1613. doi: 10.1007/s00300-019-02547-8

Langenheder, S., and Lindström, E. S. (2019). Factors influencing aquatic and terrestrial bacterial community assembly. *Environ. Microbiol. Rep.* 11, 306–315. doi: 10.1111/1758-2229.12731

Legendre, P., and Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia* 129, 271–280. doi: 10.1007/s004420100716

Leibold, M. A., and Chase, J. M. (2017). *Metacommunity Ecology*. Princeton, NJ: Princeton University Press.

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. doi: 10.1111/j.1461-0248.2004.00608.x

Levy, J. (2013). How big are the McMurdo Dry Valleys? Estimating ice-free area using Landsat image data. *Antarct. Sci.* 25, 119–120. doi: 10.1017/S0954102012000727

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. doi: 10.1038/nrmicro1341

McKnight, D. M., Alger, A., Tate, C., Shupe, G., and Spaulding, S. (1998). "Longitudinal patterns in algal abundance and species distribution in meltwater streams in taylor valley, southern victoria land, Antarctica," in *Ecosystem Dynamics in a Polar Desert: the Mcmurdo Dry Valleys, Antarctica*, ed. J. C. Priscu (Washington, DC: American Geophysical Union), 109–127. doi: 10.1029/AR072p0109/summary

McKnight, D. M., Niyogi, D. K., Alger, A. S., Bomblies, A., Conovitz, P. A., and Tate, C. M. (1999). Dry valley streams in Antarctica: ecosystems waiting for water. *BioScience* 49, 985–995. doi: 10.1525/bisi.1999.49.12.985

McKnight, D. M., Runkel, R. L., Tate, C. M., Duff, J. H., and Moorhead, D. L. (2004). Inorganic N and P dynamics of Antarctic glacial meltwater streams as controlled by hyporheic exchange and benthic autotrophic communities. *J. North Am. Benthol. Soc* 23, 171–188. doi: 10.1899/0887-35932004023<0171:INAPDO<2.0.CO;2

McKnight, D. M., and Tate, C. M. (1997). Canada stream: a glacial meltwater stream in taylor valley, south victoria land. Antarctica. *J. North Am. Benthol. Soc.* 16, 14–17. doi: 10.2307/1468224

Michaud, A. B., Šabacká, M., and Priscu, J. C. (2012). Cyanobacterial diversity across landscape units in a polar desert: taylor valley, Antarctica. *FEMS Microbiol. Ecol.* 82, 268–278. doi: 10.1111/j.1574-6941.2012.01297.x

Milly, P. C. D., Betancourt, J., Falkenmark, M., Hirsch, R. M., Kundzewicz, Z. W., Lettenmaier, D. P., et al. (2008). Stationarity is dead: whither water management? *Science* 319, 573–574. doi: 10.1126/science.1151915

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. doi: 10.1128/MMBR.00051-12

Nylen, T. H., Fountain, A. G., and Doran, P. T. (2004). Climatology of katabatic winds in the McMurdo dry valleys, southern victoria land, Antarctica. *J. Geophys. Res.* 109:D03114. doi: 10.1029/2003JD003937

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2017). *vegan: Community Ecology Package. R Package Version 2.4-3*. Available online at: https://CRAN.R-project.org/package=vegan (accessed September 01, 2019).

Poff, N. L., and Ward, J. V. (1990). Physical habitat template of lotic systems: Recovery in the context of historical pattern of spatiotemporal heterogeneity. *Environ. Manag.* 14:629. doi: 10.1007/BF02394714

Šabacká, M., Priscu, J. C., Basagic, H. J., Fountain, A. G., Wall, D. H., Virginia, R. A., et al. (2012). Aeolian flux of biotic and abiotic material in Taylor Valley. Antarctica. *Geomorphology* 15, 102–111. doi: 10.1016/j.geomorph.2011.12.009

Sakaeva, A., Sokol, E. R., Kohler, T. J., Stanish, L. F., Spaulding, S. A., Howkins, A., et al. (2016). Evidence for dispersal and habitat controls on pond diatom communities from the McMurdo sound region of Antarctica. *Polar Biol.* 39, 2441–2456. doi: 10.1007/s00300-016-1901-6

Salvatore, M. R. (2015). High-resolution compositional remote sensing of the transantarctic mountains: application to the WorldView-2 dataset. *Antarct. Sci.* 27, 473–491. doi: 10.1017/S095410201500019X

Salvatore, M. R., Borges, S. R., Barrett, J. E., Sokol, E. R., Stanish, L. F., Power, S. N., et al. (2020). Remote characterization of photosynthetic communities in the Fryxell basin of Taylor Valley. Antarctica. *Antarct. Sci.* 32, 255–270. doi: 10.1017/S0954102020000176

Shindell, D. T., and Schmidt, G. A. (2004). Southern hemisphere climate response to ozone changes and greenhouse gas increases. *Geophys. Res. Lett* 31:L18209. doi: 10.1029/2004GL020724

Smol, J. P., and Stoermer, E. F. (2010). *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge: Cambridge University Press.

Soininen, J. (2012). Macroecology of unicellular organisms – patterns and processes. *Environ. Microbiol. Rep.* 4, 10–22. doi: 10.1111/j.1758-2229.2011.00308.x

Soininen, J., Paavola, R., and Muotka, T. (2004). Benthic diatom communities in boreal streams: community structure in relation to environmental and spatial gradients. *Ecography* 27, 330–342. doi: 10.1111/j.0906-7590.2004.03749.x

Sokol, E. R. (2019). *MCSim: An R Package for Metacommunity Simulations*. Available online at: https://github.com/sokole/MCSim/releases/tag/v0.4.9 (accessed December 12, 2019).

Sokol, E. R., Barrett, J. E., Kohler, T. J., McKnight, D. M., Salvatore, M. R., and Stanish, L. F. (2020). Metacommunity simulations for diatom assemblages residing in benthic cyanobacterial mats in Fryxell Basin in Taylor Valley in the McMurdo Dry Valleys, Antarctica ver 2. *Environmental Data Initiative* doi: 10.6073/PASTA/928879746D04E847C1B5ACFA31F1CBFA

Sokol, E. R., Brown, B. L., and Barrett, J. E. (2017). A simulation-based approach to understand how metacommunity characteristics influence emergent biodiversity patterns. *Oikos* 126, 723–737. doi: 10.1111/oik.03690

Sokol, E. R., Brown, B. L., Carey, C. C., Tornwall, B. M., Swan, C. M., and Barrett, J. E. (2015). Linking management to biodiversity in built ponds using metacommunity simulations. *Ecol. Model.* 296, 36–45. doi: 10.1016/j.ecolmodel.2014.10.022

Sokol, E. R., Herbold, C. W., Lee, C. K., Cary, S. C., and Barrett, J. E. (2013). Local and regional influences over soil microbial metacommunities in the Transantarctic Mountains. *Ecosphere* 4:art136. doi: 10.1890/ES13-00136.1

Spaulding, S. A., Van de Vijver, B., Hodgson, D. A., McKnight, D. M., Verleyen, E., and Stanish, L. F. (2010). "Diatoms as indicators of environmental change in Antarctic and subantarctic freshwaters," in *The Diatoms: Applications for the Environmental and Earth Sciences*, eds J. P. Smol and E. F. Stoermer (Cambridge: Cambridge University Press), 267. doi: 10.1017/cbo9780511763175.015

Stanish, L. F., Kohler, T. J., Esposito, R. M. M., Simmons, B. L., Nielsen, U. N., Wall, D. H., et al. (2012). Extreme streams: flow intermittency as a control on diatom communities in meltwater streams in the McMurdo Dry valleys, Antarctica. *Can. J. Fish. Aquat. Sci.* 69, 1405–1419. doi: 10.1139/f2012-022

Stanish, L. F., Nemergut, D. R., and McKnight, D. M. (2011). Hydrologic processes influence diatom community composition in Dry Valley streams. *J. North Am. Benthol. Soc.* 30, 1057–1073. doi: 10.1899/11-008.1

Van Horn, D. J., Wolf, C. R., Colman, D. R., Jiang, X., Kohler, T. J., McKnight, D. M., et al. (2016). Patterns of bacterial biodiversity in the glacial meltwater streams of the McMurdo Dry Valleys. Antarctica. *FEMS Microbiol. Ecol.* 92:fiw148. doi: 10.1093/femsec/fiw148

Verleyen, E., Vyverman, W., Sterken, M., Hodgson, D. A., De Wever, A., Juggins, S., et al. (2009). The importance of dispersal related and local factors in shaping the taxonomic structure of diatom metacommunities. *Oikos* 118, 1239–1249. doi: 10.1111/j.1600-0706.2009.17575.x

Welch, K. A., Lyons, W. B., Whisner, C., Gardner, C. B., Gooseff, M. N., McKnight, D. M., et al. (2010). Spatial variations in the geochemistry of glacial meltwater streams in the Taylor Valley. Antarctica. *Antarct. Sci.* 22, 662–672. doi: 10.1017/S0954102010000702

Whittaker, T. E., Hall, B. L., Hendy, C. H., and Spaulding, S. A. (2008). Holocene depositional environments and surface-level changes at Lake Fryxell. Antarctica. *Holocene* 18, 775–786. doi: 10.1177/0959683608091797

Wlostowski, A. N., Gooseff, M. N., McKnight, D. M., Jaros, C., and Lyons, W. B. (2016). Patterns of hydrologic connectivity in the McMurdo Dry Valleys, Antarctica: a synthesis of 20 years of hydrologic data. *Hydrol. Process.* 30, 2958–2975. doi: 10.1002/hyp.10818

Wlostowski, A. N., Gooseff, M. N., McKnight, D. M., and Lyons, W. B. (2018). Transit Times and rapid chemical equilibrium explain chemostasis in glacial meltwater streams in the McMurdo Dry valleys, Antarctica. *Geophys. Res. Lett.* 45, 13,322–13,313. doi: 10.1029/2018GL080369

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

REVIEW
published: 20 November 2020
doi: 10.3389/fmicb.2020.561427

A Landscape of Opportunities for Microbial Ecology Research

Cendrine Mony[1], Philippe Vandenkoornhuyse[1], Brendan J. M. Bohannan[2], Kabir Peay[3] and Mathew A Leibold[4]*

[1] UMR CNRS ECOBIO, Université de Rennes, Rennes, France, [2] Institute of Ecology and Evolution, University of Oregon, Eugene, ORE, United States, [3] Department of Biology, University of Stanford, Stanford, CA, United States, [4] Department of Biology, University of Florida, Gainesville, FL, United States

Microbes encompass tremendous biodiversity, provide support to all living forms, including humans, and play an important role in many ecosystem services. The rules that govern microorganism community assembly are increasingly revealed due to key advances in molecular and analytical methods but their understanding remain a key challenge in microbial ecology. The existence of biogeographic patterns within microbial communities has been established and explained in relation to landscape-scale processes, including selection, drift, dispersal and mutation. The effect of habitat patchiness on microorganisms' assembly rules remains though incompletely understood. Here, we review how landscape ecology principles can be adapted to explore new perspectives on the mechanisms that determine microbial community structure. To provide a general overview, we characterize microbial landscapes, the spatial and temporal scales of the mechanisms that drive microbial assembly and the feedback between microorganisms and landscape structure. We provide evidence for the effects of landscape heterogeneity, landscape fragmentation and landscape dynamics on microbial community structure, and show that predictions made for macro-organisms at least partly also apply to microorganisms. We explain why emerging metacommunity approaches in microbial ecology should include explicit characterization of landscape structure in their development and interpretation. We also explain how biotic interactions, such as competition, prey-predator or mutualist relations may influence the microbial landscape and may be involved in the above-mentioned feedback process. However, we argue that the application of landscape ecology to the microbial world cannot simply involve transposing existing theoretical frameworks. This is due to the particularity of these organisms, in terms of size, generation time, and for some of them, tight interaction with hosts. These characteristics imply dealing with unusual and dependent space and time scales of effect. Evolutionary processes have also a strong importance in microorganisms' response to their landscapes. Lastly, microorganisms' activity and distribution induce feedback effects on the landscape that have to be taken into account. The transposition of the landscape ecology framework to microorganisms provides many challenging research directions for microbial ecology.

Keywords: landscape ecology, metacommunity, microbial assembly-rules, dispersal, plant microbiota, human microbiota, animal microbiota

OPEN ACCESS

Edited by:
Eoin L. Brodie, Lawrence Berkeley National Laboratory, United States

Reviewed by:
Alban Ramette, University of Bern, Switzerland
Kimberley Warren-Rhodes, Ames Research Center, National Aeronautics and Space Administration (NASA), United States

****Correspondence:***
Cendrine Mony
cendrine.mony@univ-rennes1.fr

Specialty section:
This article was submitted to Terrestrial Microbiology, a section of the journal Frontiers in Microbiology

Received: *12 May 2020*
Accepted: *30 October 2020*
Published: *20 November 2020*

Citation:
Mony C, Vandenkoornhuyse P, Bohannan BJM, Peay K and Leibold MA (2020) A Landscape of Opportunities for Microbial Ecology Research. Front. Microbiol. 11:561427. doi: 10.3389/fmicb.2020.561427

INTRODUCTION

Microorganisms represent by far the largest fraction of biodiversity (Curtis and Sloan, 2005). There are 100 million times as many bacteria in the oceans (13×10^28) as there are stars in the known universe [. . .] [Editorial Nature Reviews in Microbiology, (No authors listed, 2011)]. The amazing abundance of microorganisms on earth plays a central role in the biogeochemical cycles of elements (Curtis and Sloan, 2005), affects soil fertility, organic matter decomposition and carbon storage. Microorganisms are also required to sustain all living macroorganisms, including humans (Curtis, 2006), as they are involved in the nutrition, health, reproduction and behavior of their hosts (Curtis, 2006; Boulangé et al., 2016; Vuong et al., 2017). They consequently ensure the majority of ecosystem services provided to our society (Philippot et al., 2013; Vandenkoornhuyse et al., 2015). However, microorganisms display a substantial spatial heterogeneity (**Box 1**, see reviews Etterma and Wardle, 2002; Green and Bohannan, 2006; Franklin and Mills, 2007; Bahram et al., 2015). This raises questions about how the distribution of microbes depends on different components of community assembly, its link with niche theory and coexistence mechanisms, and how community assembly is linked to the functions and functioning of these microbial ecosystems.

Drivers of microorganism assemblages have so far mostly been analyzed at the patch scale, assuming that species niches result from the effect of the abiotic environment on species selection, disturbance or biotic interactions among microbial organisms (Niche theory, **Figure 1**), or with their host (Louca et al., 2018) and ignoring dispersal effects. Because microorganisms have very high reproductive capacities and short generation time, the historical view "*everything is everywhere but the environment selects*" (Baas Becking, 1934) has been accepted for a long time. The progress in resolution of microbial communities composition obtained from mass sequencing and large-scale studies of microbial distribution (see for instance Karimi et al., 2018) provided an increased number of evidences that microorganisms are much more limited in their dispersal than previously suspected (Telford et al., 2006). A framework based on large-scale biogeography has been successfully used for understanding large-scale spatial patterns of species (e.g., Martiny et al., 2006; Hanson et al., 2012; Donaldson et al., 2016; **Figure 1**). This framework considers that community assembly in local patches considered as "islands" results from colonization and extinction processes, both processes are related to the size and distance of the patch to a source patch ("continent") (**Figure 1**). A spatially implicit approach that builds on the island biogeography theory of MacArthur and Wilson (1967) was a useful starting point to consider how dispersal can affect community assembly at the landscape level, starting at first on one species (i.e., metapopulation, Hanski, 1994; **Figure 1**), to assemblages with several species (i.e., metacommunity, Leibold et al., 2004; **Figure 1**). Metacommunities consist of sets of communities connected through dispersal. Four main processes can thus drive community variation in space, which are species selection (including both abiotic and biotic factors), speciation (analogous to mutation in population genetics), dispersal and ecological drift (**Box 1**; Vellend, 2010, 2016).

An alternative approach to spatial dynamics in ecology emerged some 30 years ago in the form of landscape ecology (Wiens et al., 1993; Turner et al., 2001; Fletcher and Fortin, 2018). Landscape ecology focused specifically on the explicit analysis of spatial ecological patterns and has determined the conceptualization of what a landscape is (**Figure 2**), and provided tools for analyzing how spatial processes influenced the assembly of biodiversity, focusing primarily on plants and animals (**Box 1**). Landscape structure, described through different metrics at the landscape scale (i.e., heterogeneity) or the habitat scale (i.e., fragmentation), has been shown to affect dispersal, and local habitat exploitation during an organism's lifecycle, but also the availability of habitat for species development and movements among local patches (**Figure 3** and **Box 1**). These two types of metrics - landscape heterogeneity and fragmentation - affect species abundance and composition (Fahrig, 2003). Landscape ecology research has, however, mostly concentrated on macroorganisms and the microbial compartment has remained understudied under this framework.

Applying landscape ecology principles to microorganisms has, until recently, been slow to develop due to our limited understanding of microbial habitat requirements, the difficulties involved in observing microorganisms movements and our limited capacity to conduct spatially extensive surveys of microbial distribution. The determination of microbial community composition is also by itself difficult given that microbial communities can be quite complex and need to be studied by mass-sequencing approaches. From the nature of the data used, the microbial species-sequence delineation is also needed and the adoption of a phylogenetic species concept (i.e., "[...] the smallest diagnosable cluster of individual organisms within which there is a pattern of ancestry and descent. [...]" (Cracraft, 1983)) is implicit. After having used cutoff of sequence identity to identify Operational Taxonomic Units, recent bioinformatics advances now allow circumventing the use of this artificial cutoff (i.e., sequence-clusters (Mahé et al., 2014) and Amplicon Sequence Variants (Callahan et al., 2017)) and define taxa at a thinner grain. This better resolution in community description provides the basis for testing new ecological concepts such as landscape ecology. Application of landscape ecology to the microbial world also requires the characterization of the landscapes in which microbes live. Such landscapes can be a set of different habitat types with varying environmental conditions, but it can be the set of hosts available for microbial colonization (**Figure 3**). These "biotic" landscapes may then be driven by the behavior and growth of macro-organisms that are hosts for microorganisms, and be dependent on these hosts' response to their own landscape characteristics. Lastly, microbial landscapes can be within a host, corresponding to different anatomical sites within the body, and even within each organ (Batten et al., 2007; Proctor and Relman, 2017), providing patches varying in their environmental conditions. In addition, individual microorganisms interact with nano and

BOX 1 | Main definitions.

Species: a group of organisms that are able to exchange genes or interbreed, and create fertile offsprings. The species is the principal taxonomic unit of classification.

Habitat: the area characterized by a given set of environmental variables (abiotic and biotic factors) required by a species for survival, growth and reproduction.

Spatial heterogeneity: Non-random distribution of species or individuals within an area. Spatial heterogeneity can be related to landscape heterogeneity or a property of the population.

Microbial Landscape: Elements hosting microbial community spatially distributed and with interactions among them (exchange of individuals, energy and matter). It can be both structural landscapes (set of patches characterized by their environmental conditions) or biotic landscapes (set of hosts of various species, genotypes and ages). Microbial landscape can be seen from kilometric to centimetric scales.

Landscape heterogeneity: Differences in landscape elements in terms of composition and configuration. Heterogeneity is reached while there are a complex composition and configurations of landscape elements in the landscape. Landscape homogenization is the process leading to a decrease in landscape heterogeneity.

Landscape composition: Number and type of landscape elements.

Landscape configuration: Spatial arrangement of landscape elements. It can be related to the size, the location and the form of the habitat patches.

Landscape fragmentation: Habitat configuration within a landscape depending on isolation and habitat patch size. Landscape fragmentation is also the process involving the loss and the breaking apart of habitat.

Habitat isolation: Distance among patches of a given habitat type. Habitat isolation refers to the ability of organisms to move among habitat patches.

Habitat amount: Total patch area of a given habitat type. Habitat amount is linked to the carrying capacity for organisms.

Dispersal: Movement of organisms that has an effect on the genetic structure of populations, and communities (emigration-immigration process from one patch to another).

Species selection: Selection of species depending on their traits that promote their fitness in a given environment.

Ecological drift: Random change in demographic rates of survival and reproduction.

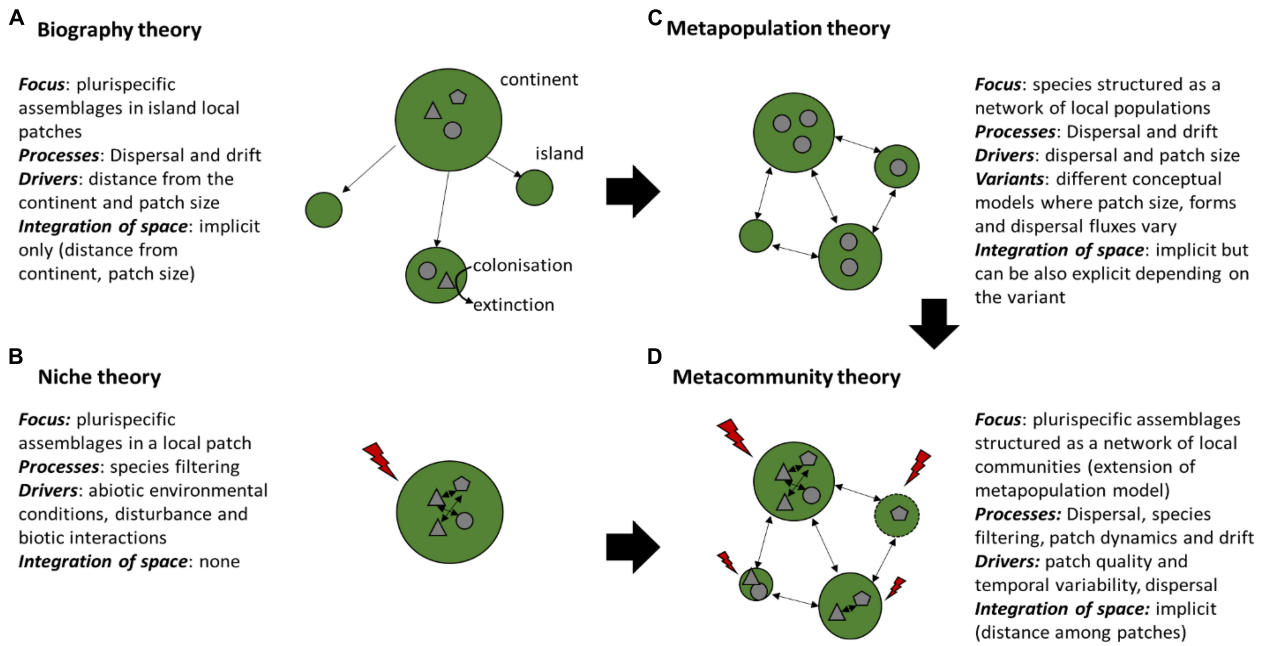


FIGURE 1 | The main conceptual frameworks of species assembly: **(A)** Biogeography theory, **(B)** Niche theory, **(C)** Metapopulation theory, **(D)** Metacommunity theory. **(A,B)** consider drivers at the local patch scale, while **(C,D)** consider the network structure of populations and communities. Green forms represent habitat patches, arrows among green forms represent dispersal fluxes, arrows within green forms represent species interactions, gray geometrical forms represent species, and red lightning represent abiotic conditions. When several types of geometrical forms are present in a given patch, it means that assemblages are plurispecific (i.e., include different species). Patches that are getting disturbed are delimited with dotted lines.

microscale surface features and volumes (Hol and Dekker, 2014). Unusual small-scale landscapes have then to be taken into consideration due to the very small size of microorganisms. Within these microscale landscapes as in terrestrial or aquatic environments, microorganisms' distribution is tightly related to patch heterogeneity (Nunan et al., 2003; Vos et al., 2013). However, most authors did not ground their work in the landscape ecology framework, and it is only recently that microbial ecology developed explicit integration of landscape ecology principles for understanding the drivers of microbial distribution (Batten et al., 2007). Because of these specificities of microbes - species-definition, dispersal, response to biotic heterogeneity, and small-scale responses to environment - the transposition of the existing theoretical framework in landscape ecology for analyzing assembly rules of microorganisms is likely indirect.

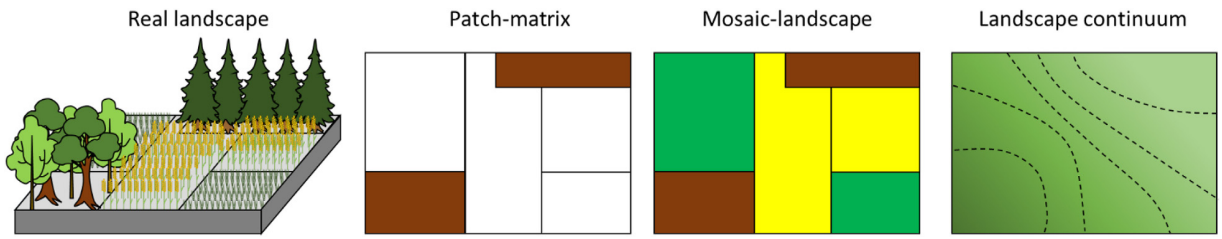


FIGURE 2 | Conceptual models of landscapes. Landscapes are represented as composed of discrete patches (patch-matrix and mosaic-landscape), or as an ecological continuum (landscape continuum). Real landscape is constituted of two kinds of forest patches, grasslands (green plants) and crops (yellow plants). Patch-matrix considers only the habitat patch and not the mosaic (e.g., only the forest patches here in brown). Mosaic-landscape considers mosaic composition and habitat patches (e.g., forest patches are in brown, grasslands in green and crops in yellow). In Patch-matrix and Mosaic-landscape models, black lines represent the limits of the discrete patches. Landscape continuum considers landscapes as described through continuous environmental variables (organic matter content for instance, or cover density). In this latter model, landscape can be linked with one or several continuous variables. Dotted lines represent different levels of the environmental gradient.

The aim of the present review was to investigate how landscape ecology concepts could apply to the microbial world, to advance our understanding of this world and to show how microorganisms can be used as new models to test and extend the existing landscape ecology framework. We accounted for aquatic, terrestrial and marine ecosystems, as well as for all kinds of host-microbiota interactions from free-living microorganisms to microorganisms associated with plants, animals and humans. Viruses were excluded from the scope of the paper because of their sub-microscopic size. If there are compelling reasons about the importance of viruses for the origin of cells and diversification (Koonin et al., 2009), there is evidence against the notion that viruses are alive (Moreira and López-García, 2009). Their dispersion, genetic changes and propagation are thus determined by specific constraints not developed herein.

FROM ENVIRONMENTAL SPATIAL PATTERNS TO LANDSCAPES

Landscape Conceptual Models

Species distribution can be related to environmental patchiness via the way abiotic (or biotic) conditions are distributed in space. The consequences of such environmental patchiness on ecological processes including species assembly can be analyzed using different conceptual models of landscapes (**Figure 2**). The very first, and simplistic, conceptual model derived from the island biogeographic theory, considered that identical habitat patches (i.e., corresponding to favorable niches) are embedded in a matrix of distinct non-habitat (Patch matrix model, **Figure 2**; Turner, 1989). In this first vision, landscapes are characterized by metrics quantifying the amount of favorable patches or their isolation, supposing that the rest of the landscape do not act on species assembly. The patch-matrix model was then rapidly extended to the mosaic landscape model (**Figure 2**; Wiens, 1995) by including the mosaic of habitats comprising the landscape, and that could be considered as composed of more or less favorable habitats for development (Fahrig et al., 2011). Species are indeed potentially dependent on different habitat patches for their life cycle (i.e., complementation concept, Dunning et al., 1992) as for instance for animals, which juvenile stages depend on one given habitat type and adult stages on another. They can also rely on alternative habitats for their development (i.e., supplementation concept, Dunning et al., 1992). The mosaic of patches shapes also dispersal by acting on the permeability of landscapes to species movement (Taylor et al., 1993). In this second vision, landscapes are characterized by metrics quantifying their heterogeneity in terms of patch composition, which defines the type, richness and relative abundance of the different patches (i.e., abiotic habitats or hosts types) (**Box 1**). Heterogeneity of configuration defines the arrangement in space of the different patches and is related to metrics measuring features such patch size, aggregation, interface types among patches (**Box 1**; Fahrig et al., 2011). A last conceptual model has recently emerged, the continuum model, considering that landscapes are a combination of several continuous environmental gradients (**Figure 2**; Fischer and Lindenmayer, 2006; Cushman et al., 2010) instead of discrete patches. For this model, metrics used to describe the landscape are continuous, and may integrate partly species response to these environmental gradients through for instance the degree of matching between the abiotic conditions and species ecological requirements.

Most microbial studies based on landscape ecology are based on the patch-matrix model, while the mosaic model is only used in particular cases where patches are very heterogenenous, or where discrete patches correspond to different hosts. Correlations between environmental factors and microbial community composition have been extensively studied (Bru et al., 2011; Vos et al., 2013; Dehkharghani et al., 2019; Martínez-Olivas et al., 2019; Muscarella et al., 2019). Even though they generally imply the continuum conceptual model, these patterns have not been very well connected to the underlying theory behind landscape conceptual models. We review the existing evidence of landscape effects in the section "Effects of Landscape Mosaic Heterogeneity and Habitat Fragmentation on Microorganisms" below.

Spatial and Temporal Scales in Community Structure Across the Landscape

A major question in landscape ecology is the scale of effect (Jackson and Fahrig, 2012; Miguet et al., 2016), i.e., at which

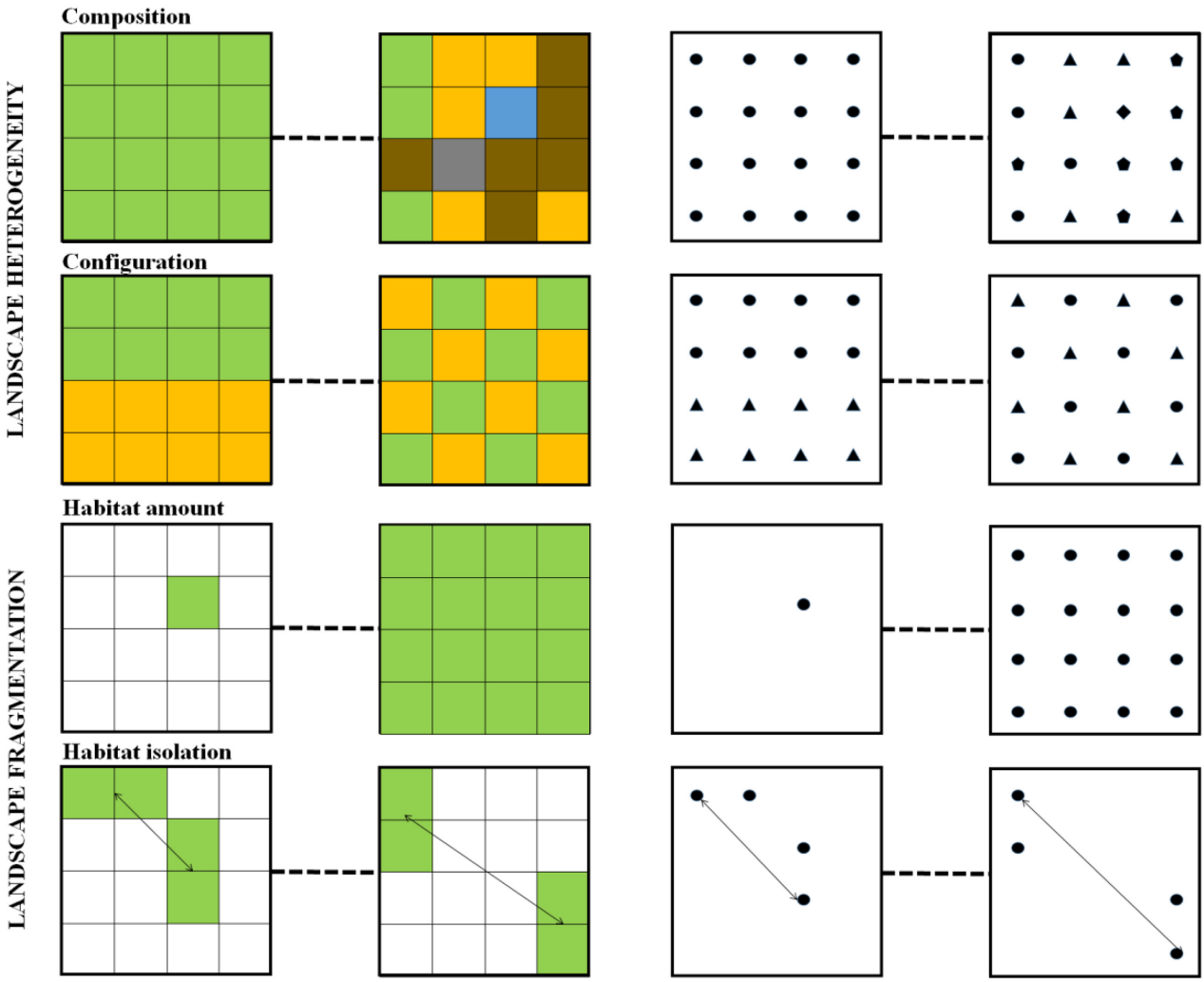


FIGURE 3 | Landscape heterogeneity and fragmentation in both structural and biotic landscapes. Gradients of low to high values of these components are represented. On the left, the colors represent different landscape elements. Landscape elements can be different land covers or land uses, different anatomical sites within a body, or different environmental patches within a given anatomical site. In this example, the focus habitat is the green one, where the microbial assemblages is studied. On the right, the geometrical shapes represent the distribution of different types of hosts. Biotic landscapes are then considered as the composition and configuration of the potential hosts. Hosts can be several species, or several genotypes within species. In this example, the focus habitat is the round host type, which microbiota is studied. See Box 1 for definitions.

scale the landscape variables has to be measured for a better prediction of the relationships between landscape variables and the biological response (e.g., species richness or diversity). This scale of effect is assumed to be related to the scale at which the species perceive and interact with the landscape. It can be analyzed both in space and over time. The standard landscape scale for macro-organisms ranges from hundreds to thousands of square meters, in relation with landscape patchiness and organism's dispersal range. The response of macro organisms can be measured over years, decades or even centuries in relation with landscape dynamics and an organism's life span. The scales of response by microorganisms is likely to differ because of their small size and short life span.

Spatial Scales of Microbial Landscapes

Existing literature on microorganism assemblages based on landscape ecology deals with large-scale landscapes (i.e., kilometric landscapes). Large-scale approaches are appropriate given the dispersal distance of many free-living organisms, thanks to the role of vectors such as wind, particles or water fluxes. For instance, the hydrological connectivity in a river floodplain system (i.e., at the kilometric scale) influences the abundance and productivity of bacteria (Luef et al., 2007). The distribution of host-associated microorganisms responds at similarly, large spatial scales given the ability of their hosts to disperse over such a distance and be a vector for these microorganisms. Such a large spatial distance has long been appropriate to assess

the dynamics and consequences of animal and plant diseases (Yuen and Mila, 2015) as fungi and bacteria pathogens are mostly spread by the long-distance vectors such as wind, human and animals mentioned above. Landscape epidemiology has developed on this background in order to help predict disease risk and disease propagation from landscape structure (see reviews by Holdenrieder et al., 2004; Suzan et al., 2012).

Although the conceptual framework of landscape ecology has not been explicitly used, a number of studies have demonstrated that microbial assemblages can be shaped by spatial heterogeneities that occur at very small spatial scales. For example, experimental studies that manipulated very small-scale differences in resource supply can produce correspondingly small landscape-induced microbial community changes (Keymer et al., 2006). In an experiment using microfluidic device where patchy and continuous landscapes were modeled, bacterial prey and predator relationships displayed different patterns: prey population in the continuous landscape progressively declined toward extinction, whereas significant stable prey population remained in the patchy landscape, indicating that microscale fragmentation significantly influenced bacterial composition and interactions (Hol et al., 2016). In response to chemical gradients, motile cells have evolved chemotaxis and chemotactic decision and behavior to reach favorable environments (Salek et al., 2019). Chemotactic velocity and performance capabilities in bacteria in response to environmental heterogeneity was habitat-of-origin dependent, seemingly higher for bacteria from the ocean in comparison to bacteria from gut for example (Son et al., 2016). More recently, landscape ecology principles were applied successfully to biotic landscapes, i.e., landscapes viewed as sets of hosts. Biotic landscape heterogeneity shaped endophytic fungal assemblages in plant roots at centimetric scales (Bittebiere et al., 2020; Mony et al., 2020a), with contrasted responses: Ascomycota depended on the floristic landscape composition through plant evenness and richness, while Basidiomycota depended on the floristic landscape configuration through host plant aggregation and connectivity (Mony et al., 2020a).

In the particular case of a microbial landscape in a host, the biological scale considered, for instance the whole body or the specific anatomical site, defines the landscape boundaries. Intra-host spatial patchiness has so far mainly been studied on human hosts and less on other organisms. For instance, the centimetric landscape mosaic of heterogeneous environmental patches has been described in different anatomical sites including the human nose, mouth, and throat, mostly with the objective to predict on microbial distribution and composition and their consequences for disease (See examples in the review of Proctor and Relman, 2017). Extending these investigations to other animal or plant hosts would be an interesting direction for future research.

Temporal Scales in Microbial Landscape Dynamics

Most existing work only implicitly accounts for time, for instance, when studying the microbial succession along series of past occupations of a given patch (Soledad Faggioli et al., 2019) or across the different developmental stages of a host (Bahram et al., 2015; Charbonneau et al., 2016; Chesneau et al., 2020). Explicitly accounting for the spatial scale in these temporal dynamics, i.e., analyzing the effects of temporal changes in the landscape has, however, been clearly overlooked (but see the studies on patch dynamics effect on planktonic and bacteria assemblages, Pringle et al., 1988).

Yet, landscapes change over time, at different scales ranging from hours to years, and likely modifying the dynamics of microorganism assemblages. Such landscape dynamics may be due to simultaneous changes in the land cover, land use or environmental conditions in the local patches that together form the landscape. In host-associated microbial communities, composition is strongly linked to the phenology of their host. For instance, in honey bees, the diversity of the bacterial community and in the type of genera colonizing the gut microbiota of young workers differs from that in 1-month older workers (Dong et al., 2020), likely due to changes in the diet and in the developmental environment, especially the social tasks and contacts attributed to older workers. In this particular case, the microbial landscape constituted by the distribution of individual bees is likely driven by population dynamics and their resulting host age-distribution and associated social interactions. Such population-dynamics driven biotic landscapes need to be investigated in many host species, including plants, animals and humans, where microbial community succession has been demonstrated to depend on the developmental stage of the host. These changes can happen in days as is the case for insects (e.g., Duguma et al., 2015; Dong et al., 2020), to several years in the case of hosts with longer life span (e.g. examples in Bahram et al., 2015; Dzidic et al., 2018).

In the particular case of landscapes within hosts, time also plays an important role in changes in the type and spatial arrangement of the environmental patchiness. For instance, children's teeth erupt at different developmental stages–from milk teeth to permanent teeth, and the sequence of eruption of the different classes of teeth (molars, incisors, canines), leads to changes in the spatial distribution of the microorganisms that inhabit the oral cavity (Dzidic et al., 2018). Teeth patch dynamics indeed had an impact on the occurrence of new patch types to be colonized and on local abiotic factors that induced modifications in species dominance, even within the same genus (for instance *Streptococcus* species, Carlsson et al., 1975). Changes in landscape heterogeneity at a finer temporal scale have also been reported to result from slight modification of saliva fluxes, or local inflammation patches in the oral cavity, likely inducing changes in bacterial composition within the course of a single day.

Overall, microbial modifications can be induced by landscape characteristics at different time and space scales, potentially in interaction, and in many situations nested within each other.

Nested Spatial and Temporal Scales

In landscape ecology, nested relationships among spatial scales and to a lesser extent among temporal scales within a landscape are accounted for in the hierarchy theory (Allen and Starr, 1982; O'Neill et al., 1986), which states that ecosystem processes are organized in discrete scales of interaction. Wu and Loucks (1995) proposed including time in this theoretical framework through the hierarchical patch dynamics concept that integrates patch dynamics in the hierarchy theory and provides a conceptual

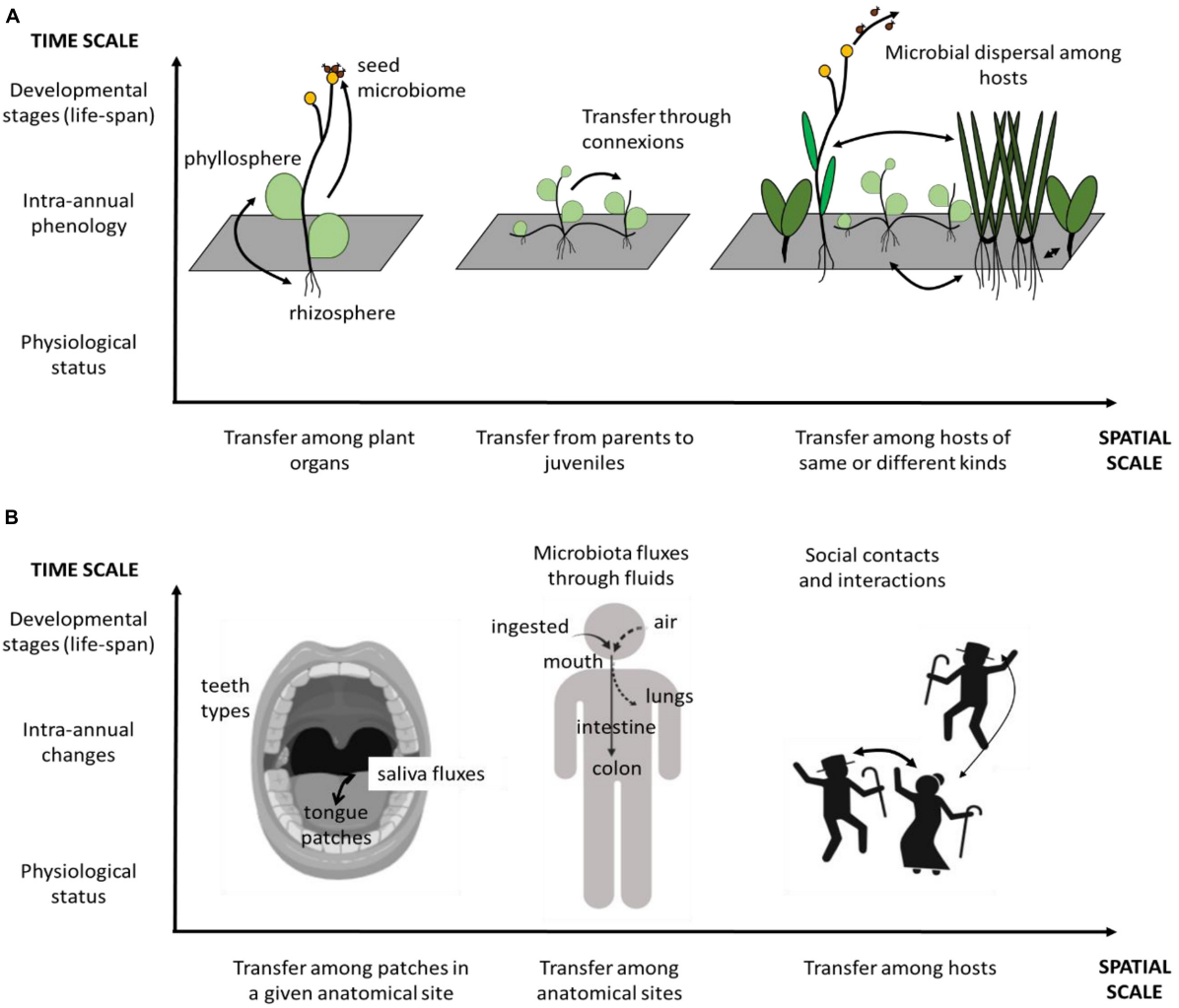


FIGURE 4 | Examples of nested scales in space and time of microbiota distribution. **(A)** microbiota-associated with host plants, micro-scale landscape corresponds to the organ partitioning within the individual plant. Meso-scale landscape corresponds to the distribution of individual plants at the local scale due to the effect of neighborhood and vegetative multiplication. Large-scale landscapes correspond to microbial transmission among individual plants and through seed dispersal. **(B)** Microbiota-associated with humans. Micro-scale landscape corresponds to the different patches within an anatomical site, here, the oral cavity. Meso-scale corresponds to the different anatomical sites within the human body, connected through dispersal. Large scale corresponds to social interactions and contacts among different humans. In both examples, microbiota changes occur at different temporal scales: short scale of an hour or a day (e.g., changes in physiological status), medium scale of a year (e.g., intra-annual phenological stages), and long scale covering decades (e.g., developmental stages in an individual life cycle). These temporal scales affect all the spatial scales illustrated in the figure.

framework for analyzing interactions among spatial and temporal scales in landscapes.

The nested relationship among spatial and temporal scales of landscape probably also applies to microorganisms. Here we provide two examples of such potential nestedness (**Figure 4**). First example is linked with microorganism distribution in the human body. Such distribution depends on the local landscape of a given anatomic site, in the nose, for instance (Proctor and Relman, 2017) or in the oral cavity (Proctor et al., 2018). However, microorganisms can also disperse across anatomic sites (von Eiff et al., 2001) as the nasal and oral cavities both drain into the pharynx, which ultimately connects through the trachea to the lungs or through the esophagus to the stomach. Microbiota can then disperse among human hosts depending on types of social contacts and behavior: for instance, microbiota exchanges between oral cavities depend on human partners kissing habits (Kort et al., 2014). These three spatial scales – within anatomic sites, among anatomic sites within a body, and among individuals – are then nested and potentially in interaction with each other.

We can also identify nested spatial scales in another example linked with plant-associated symbiotic fungi. A fraction of fungi recruited from the plant roots are transmitted to the other organs – leaves and seeds – of the plant (i.e., systemic distribution) (e.g., Vandenkoornhuyse et al., 2015), likely depending on the architecture and energy trade-offs of the

individual. Fungi have recently been shown to disperse through plant-vegetative multiplication, colonizing young individual offsprings developing along the stolons (Vannier et al., 2018). But the fungal microbiota of a given individual plant is also influenced by the neighboring host composition (Bittebiere et al., 2020) and isolation from hosts of the same species at the centimetric scale (Mony et al., 2020b). At a larger scale, fungal spores and propagules can disperse over much longer distances, for instance with birds as vectors (Correia et al., 2019), thus at least in part, being under the influence of the macro-landscape scale. This example presents another illustration of a nested spatial scale structure, based on biotic landscapes constituted as host distribution, which likely affects microbial composition. In this example as for the first one, there is in addition a potential interaction between the landscape of microbes and the landscape of the hosts, to which microbes are associated. Disentangling the respective effect of each scale of landscapes in the microorganism distribution, and the dependency between microbial landscape and host landscape has not yet been done. Many interesting questions could then be raised among which the analysis of the respective effect of each spatial scale in shaping the distribution of microorganisms, the effect of the intensity of dispersal fluxes among scales, and their dynamics over time.

Considering time scales, few studies have demonstrated these nested scales, probably because studies that investigate the impact of temporal changes in landscape structure on microbiota are rare. However, there are many cases where such nestedness among temporal scales can be assumed, especially when a landscape is based on host distribution, and hence host phenology. To go even further, it is likely that both spatial and temporal multi-scales interact, making it even more complex to address these processes.

Feedback Loops Within Microbial Landscapes

Landscape ecology generally analyzes how landscape structure shapes species distribution and abundance, assuming that there is no reverse effect. Yet, in contrast to macroorganisms, the activity of microorganisms is likely to reshape the structure of their own landscape. We can cite three examples of such potential feedback. The first example is linked to the huge role played by microorganisms in soil chemistry and structure (e.g., Bardgett and van der Putten, 2014). Decomposition of organic matter, as well as many biological cycles, are linked to bacterial or fungal activity (e.g., Schimel and Schaeffer, 2012). The patchiness of microorganisms in the soil may then lead to further changes in environmental patchiness, thereby affecting future generations of microorganisms in their foraging activity and dispersal. The second example is the microbial communities forming biofilms, groups of surface-adhering or free-floating cells, a case where free living microorganisms are interacting with each other to form a new environment and ecological habitat. These self-organized biofilms are mediated by interaction networks, which makes a feedback consequence on nutrient fluxes and spatial structure of the biofilms themselves (Nadell et al., 2016), social interactions and cross feeding which can modify microbial population spatial structure (West et al., 2007; Mitri et al., 2011; Mas et al., 2016). This cross feeding and cooperation among biofilm-members is supposed to be key for the biofilm stability and is likely a consequence of evolution of metabolic dependencies and specialization leading to a steady state among microbial populations (Mas et al., 2016). The third example is linked to biotic landscapes (**Box 1**). The interplay between microorganisms and all the biological functions of their hosts, i.e., growth, behavior and reproduction, affect their fitness (e.g., Rosshart et al., 2017). In the host-pathogen system, pathogen colonization of hosts may cause a drastic change in their host physiology and even their death. The way microorganisms are distributed among host patches is then likely to contribute to host population dynamics. Host patches could disappear, increase, or even move in case of mobile hosts, under the action of microorganisms, thereby modifying the spatial structure of the biotic landscape. This feedback, which is generally analyzed at the patch level (local conditions or host level), has not been investigated at the scale of multi-patches (i.e., landscape scale) and should be considered as a key particularity of microorganisms compared to macroorganisms.

EFFECTS OF LANDSCAPE MOSAIC HETEROGENEITY AND HABITAT FRAGMENTATION ON MICROORGANISMS

Landscape Spatial Heterogeneity

Studies that investigate the effect of landscape heterogeneity on microbiota are generally focused on analyzing the effect of composition or configuration at the scale of a given habitat patch, rather than investigating the effect of heterogeneity as a whole. This is done by concentrating on focal patches and then accounting for heterogeneity in the close neighborhood to the microbial assemblage under study (**Figure 3**).

Heterogeneity of Composition

Composition can be assessed while taking other types of elements in the landscape into account: for instance, the composition of the fungal microbiota associated with trees was shown to depend on the composition of plant species in the vicinity (Bogar and Kennedy, 2013), each plant species representing a particular habitat. The heterogeneity of composition can also be due to genetic differences within a host. Decreasing the frequency of susceptible host genotype compared to resistant ones in the landscape mosaic decreased the spread of a bacterial leaf streak in wheat (Mundt et al., 2011).

Heterogeneity of Configuration

Similarly, the impact of spatial configuration on the spread of pathogens at the landscape scale has been demonstrated with *Leptosphaeria maculans* (Bousset et al., 2018) that causes "blackleg" disease in canola (*Brassica napus*) and with the fusiform rust *Cropartium quercuum* in pine plantations (Perkins and Matlack, 2002). In both cases, the proximity of more susceptible stands of hosts and the absence of barriers to dispersal

of the pathogen, such as non-host plants or particular land-use types facilitated the spread of the disease. The density and proximity of other particular landscape elements, such as roads, may also be important. Roads facilitate access to their host by the pathogens because roadsides are mowed regularly promoting pathogen spread (Laine and Hanski, 2006) but also because it is dispersed through the movement of cars or animals. For example, Laine and Hanski (2006) showed that *Plantago lanceolata* and its wind-dispersed obligate pathogen *Podosphaera plantaginis* were dispersed by the currents of air created by cars, and Jules et al. (2002) showed that the exotic root pathogen *Phytophthora lateralis* spreading on *Chamaecyparis lawsoniana* was dispersed by mud transported via vehicles and on people's feet and animals' hooves. Overall landscape configuration has thus mostly been seen as a driver of microorganism dispersal while other mechanisms, such as supplementation or complementation processes (Dunning et al., 1992) have not yet been studied.

Landscape Habitat Fragmentation

Landscape effects are also linked to fragmentation, which includes both the effect of the reduction in habitat amount and/or the increase in isolation of habitat patches (Fahrig, 2003). The reduction in habitat amount affects species ability to survive and develop, due to an increase in extinction rate and the habitat's limited carrying capacity. At the patch level, small patch size increases the effect of patch edges. Edges are indeed at the center of active exchange of energy, matter, and species from one patch to another. They may act as barriers or filters to the movement but also contribute to changes in abiotic conditions (nutrients, microclimatic conditions) inside the patches (Saunders et al., 1991; Murcia, 1995). These effects may be beneficial or detrimental to species, depending on the species' ecological requirements. Isolation limits an organism's ability to disperse and to colonize other patches in the landscape. Because of these effects, fragmentation is assumed to reduce biodiversity by increasing the susceptibility of species to environmental stochasticity leading to an increased risk of extinction (Fahrig, 2003). A wide range of studies deals with the effects of habitat fragmentation on microorganism assemblages, the effect either of habitat size or of habitat isolation, or both. In the case of biotrophs, the host determines the available habitat. Fragmentation is linked to the size of the host population and its distribution across the landscape, yet few studies have tried to disentangle the respective effects of patch size, isolation and edges. The combination of small and isolated patches generally increases the prevalence of pathogens, including fungal infections in plants (Groppe et al., 2001; Colling and Matthies, 2004), although the reverse effect has also been found, for example, Linert and Fischer (2003) reported higher prevalence of the fungal *Urocystis primulicola* on the plant *Primula farinosa* in fragmented landscapes.

Patch Size

The effect of habitat size has been widely studied but contrasted patterns have been demonstrated even within the same taxonomic group. Penttila et al. (2006) found that species richness and wood-decay fungi increase rapidly with an increase in area. On the other hand, no particular effect of habitat size was found to determine the composition of spores of arbuscular mycorrhizal (AM) fungi in forest soils (Mangan et al., 2004), whereas the colonization intensity of AM fungi was positively correlated with the size of the forest fragment (Grilli et al., 2012). The spread of pathogens has been associated with the presence and amount of edges. For instance, fungi were reported to colonize the leaves of woody seedlings three times faster in edge plots than in interior plots, perhaps due to interactions with damage caused by herbivory (Benitez-Malvido and Lemus-Albor, 2005).

Habitat Isolation

Habitat isolation is related to the distance between neighboring habitats (**Box 1**). The effects of isolation were originally studied by investigating the effects of geographic distance on community composition following the biogeography theoretical framework. Distances can range from one meter to the continental scale (Fierer and Jackson, 2006). First focusing on pathogens, research on habitat isolation has accumulated evidence that geographical distances among hosts determines the severity and incidence of disease, and likely also affects its spread (Thrall et al., 2003; Laine and Hanski, 2006). One of the very first works to investigate the effect of isolation at the community scale demonstrated a 50% decrease in ectomycorrhizal fungi richness associated with individual *Pinus* trees located at a distance of 1 000 m from the forest edge (Peay et al., 2010). This decrease was likely driven by dispersal-limitation mechanisms as demonstrated by Peay et al. (2012) who used a trap experiment and showed that the quantity and richness of spores of ectomycorrhizal fungi in the trap, and their colonization of sterile pine seedlings decreased rapidly with increased spatial distance from the host vegetation. Conceptual development in landscape ecology considers that the isolation effect is not only driven by geographic distance, but also by landscape structure (i.e., using patch-matrix or landscape mosaic model, **Figure 2**). By accounting for how landscape can facilitate or impede the dispersal of organisms (Taylor et al., 1993), landscape connectivity is suggested to be a key component of isolation metrics, even in microorganism studies. One simple metric used is the distance to the nearest patches of similar habitat. For instance, Vannette et al. (2016) demonstrated that fungal species composition associated with the tree, *Metrosideros polymorpha*, was more similar among highly connected habitat patches, i.e., patches with a habitat of same type in the close vicinity, than among poorly connected ones, i.e., patches with the same type of habitat located far away. A study by Peay et al. (2010) demonstrated the importance of particular fungal reservoirs as the composition of ectomycorrhizal fungi associated with *Pinus muricata* isolated trees embedded in a non-forested matrix, depended on the distance to large forest patches but not necessarily to the nearest isolated tree. These results suggest an effect of population size or age in the connectivity effect. On the contrary, connectivity is also determined by the existence of barriers to dispersal: for instance, in polar environments, the occurrence of mud boils due to frost was shown to modify microbial co-occurrence networks in bacteria in the soil

(Ferrari et al., 2016). This study unexpectedly demonstrated that patches isolated through these barriers harbored higher species richness, probably due to a sheltering effect from the predators. Interestingly, these results suggest that biotic interactions may interplay with the connectivity effect and should thus be taken into account in future studies.

At a much smaller scale, for instance, in human microbiomes, the concept of connectivity within the body is still in its infancy, even though many observations support the validity of the concept applied to the distribution of microorganisms. For instance, the microbiota in the mouth, nose and stomach were shown to resemble each other more than they resembled lung communities, suggesting that the esophagus acts as a corridor that promotes microorganism dispersal (Bassis et al., 2015). The role of other components as corridors has been suggested as symptomatic patterns associated with diseases, such as the connection between nasal canal and the middle ear through the Eustachian tube that facilitates the spread of the bacterial agents of otitis (Chan et al., 2016). At an even smaller spatial scale, in the lung, microorganism community richness has been found to be a function of increasing distance to the supraglottis, seen as a reservoir of microorganisms (Dickson et al., 2015). Other studies suggest that microbial dispersal along corridors might be related to fluids like mucus or saliva. For instance, the velocity of the salivary film and the position of the teeth were reported to control the microbiota present on teeth and their susceptibility to be colonized by caries-associated bacteria (Proctor et al., 2018). The nasal mucus present in the nasal cavity transported microorganisms to the paranasal sites, together with an input of nutrients (Abreu et al., 2012; Aurora et al., 2013). In these particular studies, connectivity is mostly linked to the occurrence of a physical connection associated with a fluid vector (a corridor viewed as a conduit), which is a restricted case study of landscape connectivity.

DISPERSAL AND METACOMMUNITIES WITHIN THE LANDSCAPE ECOLOGY FRAMEWORK

Dispersal for Microorganisms and Interactions With Landscape Parameters

Microorganisms can disperse either passively or actively. While some taxa disperse over long distances, others only disperse over very short distances, generating non-random distributions. Species also display different modes of dispersal leading to a wide range of dispersal distances, for instance, fungi can disperse at the centimetric scale through expansion of the vegetative mycelium but also at much larger spatial distance through aerial dispersal of spores. In addition, the dispersal of microorganisms often depends on the vector involved, wind (Allen et al., 1989), water, or host movement. Inside the human body, microorganisms often disperse in mucus (Proctor and Relman, 2017).

Microorganism dispersal can thus be indirectly linked to landscape characteristics through their effect on the vector. For instance, microbial dispersal, and especially pathogens' has been shown to strongly depend on particular layouts of air conditioning ducts in public buildings (Fernstrom and Goldblatt, 2013). When it comes to dispersal mediated by hosts, the distribution and movement of microbes across the landscape is also tightly linked with the response of their host to the landscape structure. For instance, proximity to cattle and to urban zones modifies the behavior of wildlife and affects the spread of antimicrobial resistance, presumably due to effects on microbial distribution (Arnold et al., 2016). In a biogeography study of public restrooms, the composition of microbiota sampled on open surfaces, and its origin in the human body (annal, vaginal or skin) depended on the behavior of the restroom users, suggesting that the restroom can be considered as a landscape made up of different elements corresponding to different ecological uses (Flores et al., 2011). In plants, the propagation of microorganisms associated with seed dispersal is less well known, especially because only a small part of the plant microbiota can colonize and be transmitted by seeds (Shade et al., 2017). Although the effect of landscape characteristics, particularly connectivity, on seed dispersal has been demonstrated in many ecosystems (Uroy et al., 2019), their consequences for microorganism dispersal have just started being demonstrated (Correia et al., 2019).

Metacommunity Structure in Microorganisms: Toward Landscape Explicit Consideration?

From a biogeographic perspective, many species can be structured as metapopulations (Hanski, 1994) in which distinct local populations are assumed to be linked by dispersal fluxes. This concept has been extended to the concept of metacommunities that simultaneously considers the role of species interaction (Leibold et al., 2004; Leibold and Chase, 2018). Until recently, four main models have been described depending on how species respond to local environmental conditions, dispersal limitation and disturbances (species sorting, patch dynamics, mass effects and neutral dynamics). This conceptual framework can be applied at a wide range of spatial scales. Although many authors have considered the individual components of the theory including the effect of local factors or interactions between species in shaping assemblages, there has been relatively few works done to organize this within a unified framework (Christian et al., 2015; Miller et al., 2018; Langenheder and Lindström, 2019).

Langenheder and Lindström (2019) review the literature for non-host associated microbes and conclude that environmental heterogeneity (influencing the sorting of species among habitat types) is very generally important. However they also review studies showing that dispersal limitation (large-scale distance effects), dispersal excess (small-scale distance effects), priority effects in species interactions (independent of environment), and stochasticity (including apparent neutrality or near-neutrality) are evident in different systems under different conditions. They also show that these effects are linked to other community and ecosystem attributes such as overall productivity, stability and scale effects, but that all of these effects vary, often inconsistently, among studies in ways that are still unresolved. Some of these

effects have been additionally demonstrated more rigorously by manipulative experiments (e.g., Thrall et al., 2003; Livingston et al., 2013; Berga et al., 2015). Other effects that have been identified but not extensively studied include interactions with "macrobes" (e.g., Verreydt et al., 2012). Similarly, Miller et al. (2018) evaluate how metacommunity ecology can inform (and be informed by) the study of host-associated microbiomes. They also conclude that there is evidence of the same set of metacommunity processes as were found in non-host associated microbes, including habitat (i.e., host types) heterogeneity, dispersal limitation, priority effects, and stochasticity, and that the importance of these effects can be highly context dependent in ways that are not fully resolved. However, they also highlight the additional importance of host-microbiome feedback as potentially important factors to incorporate into metacommunity ecology. One set of mechanisms that have yet to be adequately addressed include the role of local genetic evolution that may also be responsible for legacy effects (Urban et al., 2008; Bahl et al., 2011; Vass and Langenheder, 2017) and these may be particularly relevant in microbes due to their large population sizes and short generation times (Miller et al., 2018).

Nevertheless, even though there are clear conceptual connections between them, the link between metacommunity and landscape ecology remains poorly resolved (Almeida-Gomes et al., 2020). To a large degree, this is because metacommunity ecology has focused more on species attributes and how they contribute to community assembly than to site attributes. A promising step toward reconciling the two involves the modification of joint species distribution models and related methods (primarily focused on the distribution of species) to address landscape distributions (e.g., Fournier et al., 2017; Leibold et al., 2020).

BIOTIC INTERACTIONS WITHIN THE MICROBIOTA AND EVOLUTIONARY EFFECTS ON THE MICROBIAL LANDSCAPE

Landscapes for microbes can be shaped by a feedback process due to microbes' distribution and activity, and to their biotic interactions. Below, we review different examples of such interactions that can influence landscape structure and its dynamics.

Competition

If at the microscale, landscape patches of nutrients are ephemeral in space and over time, as is the case in aquatic environments (Yawata et al., 2014), these transient patches likely lead to high heterogeneity in microbial communities. This will be even more the case when foraging behavior differs among microorganisms, some producing a biofilm (a multicellular bacterial community embedded in an extracellular matrix) while others explore more patches (free living cells). Short term changes in micro-landscapes could then explain the fine-scale ecological differentiation of microbial communities (Yawata et al., 2014) as well as the temporal dynamics of meta-communities related to the dispersal tradeoff (i.e., forming a biofilm but promoting kin competition vs. high dispersion to limit competition although with the risk of not finding a new patch). As demonstrated by Yawata et al. (2014), these different behaviors and the associated tradeoff lead to population segregation at a small spatial scale. The same tradeoff could be expected in all microbial communities that form biofilms, possibly through enforcement processes related to cooperation (Agren et al., 2019) and where bacterial foraging could deeply affect meta-community changes and dynamics.

Prey-Predator

In nature, microbial communities are also the subject of prey-predator relationships. A recent elegant study demonstrated that a protist species can strongly affect the spatial patterns of two other protist species it predated by prey sorting (i.e., prey preference), thereby affecting their response to the patches of resources that comprise the micro-landscape. This prey-predator relationship ultimately had a feedback effect on the distribution of the predator within the landscape (Hol et al., 2016; Livingston et al., 2017). Although to our knowledge, this topic has not yet been studied, microbial viruses can act in a similar way as protist predators by increasing microbial landscape complexity. For instance, the regulation of microbial populations density at macro-landscape scale has been demonstrated, with highly successful microorganisms being attacked by the proliferation of their specific viruses (i.e., "killing the winner hypothesis") (Miki and Yamamura, 2005) leaving the habitat free for other less successful microorganisms. Because microbial predators regulate population size, microbial fitness decreases with an increase in its relative abundance. Thus, microbial predators do engineer the landscapes of bacterial communities but simultaneously depend on such landscape structure. Similar phenomenon was also demonstrated on biofilms. Grazing from protozoans was shown to shape biofilm volume and spatial heterogeneity (Bohme et al., 2009; Weeman et al., 2011).

Mutualist Interactions

Symbiotic interactions among free-living members of the microbial community also drive the microbial community structure. Cooperative behaviors can emerge as an evolutionary process to escape competition with a member of the community that produces a shared public good while cheaters (i.e., those no longer able to produce it) become dependent on the producer and are fitter than the wild-type non-cheater (Morris et al., 2012; Mas et al., 2016). This evolutionary trajectory of dependencies through gene loss (e.g., Mas et al., 2016) can at least partly explain the complexity of co-occurring microbial communities and their spatial heterogeneity, but this evolutionary pathway also triggers a feedback process on the microbial landscape made up of the producer patches. In humans and animals, cellular disorder can induce or be induced by microbiota: members of the microbiota (pathobiome) can be involved in shaping inflammatory environments and in some cases could promote tumor growth and spread (Brennan and Garrett, 2016). Evolutionary processes can thus contribute to the spatial dynamics of both interactors at micro-scale and impact the fate and success of dispersal among patches of the microbial landscape.

Bacterial Coexistence and Rapid Evolution

Diversity in communities is often viewed in light of ecological processes but, by modifying the microbial landscape, rapid evolutionary processes may also matter (Hart et al., 2019). To illustrate this issue, let us use the rapid evolution of resistance to antibiotics, which is among the best-documented cases of recent evolution in microorganisms (Nesme and Simonet, 2015; Hiltunen et al., 2017). The example of the evolution of antibiotic resistance can be summarized as a rapid evolutionary process of gene acquisition conferring new ecological abilities. As a result, the related eco-evolutionary processes modify the competitive hierarchy and in turn, may affect the coexistence outcome and realized niche. The dissemination of these new functional abilities will likely result in the rapid evolution of the microbial landscape. The eco-evolutionary processes that take place in the context of microbial landscapes is a fundamental frontier of knowledge that could be reached through a more holistic perception of the factors driving bacterial coexistence.

CONCLUSION

The application of landscape ecology to the microbial world is still in its infancy despite an important set of works on microbial spatial ecology and biogeography. On the one hand, studies analyzing the effect of environmental heterogeneity on microorganisms have been for long developed without clearly using the concepts and methods of landscape ecology. On the other hand, the current research effort on landscape ecology for microorganisms is mostly focused on pathogens and disease risk assessment. These latter studies, despite their strong interest, may limit our knowledge on microbial landscape ecology toward specific host-pathogens systems, and methods used to investigate symptoms rather than species presence and abundance. We demonstrated, however, through this review an increasing interest to fill the gap between both approaches, and transpose concepts and methods of landscape ecology for analyzing the structure of microbial assemblages.

If most existing literature on the topic describes the landscape using the continuum landscape model (i.e., continuous environmental heterogeneity), there is an emerging set of works that use the patch-matrix model, i.e., consider landscapes as constituted of discrete favorable habitat patches. Integrating the landscape mosaic in the description of the microbial landscape is poorly done yet. However, there is an obvious interest of using the mosaic landscape model for microbes, especially because some of these microorganisms develop in biotic landscapes constituted of hosts (i.e., discrete habitat patches of different kinds), but also because microbial dispersal likely depends on the permeability of the landscape matrix. There is then a strong need to develop the dedicated metrics for using this conceptual model to microorganisms.

Through this review, we highlighted some convergences in organisms' response to landscape features, among free-living organisms and microorganisms associated with plants, animals or humans. We demonstrated especially the importance of landscape configuration (and not only composition) as a driver of microbial community heterogeneity in space and time; and the key role of dispersal mechanisms - both active and passive - in this relationship. Some ecological processes and their influence differ however, for microorganisms compared to macroorganisms. Microorganisms' small size and short generation time affect their responses to landscape characteristics. These responses can occur at a very small spatial scale, and across several generations promoting the importance of evolutionary processes in species assembly. The scale of effect for microorganisms is then more complex than for macroorganisms, involving potentially nested-spatial and time scales. These nested-scales depend on the dispersal abilities of microorganisms, and on microorganisms' potential interactions with a host or with other microbes. Studying landscape ecology of microbes should then involve sampling or experimental designs across multiple scales. In host-associated microorganisms, it should also take into account the "host-microbes" system as a whole, for designing the study and in interpreting the results. Another important point is the existence of feedback effects of microbes on their own landscape. Thanks to their distribution and activity, microorganisms modify the abiotic conditions, or act on their host fitness and behavior. They shape then their future landscape. Macroorganisms affecting local environmental conditions at the patch scale is a well-known feedback; we demonstrated here that it could be up scaled at the landscape level for microorganisms. The existence of this landscape feedback effect opens a large array of hypotheses on its influence on the metacommunity internal processes, and on a possible coevolution of microbes with their landscape.

These specificities listed above may then call for further developments on the theoretical framework of landscape ecology for microbial organisms. Such development may overall help to reach a comprehensive view of stochastic and deterministic processes in their assembly, and develop approaches that are more functional. Because of their pivotal role in many ecosystem services, from health to food production, the development of landscape ecology for microorganisms should have major consequences for our understanding of their assembly and potentially for their manipulation in anthropogenic and natural ecosystems.

AUTHOR CONTRIBUTIONS

All authors discussed about the content of the article and reviewed the draft, and gave final approval for publication. CM and PV wrote the first draft of the manuscript with inputs from ML, BB, and KP.

FUNDING

This work was supported by the French National program EC2CO (Ecosphère Continentale et Côtière) of the CNRS.

REFERENCES

Abreu, N. A., Nagalingam, N. A., Song, Y., Roediger, F. C., Pletcher, S. D., Goldberg, A. N., et al. (2012). Sinus microbiome diversity depletion and corynebacterium tuberculostearicum enrichment mediates rhinosinusitis. *Sci. Transl. Med.* 4:151ra124. doi: 10.1126/scitranslmed.3003783

Agren, J. A., Davies, N. G., and Foster, K. R. (2019). Enforcement is central to the evolution of cooperation. *Nat. Ecol. Evol.* 3, 1018–1029. doi: 10.1038/s41559-019-0907-1

Allen, M. F., Hipps, L. E., and Wooldridge, G. L. (1989). Wind dispersal and subsequent establishment of VA mycorrhizal fungi across a successional arid landscape. *Landsc. Ecol.* 2, 165–171. doi: 10.1007/bf00126016

Allen, T. F. H., and Starr, T. B. (1982). *Hierarchy: Perspectives for Ecological Complexity*. Chicago, IL: University of Chicago Press.

Almeida-Gomes, M., Valente-Neto, F., Pacheco, E. O., Ganci, C. C., Leibold, M. A., Melo, A. S., et al. (2020). How does the landscape affect metacommunity structure ? A quantitative review for lentic environments. *Curr. Landsc. Ecol. Rep.* 5, 68–75. doi: 10.1007/s40823-020-00049-6

Arnold, K. E., Williams, N. J., and Bennett, M. (2016). Disperse abroad in the land: the role of wildlife in the dissemination of antimicrobial resistance. *Biol. Lett.* 12:20160137. doi: 10.1098/rsbl.2016.0137

Aurora, R., Chatterjee, D., Hentzleman, J., Prasad, G., Sindwani, R., and Sanford, R. (2013). Contrasting the microbiomes from healthy volunteers and patients with chronic rhinosinusitis. *JAMA Otolaryngol. Head Neck Surg.* 139, 1328–1338. doi: 10.1001/jamaoto.2013.5465

Baas Becking, L. G. M. (1934). *Geobiologie of Inleiding Tot de Milieukunde*. The Hague: W. P. Van Stockum and Zoon.

Bahl, J., Lau, M. C. Y., Smith, G. J. D., Vijaykrishna, D., Cary, S. C., Lacap, D. C., et al. (2011). Ancient origins determine global biogeography of hot and cold desert cyanobacteria. *Nat. Commun.* 2:163.

Bahram, M., Peay, K. G., and Tedersoo, L. (2015). Local-scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. *New Phytol.* 205, 1454–1463. doi: 10.1111/nph.13206

Bardgett, R. D., and van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature* 515, 505–511. doi: 10.1038/nature13855

Bassis, C. M., Erb-Downward, J. R. E., Dickson, R. P., Freeman, C. M., Schmidt, T. M., Young, V. B., et al. (2015). Analysis of the upper respiratory tract microbiotas as the source of the lung and gastric microbiotas in healthy individuals. *mBio* 6:e00037-15.

Batten, T. J., Sloan, W. T., Kjelleberg, S., Daims, H., Head, I. M., Curtis, T. P., et al. (2007). Microbial landscapes:new paths to biofilm research. *Nat. Rev. Microbiol.* 5, 76–81. doi: 10.1038/nrmicro1556

Benitez-Malvido, J., and Lemus-Albor, A. (2005). The seedling community of tropical rain forest edges and its interaction with herbivores and pathogens. *Biotropica* 37, 301–313. doi: 10.1111/j.1744-7429.2005.00031.x

Berga, M., Östman, Ö, Lindström, E. S., and Langenheder, S. (2015). Combined effects of zooplankton grazing and dispersal on the diversity and functioning of bacterial metacommunities. *Environ. Microbiol.* 17, 2275–2287. doi: 10.1111/1462-2920.12688

Bittebiere, A. K., Vandenkoornhuyse, P., Maluenda, E., Gareil, A., Dheilly, A., Coudouel, S., et al. (2020). Past spatial structure of plant communities determines arbuscular mycorrhizal fungal community assembly. *J. Ecol.* 108, 546–560. doi: 10.1111/1365-2745.13279

Bogar, L. M., and Kennedy, P. G. (2013). Neighborhood effect between host-plants. New wrinkles in an old paradigm: neighborhood effects can modify the structure and specificity of Alnus-associated ectomycorrhizal fungal communities. *FEMS Microbiol. Ecol.* 83, 767–777. doi: 10.1111/1574-6941.12032

Bohme, A., Risse-Buhl, U., and Kusel, K. (2009). Protists with different feeding modes change biofilm morphology. *FEMS Microbiol. Ecol.* 69, 158–169. doi: 10.1111/j.1574-6941.2009.00710.x

Boulangé, C. L., Neves, A. L., Chilloux, J., Nicholson, J. K., and Dumas, M. E. (2016). Impact of the gut microbiota on infammation, obesity, and metabolic disease. *Genome Med.* 8:42.

Bousset, L., Sprague, S. J., Thrall, P. H., and Barrett, L. G. (2018). Spatio-temporal connectivity and host resistance influence evolutionary and epidemiological dynamics of the canola pathogen *Leptosphaeria maculans*. *Evol. Appl.* 11, 1354–1370. doi: 10.1111/eva.12630

Brennan, C. A., and Garrett, W. S. (2016). Gut microbiota, inflammation, and colorectal cancer. *Annu. Rev. Microbiol.* 70, 395–411.

Bru, D., Ramette, A., Saby, N. P., Dequiedt, S., Ranjard, L., Jolivet, C., et al. (2011). Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. *ISME J.* 5, 532–542. doi: 10.1038/ismej.2010.130

Callahan, B., McMurdie, P., and Holmes, S. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J.* 11, 2639–2643. doi: 10.1038/ismej.2017.119

Carlsson, J., Grahnen, H., and Jonsson, G. (1975). Lactobacilli and Streptococci in the mouth of children. *Caries Res.* 9, 333–339. doi: 10.1159/000260166

Chan, C. L., Wabnitz, D., Bardy, J. J., Bassiouni, A., Wormald, P. J., Vreugde, S., et al. (2016). The microbiome of otitis media with effusion. *Laryngoscope* 126, 2844–2851. doi: 10.1002/lary.26128

Charbonneau, M. R., Blanton, L. V., DiGiulio, D. B., Relman, D. A., Lebrilla, C. B., Mills, D. A., et al. (2016). Human developmental biology viewed from a microbial perspective. *Nature* 535, 48–55. doi: 10.1038/nature18845

Chesneau, G., Toress-Cortes, G., Briand, M., Darrasse, A., Preveaux, A., Marais, C., et al. (2020). Temporal dynamics of bacterial communities during seed development and maturation. *FEMS Microbiol. Ecol.* fiaa190.

Christian, N., Whitaker, B. K., and Clay, K. (2015). Microbiomes: unifying animal and plant systems through the lens of community ecology theory. *Front. Microbiol.* 6:869. doi: 10.3389/fmicb.2015.00869

Colling, G., and Matthies, D. (2004). The effects of plant population size on the interactions between the endangered plant Scorzonera humilis, a specialized herbivore, and a phytopathogenic fungus. *Oikos* 105, 71–78. doi: 10.1111/j.0030-1299.2004.12783.x

Correia, M., Heleno, R., Pascoal da Silva, L., Miguel Costa, J., and Rodriguez-Echeverria, S. (2019). First evidence for the joint dispersal of mycorrhizal fungi and plant disapores by birds. *New Phytol.* 222, 1054–1060. doi: 10.1111/nph.15571

Cracraft, J. (1983). *Species Concepts and Speciation Analysis. In Current Ornithology*. New York, NY: Plenum Press, 159–187.

Curtis, T. (2006). Microbial ecologists: it's time to 'go large'. *Nat. Rev. Microbiol.* 4:488. doi: 10.1038/nrmicro1455

Curtis, T. P., and Sloan, W. T. (2005). Exploring microbial diversity – A vast below. *Science* 309, 1331–1333. doi: 10.1126/science.1118176

Cushman, S. A., Evans, J. S., and McGarigal, K. (2010). "Landscape ecology: past, present, and future," in *Spatial Complexity, Informatics, and Wildlife Conservation*, eds S. A. Cushman and F. Huettmann (Tokyo: Springer).

Dehkharghani, A., Waisbord, N., Dunkel, J., and Guasto, J. S. (2019). Bacterial scattering in microfluidic crystal flows reveals giant active Taylor-Aris dispersion. *PNAS* 116, 11119–11124. doi: 10.1073/pnas.1819613116

Dickson, R. P., Erb-Downward, J. R., Freeman, C. M., McCloskey, L., Beck, J. M., Huffnagle, G. B., et al. (2015). Spatial variation in the healthy human lung microbiome and the adapted island model of lung biogeography. *Ann. Am. Thorac. Soc.* 12, 821–830. doi: 10.1513/annalsats.201501-029oc

Donaldson, G. P., Lee, S., and Mazmanian, S. K. (2016). Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* 134, 20–32. doi: 10.1038/nrmicro3552

Dong, Z.-X., Li, H.-Y., Chen, Y.-F., Wang, F., Deng, X.-Y., Lin, L.-B., et al. (2020). Colonization of the gut microbiota of honey bee (apis mellifera) workers at different developmental stages. *Microbiol. Res.* 231:126370. doi: 10.1016/j.micres.2019.126370

Duguma, D., Hall, M. W., Rugman-Jones, P., Stouthamer, R., Terenius, O., Neufeld, J. D., et al. (2015). Developmental succession of the microbiome of *Culex* mosquitoes. *BMC Microbiol.* 15:140. doi: 10.1186/s12866-015-0475-8

Dunning, J. B., Danielson, B. J., and Pulliam, H. R. (1992). Ecological processes that affect populations in complex landscapes. *Oikos* 65, 169–175. doi: 10.2307/3544901

Dzidic, M., Collado, M. C., Abrahamsson, T., Artacho, A., Stensson, M., Jenmalm, M. C., et al. (2018). Oral microbiome development during childhood: an ecological succession influenced by postnatal factors and associated with tooth decay. *ISME J.* 12, 2292–2306. doi: 10.1038/s41396-018-0204-z

Etterma, C. H., and Wardle, D. A. (2002). Spatial soil ecology. *Trends Eco. Evol.* 17, 177–183. doi: 10.1016/s0169-5347(02)02496-5

Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. *Annu. Rev. Ecol. Evol. Syst.* 34, 487–515.

Fahrig, L., Baudry, J., Brotons, L., Burel, F. G., Crist, T. O., Fuller, R. J., et al. (2011). Functional landscape heterogeneity and animal biodiversity in agricultural

landscapes. *Ecol. Lett.* 14, 101–112. doi: 10.1111/j.1461-0248.2010.01559.x

Fernstrom, A., and Goldblatt, M. (2013). Aerobiology and its role in the transmission of infectious diseases. *J. Pathol.* 2013:493960.

Ferrari, B., Bissett, A., Snape, I., van Dorst, J., Palmer, A. S., Ji, M., et al. (2016). Geological connectivity drives microbial community structure and connectivity in polar, terrestrial ecosystems. *Environ. Microbiol.* 18, 1834–1849. doi: 10.1111/1462-2920.13034

Fierer, N., and Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. U.S.A.* 103, 626–631. doi: 10.1073/pnas.0507535103

Fischer, J., and Lindenmayer, D. B. (2006). Landscape modification and habitat fragmentation. *Glob. Ecol. Biogeogr.* 16, 265–280. doi: 10.1111/j.1466-8238.2007.00287.x

Fletcher, R., and Fortin, M.-J. (2018). *Spatial Ecology and Conservation Modeling*. Berlin: Sringer.

Flores, G. E., Bates, S. T., Knights, D., Laubre, C. L., Stombaugh, J., Knight, R., et al. (2011). Microbial biogeography of public restroom surface. *PLoS One* 6:e28132. doi: 10.1371/journal.pone.0028132

Fournier, B., Mouquet, N., Leibold, M. A., and Gravel, D. (2017). An integrative framework of coexistence mechanisms in competitive metacommunities. *Ecography* 40, 630–641. doi: 10.1111/ecog.02137

Franklin, R., and Mills, A. (2007). *The Spatial Distribution of Microbes in the Environment*. Berlin: Springer.

Green, J., and Bohannan, B. J. M. (2006). Spatial scaling of microbial biodiversity. *Trends Ecol. Evol.* 21, 501–507. doi: 10.1016/j.tree.2006.06.012

Grilli, G., Urcelay, C., and Galetto, L. (2012). Forest fragment size and nutrient availability : complex responses of mycorrhizal fungi in native-exotic hosts. *Plant Ecol.* 213, 155–165. doi: 10.1007/s11258-011-9966-3

Groppe, K., Steinger, T., Schmid, B., Bar, B., and Boller, T. (2001). Effects of habitat fragmentation on choke disease (Epichloe bromicola) in the grass *Bromus erectus*. *J. Ecol.* 89, 247–255. doi: 10.1046/j.1365-2745.2001.00538.x

Hanski, I. (1994). A practical model of metapopulation dynamics. *J. Anim. Ecol.* 63, 151–162. doi: 10.2307/5591

Hanson, C. A., Fuhrman, J. A., Horner-Devine, C., and Martiny, J. B. H. (2012). Beyond biogeographic patterns : processes shaping the microbial landscape. *Nat. Rev. Microbiol.* 10, 497–506. doi: 10.1038/nrmicro2795

Hart, S. P., Turcotte, M. M., and Levine, J. M. (2019). Effects of rapid evolution on species coexistence. *Proc. Natl. Acad. Sci. U.S.A.* 113, 2112–2117. doi: 10.1073/pnas.1816298116

Hiltunen, T., Virta, M., and Laine, A.-L. (2017). Antibiotic resistance in the wild: an eco-evolutionary perspective. *Philos. Trans. R. Soc. B Biol. Sci.* 372:20160039. doi: 10.1098/rstb.2016.0039

Hol, F. J. H., and Dekker, C. (2014). Zooming in to see the bigger picture: microfluidic and nanofabrication tools to study bacteria. *Science* 346:1251821. doi: 10.1126/science.1251821

Hol, F. J. H., Rotem, O., Jurkevitch, E., Dekker, C., and Koster, D. A. (2016). Bacterial predator-prey dynamics in microscale patchy landscapes. *Proc. R. Soc. B* 283:20152154. doi: 10.1098/rspb.2015.2154

Holdenrieder, O., Pautasso, M., Weisberg, P. J., and Lonsdale, D. (2004). Tree diseases and landscape processes: the challenge of landscape pathology. *Trends Ecol. Evol.* 19, 446–452. doi: 10.1016/j.tree.2004.06.003

Jackson, H. B., and Fahrig, L. (2012). What size is a biologically relevant landscape ? *Landsc. Ecol.* 27, 929–941. doi: 10.1007/s10980-012-9757-9

Jules, E. S., Kauffman, M. J., Ritts, W. D., and Carroll, A. L. (2002). Spread of an invasive pathogen over a variable landscape: a nonnative root rot on port orford cedar. *Ecology* 83, 3167–3181. doi: 10.1890/0012-9658(2002)083[3167:soaipo]2.0.co;2

Karimi, B., Terrat, S., Dequiedt, S., Saby, N. P. A., Horrigue, W., Lelièvre, M., et al. (2018). Biogeography of soil bacteria and archaea across France. *Sci. Adv.* 4:eaat1808. doi: 10.1126/sciadv.aat1808

Keymer, J. E., Galajda, P., Muldoon, C., Park, S., and Austin, R. H. (2006). Bacterial metapopulations in nanofabricated landscapes. *Proc. NatL. Acad. Sci. U.S.A.* 103, 17290–17295. doi: 10.1073/pnas.0607971103

Koonin, E. V., Senkevich, T. G., and Dolja, V. V. (2009). The ancient virus world and evolution of cells. *Biol. Direct.* 1:29.

Kort, R., Caspers, M., van de Graaf, A., van Egmond, W., Keijser, B., and Roeselers, G. (2014). Shaping the oral microbiota through intimate kissing. *Microbiome* 2:41.

Laine, A.-L., and Hanski, I. (2006). Large-scale spatial dynamics of a specialist plant pathogen in a fragmented landscape. *J. Ecol.* 94, 217–226. doi: 10.1111/j.1365-2745.2005.01075.x

Langenheder, S., and Lindström, E. S. (2019). Factors influencing aquatic and terrestrial bacterial community assembly. *Environ. Microbiol. Rep.* 11, 306–315. doi: 10.1111/1758-2229.12731

Leibold, M. A., and Chase, J. M. (2018). *Metacommunity Ecology*, Vol. 59. Princeton University Press.

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. doi: 10.1111/j.1461-0248.2004.00608.x

Leibold, M. A., Rudolph, J., Blanchet, F. G., De Meester, L., Gravel, D., Hartig, F., et al. (2020). The internal structure of metacommunities. *bioRxiv* [Preprint]. doi: 10.1101/2020.07.04.187955

Linert, J., and Fischer, M. (2003). Habitat fragmentation affects the common wetland specialist Primula farinose in north-east Switzerland. *J. Ecol.* 91, 587–599. doi: 10.1046/j.1365-2745.2003.00793.x

Livingston, G., Fukumori, K., Provete, D. B., Kawachi, M., Takamura, N., and Leibold, M. A. (2017). Predators regulate prey species sorting and spatial distribution in microbial landscapes. *J. Anim. Ecol.* 86, 501–510. doi: 10.1111/1365-2656.12639

Livingston, G., Jiang, Y., Fox, J. W., and Leibold, M. A. (2013). The dynamics of community assembly under sudden mixing in experimental microcosms. *Ecology* 94, 2898–2906. doi: 10.1890/12-1993.1

Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Hubere, J. A., O'Connor, M., et al. (2018). Function and functional redundancy in microbial systems. *Nat. Ecol. Evol.* 2, 936–943. doi: 10.1038/s41559-018-0519-1

Luef, B., Aspetsberger, F., Hein, T., Huber, F., and Peduzzi, P. (2007). Impact of hydrology on free-living and particle-associated microorganisms in a river floodplain system. *Freshw. Biol.* 52, 1043–1057. doi: 10.1111/j.1365-2427.2007.01752.x

MacArthur, R. H., and Wilson, E. O. (1967). *The Theory of Island Biogeography*. Princeton, NJ: Princeton University Press.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., and Dunthorn, M. (2014). Swarm: robust and fast clustering method for amplicon-based studies. *PeerJ* 2:e593. doi: 10.7717/peerj.593

Mangan, S. A., Eom, A.-H., Adler, G. H., Yavitt, J. B., and Herre, E. A. (2004). Diversity of arbuscular mycorrhizal fungi across a fragmented forest in Panama: insular spore communitities differ from mainland communities. *Oecologia* 141, 687–700. doi: 10.1007/s00442-004-1684-2

Martínez-Olivas, M., Jiménez-Bueno, N. G., Hernandez-Garcia, J. A., Fusaro, C., Luna-Guido, M., Navarro-Noya, Y. E., et al. (2019). Bacterial and archaeal spatial distribution and its environmental drivers in an extremely haloalkaline soil at the landscape scale. *PeerJ* 7:e6127. doi: 10.7717/peerj.6127

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. doi: 10.1038/nrmicro1341

Mas, A., Jamshidi, S., Lagadeuc, Y., Eveillard, D., and Vandenkoornhuyse, P. (2016). Beyond the black queen hypothesis. *ISME J.* 10, 2085–2091. doi: 10.1038/ismej.2016.22

Miguet, P., Jackson, H. B., Jackson, N. D., Martin, A. E., and Fahrig, L. (2016). What determines the spatial extent of landscape effects on species ? *Landsc. Ecol.* 31, 1177–1194. doi: 10.1007/s10980-015-0314-1

Miki, T., and Yamamura, N. (2005). Intraguild predation reduces bacterial species richness and loosens the viral loop in aquatic systems: 'kill the killer of the winner' hypothesis. *Aquat. Microb. Ecol.* 40, 1–12. doi: 10.3354/ame040001

Miller, E. T., Svanbäck, R., and Bohannan, B. J. M. (2018). Microbiomes as metacommmunities: understanding host-associated microbes through metacommunity ecology. *Trends Ecol. Evol.* 33, 926–935. doi: 10.1016/j.tree.2018.09.002

Mitri, S., Xavier, J. B., and Foster, K. R. (2011). Social evolution in multispecies biofilms. *Proc. Natl Acad. Sci. U.S.A.* 108, 10839–10846. doi: 10.1073/pnas.1100292108

Mony, C., Brunellière, P., Vannier, N., Bittebière, A.-K., and Vandenkoornhuyse, P. (2020a). Effect of floristic composition and configuration on plant root mycobiota: a landscape transposition at a small scale. *New Phytol.* 225, 1777–1787. doi: 10.1111/nph.16262

Mony, C., Vannier, N., Brunellière, P., Biget, M., Coudouel, S., and Vandenkoornhuyse, P. (2020b). The influence of host-plant connectivity on fungal assemblages in the root microbiota of Brachypodium pinnatum. *Ecology* 101:e02976.

Moreira, D., and López-García, P. (2009). Ten reasons to exclude viruses from the tree of life. *Nat. Rev. Microbiol.* 7, 306–311. doi: 10.1038/nrmicro2108

Morris, J. J., Lenski, R. E., and Zinser, E. R. (2012). The black queen hypothesis: evolution of dependencies through adaptive gene loss. *mBio* 3:e00036-12.

Mundt, C. C., Sackett, K. E., and Wallace, L. D. (2011). Landscape heterogeneity and disease spread; experimental approaches with a plant pathogen. *Ecol. Appl.* 21, 321–328. doi: 10.1890/10-1004.1

Murcia, C. (1995). Edge effects in fragmented forests: implications for conservation. *Trends Ecol. Evol.* 10, 58–62. doi: 10.1016/s0169-5347(00)88977-6

Muscarella, M. E., Boot, C. M., Broeckling, C. D., and Lennon, J. T. (2019). Resource heterogeneity structures aquatic bacterial communities. *ISME J.* 13, 2183–2195. doi: 10.1038/s41396-019-0427-7

Nadell, C. D., Drescher, K., and Foster, K. R. (2016). Spatial structure, cooperation, and competition in biofilms. *Nat. Rev. Microbiol.* 14, 589–600. doi: 10.1038/nrmicro.2016.84

Nesme, J., and Simonet, P. (2015). The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. *Environ. Microbiol.* 17, 913–930. doi: 10.1038/nrmicro2644

No authors listed (2011). Microbiology by numbers. *Nat. Rev. Microbiol.* 9:628.

Nunan, N., Wu, K. J., Young, I. M., Crawford, J. W., and Ritz, K. (2003). Spatial distribution of bacterial communities and their relationships with the micro-architecture of soil. *FEMS Microbiol. Ecol.* 44, 203–215. doi: 10.1016/s0168-6496(03)00027-8

O'Neill, R. V., DeAngelis, D. L., Waide, J. B., and Allen, T. F. H. (1986). *A Hierarchical Concept of Ecosystems*. Princeton, NJ: Princeton University Press.

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. doi: 10.1890/09-2237.1

Peay, K. G., Schubert, M. G., Guyen, N. H., and Bruns, T. D. (2012). Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. *Mol. Ecol.* 21, 4122–4136. doi: 10.1111/j.1365-294x.2012.05666.x

Penttila, R., Lindgren, M., Miettinen, O., Rita, H., and Hanski, I. (2006). Consequences of forest fragmentation for polyporous fungi at two spatial scales. *Oikos* 114, 225–240. doi: 10.1111/j.2006.0030-1299.14349.x

Perkins, T. E., and Matlack, G. R. (2002). Human-generated pattern in commercial forests of southern Mississippi and consequences for the spread of pests and pathogens. *For. Ecol. Manag.* 157, 143–154. doi: 10.1016/s0378-1127(00)00645-9

Philippot, L., Spor, A., Hénault, C., Bru, D., Bizouard, F., Jones, C. M., et al. (2013). Loss in microbial diversity affects nitrogen cycling in soil. *ISME J.* 7, 1609–1619. doi: 10.1038/ismej.2013.34

Pringle, C. M., Naiman, R. J., Bretschko, G., Karr, J. R., Oswood, N. W., Webster, J. R., et al. (1988). Patch dynamics in lotic systems: the stream as a mosaic. *J. N. Am. Bentho. Soc.* 7, 503–524. doi: 10.2307/1467303

Proctor, D. M., Fukuyama, J. A., Loomer, P. M., Armitage, G. C., Lee, S. A., Davis, N. M., et al. (2018). A spatial gradient of bacterial diversity in the human oral cavity shaped by salivary flow. *Nat. Commun.* 9:681.

Proctor, D. M., and Relman, D. A. (2017). The landscape ecology and microbiota of the human nose, mouth and throat. *Cell Host Microbe* 21, 421–432. doi: 10.1016/j.chom.2017.03.011

Rosshart, S. P., Vassallo, B. G., Angeletti, D., Hutchinson, D. S., Morgan, A. P., Takeda, K., et al. (2017). Wild mouse gut microbota promotes host fitness and improves disease resistance. *Cell* 171, 1015–1028. doi: 10.1016/j.cell.2017.09.016

Salek, M. M., Carrara, F., Fernandez, V., Guasto, J. S., and Stocker, R. (2019). Bacterial chemotaxis in a microfluidic T-maze reveals strong phenotypic heterogeneity in chemotactic sensitivity. *Nat. Commun.* 10:1877.

Saunders, D. A., Hobbs, R. J., and Margules, C. R. (1991). Biological consequences of ecosystem fragmentation : a review. *Conserv. Biol.* 5, 18–32. doi: 10.1111/j.1523-1739.1991.tb00384.x

Schimel, J. P., and Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Front. Microbiol.* 3:348. doi: 10.3389/fmicb.2012.00348

Shade, A., Jacques, M.-A., and Barret, M. (2017). Ecological patterns of seed microbiome diversity, transmission, and assembly. *Curr. Opin. Microbiol.* 37, 15–22. doi: 10.1016/j.mib.2017.03.010

Soledad Faggioli, V., Cabello, M. N., Grilli, G., Vasar, M., Covacevich, F., and Opik, M. (2019). Root colonization and soil borne communities of arbuscular mycorrhizal fungi differ among soybean fields with contrasting historical land use. *Agric. Ecosyst. Environ.* 269, 174–182. doi: 10.1016/j.agee.2018.10.002

Son, K., Menolascina, F., and Stocker, R. (2016). Speed-dependent chemotactic precision in marine bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 113, 8624–8629. doi: 10.1073/pnas.1602307113

Suzan, G., Esponda, F., Carrasco-Hernandez, R., and Alonso Aguirre, A. (2012). "Habitat fragmentation and infectious disease ecology," in *New Directions in Conservation Medicine : Applied Cases of Ecological Health*, eds A. Alonso Aguirre, R. Ostfeld, and P. Daszak (New York, NY: Owford University Press).

Taylor, P. D., Fahrig, L., Henein, K., and Merriam, G. (1993). Connectivity is a vital element of landscape structure. *Oikos* 68, 571–573. doi: 10.2307/3544927

Telford, R. J., Vandvik, V., and Birks, H. J. B. (2006). Dispersal limitations matter for microbial morphospecies. *Science* 312:1015. doi: 10.1126/science.1125669

Thrall, P. H., Godfree, R., and Burdon, J. J. (2003). Influence of spatial structure on pathogen colonization and extinction: a test using an experimental metapopulation. *Plant Pathol.* 52, 350–361. doi: 10.1046/j.1365-3059.2003.00863.x

Turner, M. G. (1989). Landscape ecology: the effect of pattern on process. *Annu. Rev. Ecol. Syst.* 20, 171–197. doi: 10.1146/annurev.es.20.110189.001131

Turner, M. G., Gardner, R. H., and O'Neill, R. V. (2001). *Landscape Ecology in Theory and Practice*. New York, NY: Springer-Verlag.

Urban, M. C., Leibold, M. A., Amarasekare, P., De Meester, L., Gomulkiewicz, R., Hochberg, M. E., et al. (2008). The evolutionary ecology of metacommunities. *Trends Ecol. Evol.* 23, 311–317.

Uroy, L., Ernoult, A., and Mony, C. (2019). Effect of landscape connectivity on plant communities: a review of response patterns. *Landsc. Ecol.* 34, 203–225. doi: 10.1007/s10980-019-00771-5

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A., and Dufresne, A. (2015). The importance of the microbiome of the plant holobiont. *New Phytol.* 206, 1196–1206. doi: 10.1111/nph.13312

Vannette, R., Leopold, D. R., and Fukami, T. (2016). Forest area and connectivity influence root-associated fungal communities in a fragmented landscape. *Ecology* 7, 2374–2383. doi: 10.1002/ecy.1472

Vannier, N., Mony, C., Bittebiere, A.-K., Michon-Coudouel, S., Biget, M., and Vandenkoornhuyse, P. (2018). A microorganisms' journey between plant generations. *Microbiome* 6:79.

Vass, M., and Langenheder, S. (2017). The legacy of the past: effects of historical processes on microbial metacommunitites. *Aquat. Microb. Ecol.* 79, 13–19. doi: 10.3354/ame01816

Vellend, M. (2010). Conceptual synthesis in community ecology. *Q. Rev. Biol.* 85, 183–206. doi: 10.1086/652373

Vellend, M. (2016). *The Theory of Ecological Communities*. Princeton, NJ: Princeton university Press.

Verreydt, D., De Meester, L., Decaestecker, E., Villena, M. J., Van der Gucht, K., Vannormelingen, P., et al. (2012). Dispersal-mediated trophic interactions can generate apparent patterns of dispersal limitation in aquatic metacommunities. *Ecol. Lett.* 15, 218–226. doi: 10.1111/j.1461-0248.2011.01728.x

von Eiff, C., Becker, K., Machka, K., Stammer, H., and Peters, G. (2001). Nasal carriage as a source of Staphylococcus aureus bacteremia. *N. Engl. J. Med.* 344, 11–16.

Vos, M., Wolf, A. B., Jennings, S. J., and Kowalchuk, G. A. (2013). Micro-scale determinants of bacterial diversity in soil. *FEMS Microbiol. Rev.* 37, 936–954. doi: 10.1111/1574-6976.12023

Vuong, H. E., Yano, J. M., Fung, T. C., and Hsiao, E. Y. (2017). The Microbiome and Host Behavior. *Annu. Rev. Neurosci.* 40, 21–49.

Weeman, E. J., van der Geest, H. G., van der Meulen, M. D., Manders, E. M. M., van de Koppel, J., Herman, P. M. J., et al. (2011). Ciliates as engineers of phototrophic biofilms. *Freshw. Biol.* 56, 1358–1369. doi: 10.1111/j.1365-2427.2011.02574.x

West, S. A., Diggle, S. P., Buckling, A., Gardner, A., and Griffins, A. S. (2007). The social lives of microbes. *Annu. Rev. Ecol. Evol. Syst.* 38, 53–77. doi: 10.1146/annurev.ecolsys.38.091206.095740

Wiens, J. A. (1995). Habitat fragmentation: island versus landscape perspectives on bird conservation. *Ibis* 137, 97–104.

Wiens, J. A., Stenseth, N. C., Van Horne, B., and Anker Ims, R. (1993). Ecological mechanisms and landscape ecology. *Oikos* 66, 369–380. doi: 10.2307/3544931

Wu, J., and Loucks, O. L. (1995). From balance of nature to hierarchical patch dynamics: a paradigm shift in ecology. *Q. Rev. Biol.* 70, 439–466. doi: 10.1086/419172

Yawata, Y., Cordero, O. X., Menolascina, F., Hehemann, J.-H., Polz, M. F., and Stocker, R. (2014). Competition-dispersal tradeoff ecological differentiates recently speciated marine bacterioplankton populations. *Proc. Natl. Acad. Sci. U.S.A.* 111, 5622–5627. doi: 10.1073/pnas.1318943111

Yuen, J., and Mila, A. (2015). Landscape-scale disease risk quantification and prediction. *Annu. Rev. Phytopathol.* 53, 471–484. doi: 10.1146/annurev-phyto-080614-120406

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 09 December 2020
doi: 10.3389/fevo.2020.517434

Evolutionary Rescue Is Mediated by the History of Selection and Dispersal in Diversifying Metacommunities

Louise M. J. O'Connor[1,2]*, *Vincent Fugère*[1,3] *and Andrew Gonzalez*[1,4]*

[1] Department of Biology, McGill University, Montreal, QC, Canada, [2] Université Grenoble Alpes, University Savoie Mont Blanc, CNRS, LECA, Laboratoire d'Ecologie Alpine, Grenoble, France, [3] Département des Sciences de l'Environnement, Université du Québec à Trois-Rivières, Trois-Rivières, QC, Canada, [4] Québec Centre for Biodiversity Science, Montreal, QC, Canada

Rapid evolution can sometimes prevent population extirpation in stressful environments, but the conditions leading to "evolutionary rescue" in metacommunities are unclear. Here we studied the eco-evolutionary response of microbial metacommunities adapting to selection by the antibiotic streptomycin. Our experiment tested how the history of antibiotic selection and contrasting modes of dispersal influenced diversification and subsequent evolutionary rescue in microbial metacommunities undergoing adaptive radiation. We first tracked the change in diversity and density of *Pseudomonas fluorescens* morphotypes selected on a gradient of antibiotic stress. We then examined the recovery of these metacommunities following abrupt application of a high concentration of streptomycin lethal to the ancestral organisms. We show that dispersal increases diversity within the stressed metacommunities, that exposure to stress alters diversification dynamics, and that community composition, dispersal, and past exposure to stress mediate the speed at which evolutionary rescue occurs, but not the final outcome of recovery in abundance and diversity. These findings extend recent experiments on evolutionary rescue to the case of metacommunities undergoing adaptive diversification, and should motivate new theory on this question. Our findings are also relevant to evolutionary conservation biology and research on antimicrobial resistance.

Keywords: evolutionary rescue, metacommunity, spatially explicit dispersal, biodiversity, eco-evolutionary dynamics, stress gradient, antibiotic resistance, *Pseudomonas fluorescens*

OPEN ACCESS

Edited by:
Philippe Vandenkoornhuyse, University of Rennes 1, France

Reviewed by:
David K. Skelly, Yale University, United States
Cendrine Mony, University of Rennes 1, France

***Correspondence:**
Louise M. J. O'Connor
louise.mj.oconnor@gmail.com
Andrew Gonzalez
andrew.gonzalez@mcgill.ca

Specialty section:
This article was submitted to Population and Evolutionary Dynamics, a section of the journal Frontiers in Ecology and Evolution

Received: *04 December 2019*
Accepted: *13 November 2020*
Published: *09 December 2020*

Citation:
O'Connor LMJ, Fugère V and Gonzalez A (2020) Evolutionary Rescue Is Mediated by the History of Selection and Dispersal in Diversifying Metacommunities. Front. Ecol. Evol. 8:517434. doi: 10.3389/fevo.2020.517434

INTRODUCTION

Biodiversity is organized across multiple scales, from populations of individual species to communities of multiple species, that evolve and move across a range of spatial scales, from local ecosystems to entire continents. Situated within this hierarchy are metacommunities, defined as a set of local communities that are connected by dispersal (Leibold et al., 2004). Metacommunities face increasing pressure from human-induced environmental degradation, often leading to population decline, local extinctions, and biodiversity loss (Pereira et al., 2010; Haddad et al., 2015). Species occupying metacommunities can respond to these pressures by moving to other communities, by adapting *in situ*, or they can undergo extinction. Under some circumstances, populations can rapidly evolve resistance to stressors and persist in severely degraded environments (Bürger and Lynch, 1995; Hufbauer et al., 2015; Reid et al., 2016). This phenomenon is described by

the theory of evolutionary rescue (Gomulkiewicz and Holt, 1995; Bell and Gonzalez, 2009; Gonzalez et al., 2013; Bell, 2017), which explains how populations can recover from extreme environmental stress through rapid adaptation.

Evolutionary rescue occurs when stress-resistant individuals are selected within a perturbed population, allowing abundance to recover and a viable population to be maintained in conditions that would otherwise cause population extirpation in the absence of evolution. Resistant types responsible for evolutionary rescue may already be present in a population before rescue is needed (due to past *in situ* evolution or to the immigration of resistant types from connected habitats), or evolve *de novo* after the onset of extreme stress. Theory and laboratory experiments have described key drivers of evolutionary rescue for single-species populations. First, evolutionary rescue is more likely if populations were previously exposed to lower levels of the same stressor (leading to an increase in the abundance of stress-resistant genotypes through selection). Second, large population sizes favor evolutionary rescue by reducing the risk of stochastic extinction (Gomulkiewicz and Holt, 1995; Bell and Gonzalez, 2009; Gienapp et al., 2013). Third, if populations are spatially connected to others, the dispersal of stress-resistant genotypes among local populations can enable rescue even in environments that were not previously contaminated by the stressor (Bell and Gonzalez, 2011; Carlson et al., 2014). Understanding the conditions that favor evolutionary rescue has clear implications for both biodiversity conservation and for the management of pests and pathogens evolving resistance to biocides (Alexander et al., 2014).

A few recent studies have expanded the scope of evolutionary rescue from populations to diverse assemblages of species and examined evolutionary rescue in entire communities confronted with severe stress (Fussmann and Gonzalez, 2013; Low-Décarie et al., 2015; Bell et al., 2019; Fugère et al., 2020). Community rescue occurs when the populations of multiple species recover rapidly following exposure to levels of stress that were lethal to the community in its ancestral form, allowing a community to recover both abundance and diversity in severely degraded environments (Low-Décarie et al., 2015). Some key factors promoting evolutionary rescue of populations were also found to favor community rescue (Low-Décarie et al., 2015; Bell et al., 2019; Fugère et al., 2020). First, a history of stress exposure increases the relative frequency of stress-resistant individuals in communities, which provides a correlated advantage at a higher dose of stress and thus facilitates rescue. Second, just as intraspecific genetic diversity can promote the evolutionary rescue of individual populations (Carlson et al., 2014), a greater diversity of species can also favor rescue, as communities holding a more diverse set of species are more likely to contain resistant types (Low-Décarie et al., 2015). Finally, when local communities are spatially connected, thus forming a metacommunity, dispersal was found to favor evolutionary rescue in local communities by moving resistant genotypes within a heterogeneous metacommunity (Low-Décarie et al., 2015). Nonetheless, only a few recent studies have tested the conditions that promote evolutionary rescue in communities of multiple species, and much remains to be uncovered.

Here, we expand on previous work testing evolutionary rescue in communities, and ask whether a history of stress, greater biodiversity, and the presence of dispersal favor evolutionary rescue in metacommunities across a heterogeneous landscape. In contrast to previous community rescue experiments that have used microbial assemblages with existing intra and interspecific variation, we examined evolutionary rescue in an experimental system in which all diversity and stress resistance is generated *de novo* through rapid evolution. We also manipulated not only the presence of dispersal prior to exposure to extreme levels of stress, but also its spatial structure, contrasting global vs. local dispersal in metacommunities. These two modes of dispersal were shown to have distinct effects on the likelihood of evolutionary rescue in metapopulations of the yeast, *Saccharomyces cerevisiae* (Bell and Gonzalez, 2011), but how the spatial structure of dispersal affects evolutionary rescue in metacommunities remains unknown. Global dispersal connects all communities to each other, mixing individuals from the whole metacommunity. This mode of dispersal brings in diversity upon which natural selection will act and potentially enable evolutionary rescue. At the same time, the resulting migrants could be maladapted to their new habitat, thus hampering adaptation and subsequent evolutionary rescue by migration load (Bell and Gonzalez, 2011; Schiffers et al., 2013; Carlson et al., 2014; Hao et al., 2015). On the other hand, local dispersal [also known as "stepping-stone" dispersal (Bell et al., 2019)] is a mode of dispersal where communities are only connected by directional migration up a gradient of environmental stress. Local dispersal is expected to favor evolutionary rescue, because it will more likely bring better-adapted individuals as they move up the stress gradient (Bell and Gonzalez, 2011). Over longer time-scales in communities undergoing adaptive diversification and speciation, dispersal may also favor the generation of diversity and productivity (Venail et al., 2008), which would also promote the likelihood of rescue following environmental degradation.

To address how stress and dispersal interact to modulate the adaptation and diversification of metacommunities across a heterogeneous landscape, and subsequent evolutionary rescue, we used the plant symbiont *Pseudomonas fluorescens SBW25* as a model system exposed to the antibiotic streptomycin. We build on previous studies with this organism (Rainey and Travisano, 1998; Kassen et al., 2000; Massin and Gonzalez, 2006; Perron et al., 2006; Venail et al., 2008, 2010; Ramsayer et al., 2013). This bacterium shows rapid and repeatable *in vitro* diversification when grown in a heterogeneous environment. As they grow, individuals of this aerobic bacterium compete for oxygen, thus creating a vertical gradient of oxygen in the liquid medium. The resulting environment favors mutants able to colonize the air-liquid interface (Rainey and Travisano, 1998) via the formation of a biofilm composed of cellulose-based polymer (McDonald et al., 2009; Lind et al., 2018). Diversification can be recorded at the phenotypic level by growing these bacteria on solid media where they display striking differences in colony morphology that relates to their niche preference. These morphotypes are easy to detect and are heritable (Rainey and Travisano, 1998). In our experiment, the ancestral cells were derived from a single isomorphic

colony ("smooth" opaque morph) which then grew asexually. This means that mutations are only transferred to the next generation by single cell division. Consequently, adaptive mutations that arise in one morphotype are independent from the evolutionary pathway of other morphotypes. Quantifying the emerging morphological diversity allows us to track this evolution occurring *in vitro*. The species concept does not readily apply to bacteria (Rosselló-Mora and Amann, 2001; Riley and Lizotte-Waniewski, 2009), but the subsequent rapid diversification, niche specialization, and growth by asexual reproduction in *P. fluorescens* allows us to consider different morphotypes analogous to different species, and to consider our diversified bacterial assemblages as a model for communities of multiple species. Previous studies have documented the capacity of *P. fluorescens* to rapidly evolve resistance to the antibiotic streptomycin (Ramsayer et al., 2013), a versatile and widely used antibiotic (World Health Organization, 2015). This work, combined with the tendency for *P. fluorescens* to adaptively radiate (MacLean and Bell, 2002; Barrett and Bell, 2006), makes it an excellent system to study the factors promoting adaptation to stressors and evolutionary rescue in the context of rapidly diversifying communities.

Following previous experiments (Bell and Gonzalez, 2011; Low-Décarie et al., 2015; Bell et al., 2019; Fugère et al., 2020), we conducted an experiment which proceeded in two phases. In the first phase, communities evolved and diversified across a gradient of streptomycin. We created replicated four-patch metacommunities with one of three modes of dispersal: local dispersal, global dispersal, and a control with no dispersal. We recorded the dynamics of adaptation by quantifying the growth and morphological diversification of each community across the gradient of stress. In the second phase, we transferred each community regardless of its history of stress to a severe dose of streptomycin which was established to be lethal to the majority of ancestral organisms, following the method employed by previous community rescue experiments (Low-Décarie et al., 2015). In this second phase, dispersal was ceased such that any recovery of abundance and diversity could be attributed to local eco-evolutionary processes – and not demographic rescue due to dispersal. We quantified the trajectory and outcome of evolutionary rescue in Phase 2 of the experiment and linked these responses to three potential drivers of rescue manipulated in Phase 1: the history of exposure to sublethal doses of streptomycin, the mode of dispersal within the metacommunity, and the morphotype diversity of the community (i.e., the outcome of diversification occurring during Phase 1). Based on previous experiments (Bell and Gonzalez, 2011; Low-Décarie et al., 2015; Fugère et al., 2020) we expected that: (1) a history of streptomycin exposure in Phase 1 would facilitate evolutionary rescue in Phase 2; (2) the presence of dispersal in heterogeneous metacommunities would increase local morphotype diversity and would spread resistant genotypes during Phase 1, both of which would facilitate adaptation to, and rescue from, severe antibiotic stress–especially in communities naive to the stressor; and (3) that local dispersal would have a greater influence on the likelihood of evolutionary rescue than global dispersal.

MATERIALS AND METHODS

Bacterial Cultures

We used the ancestral strain of *Pseudomonas fluorescens SBW25* (Rainey and Bailey, 1996) cultured in basic growth medium was King's B (KB) medium (20.00 g/L Proteose Peptone (Difco no.3), 15 mL/L glycerol, 1.50 g/L K_2HPO_4, 1.50 g/L $MgSO_4$, distilled water). Populations in stressful treatments were supplemented with streptomycin (MilliporeSigma: Montreal, Canada). We initiated bacterial cultures from a single isolate clone of *P. fluorescens SBW25* in a 125-mL glass vial supplied with 50 mL of King's B medium, grown for 24 h at 28°C and shaken at 150 rpm. One percentage of this culture was transferred to 96-well plates supplied with 200 μL KB and grown for a further 24 h at 28°C, to initiate separate experimental populations. These were not shaken to allow diversification into morphologically diverse communities of *P. fluorescens* morphotypes over the course of the experiment (Rainey and Travisano, 1998). We thus refer to the "ancestral cells" at the onset of the experiment, and then to "communities" once microwells contained a diverse assemblage of morphotypes.

Abundance was measured spectrophotometrically as the optical density at 590 nm (OD_{590}), using a microplate reader (BioTek: Winooski, USA). Correspondence between absorbance readings and cell density was verified by growing bacterial cultures with known OD_{590} values on at least 3 replicate KB-Agar plates and by counting the number of colony-forming units. The relationship between cell density and OD_{590} ($R^2 = 0.6$) was: y (cells/mL) $= -3 \times 10^8 + 5 \times 10^9 \times OD_{590}$ (**Supplementary Figure 1**). Population size of the ancestral population was recorded after 24 h of growth in benign media, where it reached an abundance of $\sim 12 \times 10^9$ cells/mL (cell density recorded on agar, after 10^6-fold dilution). This population served to initiate the experimental metapopulations with a standardized initial abundance of 10^5 cells/mL in 96-well plates supplied with 200 μL of KB medium (see below). Then, throughout the experiment, we tracked community abundance by measuring the OD_{590} of all plates every 24 h.

To determine the susceptibility of the ancestral bacteria to streptomycin, we inoculated the ancestral bacteria of *P. fluorescens SBW25* in densities of 10^5 cells/mL in wells with 200 μL of KB medium supplemented with 10 different concentrations of streptomycin, ranging from 0 to 500 μg/mL. Eight replicate populations were treated with one of 10 streptomycin concentrations for 24 h (incubated at 28°C), during which OD_{590} was read every 30 min (**Supplementary Figure 2**). This served to identify the dose of streptomycin that was lethal to the majority of ancestral cells, the precondition required for evolutionary rescue. Note that, just as in previous experiments that tested population and community rescue, this dose is not required to be lethal to all individuals (Bell and Gonzalez, 2009; Low-Décarie et al., 2015).

Experimental Design

We randomly assigned metacommunities to streptomycin or control treatments crossed with three modes of dispersal (global, local, and none). Each metacommunity consisted of

four communities. Streptomycin-treated metacommunities were composed of four communities exposed to concentrations of 0, 100, 200, and 400 μg/mL, replicated 4 times (**Figure 1A**). These concentrations partially inhibited growth and represented a selection pressure on *P. fluorescens* during the first part of the experiment, which is expected to generate a correlated genetic response conferring some degree of tolerance to severe stress *before* it was actually experienced in Phase 2 of the experiment. Control metacommunities had the same dispersal treatments but were unexposed to streptomycin. This factorial design resulted in 96 local communities arrayed into 24 metacommunities distributed evenly on 4 separate 96-well plates.

During Phase 1 of the experiment, 1% of each culture was transferred to a new plate with fresh medium every 24 h to maintain growth. Dispersal treatments within metacommunities occurred simultaneously with the transfers. In the two dispersal treatments, 2 μL of grown culture was moved from each well to a dispersal pool, to match the rate of 1% dispersal used in previous studies with this bacterial strain (Venail et al., 2008). In the local dispersal treatments, this pool contained a contribution from the well with the next lower streptomycin concentration on the old plate: 2 μL of this dispersal pool was transferred to the next higher level of streptomycin, moving migrants up the stress gradient in the case of stressed communities. In the global dispersal treatments, the pool contained equal contributions from all wells of the metacommunity, and 2 μL of this was distributed to all wells of the metacommunity. In the no dispersal treatment, each well was inoculated exclusively with 1% from the corresponding well on the old plate, so that no cross-well transfer occurred.

Phase 2 of the experiment started 24 h after the seventh transfer. We diluted the grown communities to 10^5 cells/mL in KB (matching densities of 10^5 cells/mL for which we had assayed the tolerance of *P. fluorescens SBW25* to streptomycin) supplemented with 500 μg/mL streptomycin, a concentration that was lethal to the great majority of ancestral cells (**Supplementary Figure 2**). Bacteria were incubated at 28°C for 4 days, with no transfer or dispersal events. After the transfer, the abundance of each community was recorded by absorbance readings (OD_{590}) after 24 h (day 8), 30 h, 48 h (day 9), 72 h (day 10), and 120 h (day 12). Morphological diversity was scored after plating cells on KB-Agar on two occasions: on day 8 (24 h after the beginning of Phase 2) and on day 12 (at the end of Phase 2).

Measuring Morphological Diversity

Counts of morphotypes were scored every day during Phase 1 and on two instances during Phase 2, by plating on KB-Agar after dilution in KB. Each community was sampled and grown on two replicate Petri dishes. The morphotypes of all colonies were scored visually after 3 days of growth at 28°C; this corresponded to 50–500 colonies per replicate community. The two values obtained from replicate Petri dishes were then averaged to give the composition of each community. In some cases, when colonies failed to grow or when colonies grew very quickly and fused into a continuous biofilm, morphotype composition could not be estimated reliably. Thus, some replicates had to be discarded in analyses of composition and diversity (see below).

In keeping with previous work (Rainey and Travisano, 1998), we identified three morphs: smooth morph (the planktonic, ancestral morph), wrinkly spreader (which colonizes the air-liquid interface by forming a biofilm within 1 to 2 days) and fuzzy spreader (which colonizes the bottom of the wells after 4 days). We further divided the smooth morphotype into five subclasses – all had a smooth appearance but differed in opacity and pattern: "smooth morph translucent," "smooth morph opaque" (**Figure 1B**); "shiny smooth morph;" "eclipse smooth morph;" and "radial smooth morph." We also divided the wrinkly spreader morphotype into three subclasses – all contained "wrinkles" (distinctive asperities on the surface of the colony), but differed in the extent to which these wrinkles covered them: "wrinkly spreader 1" colonies were completely covered in wrinkles, "wrinkly spreader 2" colonies had a wrinkly center and smooth edge, and "wrinkly spreader 3" colonies had a smooth center and a wrinkly edge. This diversity observed within the wrinkly spreader morph is consistent with previous work with *P. fluorescens* (Hodgson et al., 2002; Massin and Gonzalez, 2006; Bantinaki et al., 2007). Finally, fuzzy spreader colonies displayed a distinctive blurry edge. Diversity counts thus included a total pool of nine morphological types (**Figure 1B**). Diversity in a local community was computed as the exponent of the Shannon index: $H = -\sum_{i=1}^{S} p_i \log(p_i)$, where S is the number of morphotypes and p_i is the relative abundance of morphotype i in the community. The exponent of Shannon is known as the effective number of species (Jost, 2006) – or in this case the effective number of morphotypes (henceforth diversity).

Statistical Analyses

All statistical analyses were conducted in R version 3.4.3 (R Core Team, 2017). We used linear models (ANOVAs) to test the effect of dispersal mode (three levels), streptomycin concentration (four levels, in the case of exposed metacommunities only), and their two-way interaction on the abundance (OD_{590}) and diversity of communities at key time points of the experiment: at the end of Phase 1 (day 7), the beginning of Phase 2 (day 8) and the end of Phase 2 (day 12). We evaluated the response of control metacommunities separately from exposed metacommunities. We also included experimental replicates (the 4 micro-well plates) as a blocking factor in all models. The number of replicates for each day of the experiment is referenced in the Supplementary Material (**Supplementary Table 1** for abundances and **Supplementary Table 2** for morphological diversity replicates). When main effects were statistically-significant, we conducted *post hoc* pairwise comparisons of factor levels using Tukey's "Honest Significant Difference" method (function "TukeyHSD" in R). Response variables were log-transformed when it improved the normality and homogeneity of model residuals.

To visualize differences in morphotype composition among treatments, we performed non-metric multidimensional scaling (NMDS) analyses separately at the end of Phase 1 and Phase 2, combining control and exposed metacommunities (R-package "vegan" version 2.5-6, function "metaMDS"). These NMDS ordinations and all other multivariate analyses

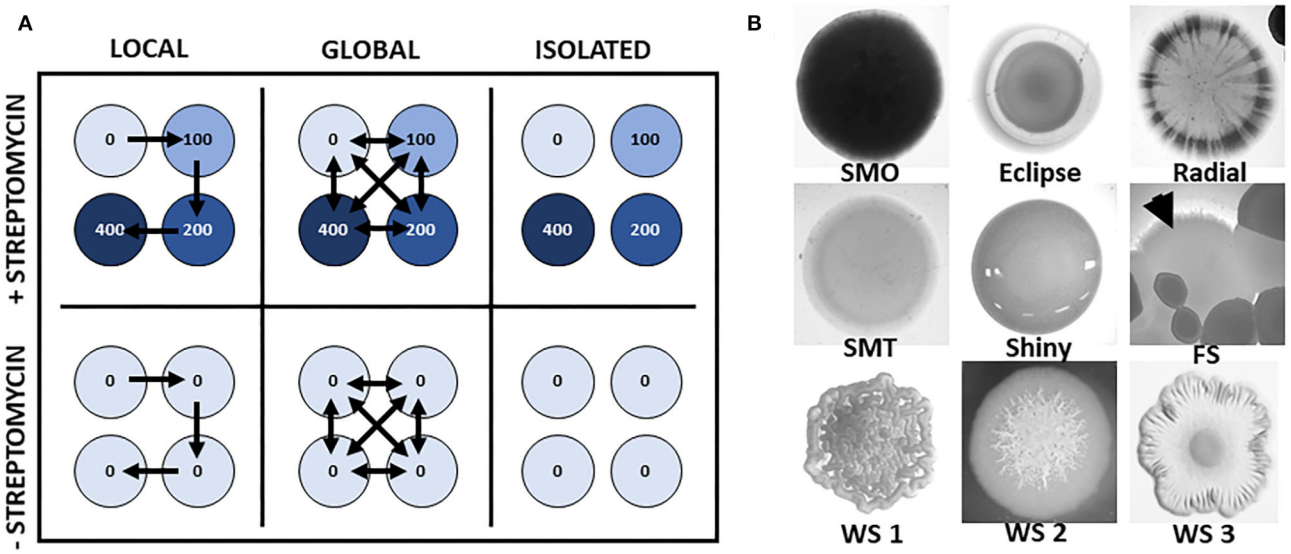


FIGURE 1 | Experimental design and morphotypes. **(A)** Experimental design for Phase 1, where each circle represents a single microwell, blue shades and numbers represent streptomycin concentrations (in μg/mL), and arrows represent the mode and direction of dispersal between communities. This design was replicated 4 times on separate multi-well plates. **(B)** Photographs of typical bacterial colonies grown on agar. We identified nine morphotypes that emerged during diversification over the course of the experiment: smooth morph opaque (SMO), Eclipse smooth morph, Radial smooth morph, smooth morph translucent (SMT), Shiny smooth morph, fuzzy spreader (FS), wrinkly spreader (WS) submorphs 1, 2, and 3. Colonies were photographed under a 10-fold magnifying lens (Leica), illuminated from below (except for WS2). The size of colonies at this stage averaged 5 mm in diameter.

used the Bray-Curtis dissimilarity index and proportional (relative abundance) data when calculating distance matrices. We tested whether morphotype composition varied among treatments using permutational multivariate analysis of variance (pMANOVA) implemented with the function "Adonis" in vegan. Separate pMANOVA models were fitted for control and streptomycin-treated metacommunities, using as a grouping factor "dispersal" (for both exposed and unexposed metacommunities) or "streptomycin concentration" (for exposed metacommunities only). We also conducted two analyses to reveal treatment effects on beta diversity, the variance in morphotype composition among local communities. We first used permutation-based tests of multivariate homogeneity of groups dispersions (the "PERMDISP" procedure implemented in function "betadisper" in vegan) to compare beta diversity at the "treatment" scale (e.g., variation in composition among all communities grown in concentrations of 100 vs. 200 μg/mL of streptomycin). These tests used "plate" and "dispersal" as grouping factors (for control metacommunities), or "plate," "dispersal," "streptomycin concentration," and their two-way interaction as factors (for exposed metacommunities). We then computed a metacommunity-scale measure of beta diversity, calculating, for each metacommunity of four microwells, the mean multivariate distance of the four local communities to their group centroid. Metacommunity-scale beta diversity was analyzed with ANOVA using "dispersal" (local vs. global vs. isolated), "metacommunity type" (control vs. streptomycin-treated), their two-way interaction, and "plate" as factors. This analysis only included the subset of metacommunities in which morphotype composition could be reliably estimated for all four local communities.

Finally, to test the hypothesis that treatment effects on community composition and alpha diversity affected the trajectory and outcome of evolutionary rescue, we used independent linear regressions to link alpha diversity and community composition on day 7 (end of Phase 1) with OD_{590} on day 8 and day 12. OD_{590} on day 8 indicates the initial potential of communities to grow at a dose of stress lethal to the ancestral cells, while OD_{590} on day 12 indicates final community abundance at this lethal dose of stress, i.e., the outcome of evolutionary rescue of multiple morphs. The measure of community composition used in this analysis were scores on the first axis of the NMDS ordination described above.

RESULTS

Ancestral Organisms

The ancestral bacterial culture was isomorphic and composed of Smooth Morph Opaque (SMO) exclusively. In these bacteria, growth was impeded by streptomycin concentrations as low as 50 μg/mL (ANOVA: $F = 2{,}680$, $p < 0.0001$), and no growth was recorded for concentrations of 400 μg/mL or more after 24 h (**Supplementary Figure 2**).

Phase 1

Growth was initially negatively affected by streptomycin, but communities displayed rapid adaptation to streptomycin during Phase 1 (**Figure 2**). During the first 24 h (∼30 generations

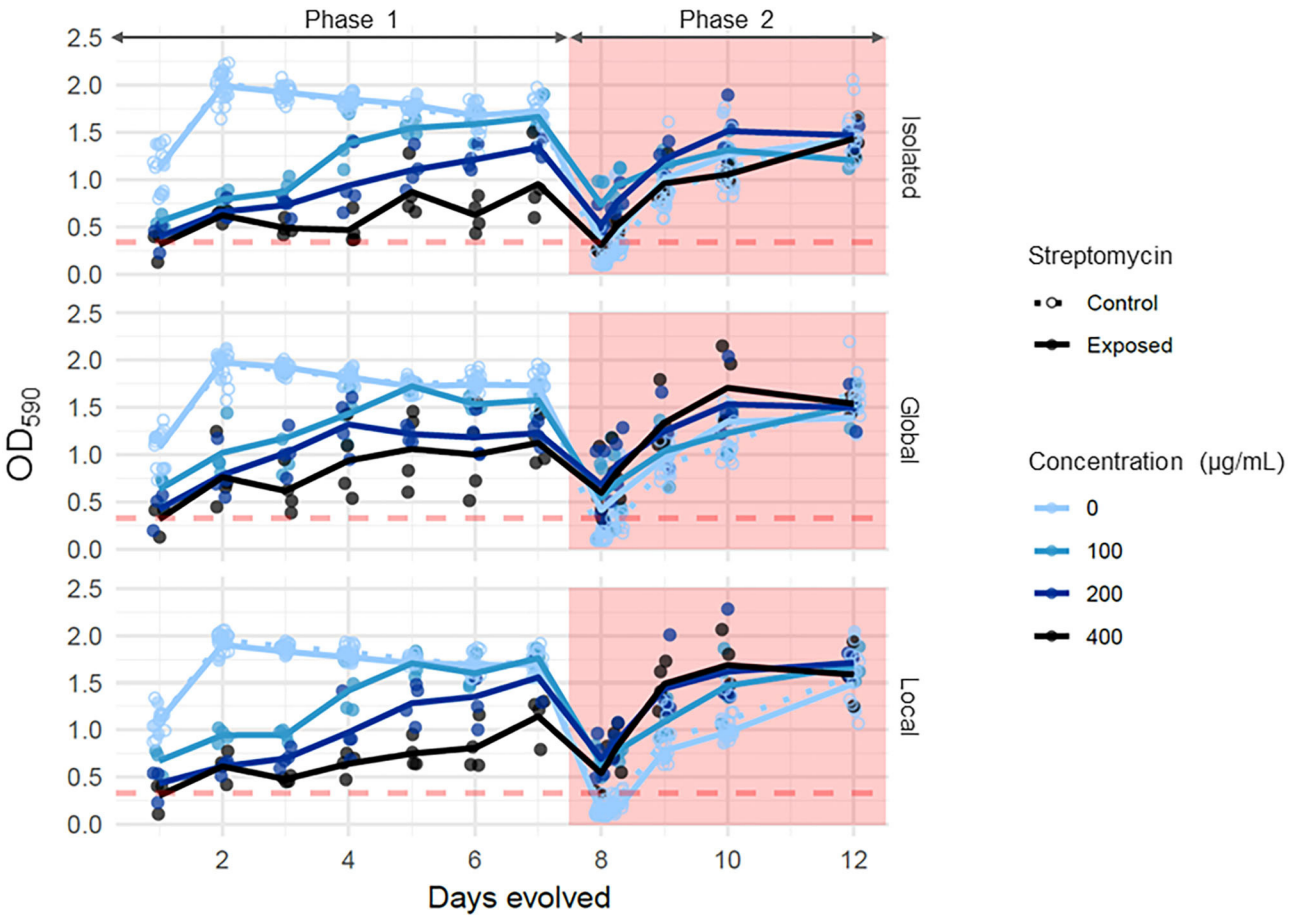


FIGURE 2 | Growth dynamics throughout the experiment. Each point represents the OD_{590} of a single community, and lines represent the mean OD_{590} across replicate communities of a given treatment combination. Shades of blue indicate Phase 1 streptomycin exposure while line types represent the type of metacommunity (control vs. streptomycin-exposed). The dashed red line represents the 95th quantile of abundance of control communities 24 h after the transfer of all communities to lethal concentrations of streptomycin. The duration of Phase 2 is shown by the pink polygon.

in benign conditions), cultures of *P. fluorescens* reacted to streptomycin in the same way as the ancestral cells: growth was significantly lower in cultures with higher concentrations of streptomycin in the environment (ANOVA: $F = 238.6$, $p < 0.0001$). However, by the end of Phase 1, while streptomycin still negatively affected growth at the highest concentrations (Tukey HSD between 0 vs. 200 or 400 µg/mL: $p < 0.0001$), communities that had evolved in concentrations of 100 µg/mL grew to abundance levels that were not significantly different than in benign environments (Tukey HSD: $p = 0.91$). By the end of Phase 1, OD_{590} of communities exposed to streptomycin at 200 and 400 µg/mL were increased by 2 or 3-fold compared with the beginning of Phase 1. In contrast to streptomycin exposure, the dispersal treatment did not significantly affect Phase 1 abundance in neither control metacommunities (ANOVA: $F = 0.812$, $p = 0.451$), nor streptomycin-exposed metacommunities ($F = 2.386$, $p = 0.108$). The interaction between dispersal mode and streptomycin concentration was not significant in streptomycin-exposed metacommunities ($F = 1.151$, $p = 0.356$).

Streptomycin exposure also influenced the dynamics of diversification. Starting from isomorphic, clonal cells, bacteria diversified into nine different morphotypes during the experiment (**Figures 1B**, **3A**). In benign conditions, communities displayed a repeatable diversification pattern where SMO first dominated the community, coexisting with shiny smooth morph and SMT in lower abundances, followed by a rise within the first 2 days in the abundance of wrinkly spreader 1 (WS1), which replaced SMO as the dominant morph throughout the rest of Phase 1. As a result, the effective number of morphotypes increased in the first few days of Phase 1, reaching a maximum of 3.6 at the end of the 2nd day (averaged across control communities, s.d. = 0.72), and subsequently decreased as WS1 then dominated the community (**Figure 3B**). These repeatable diversification dynamics were altered in

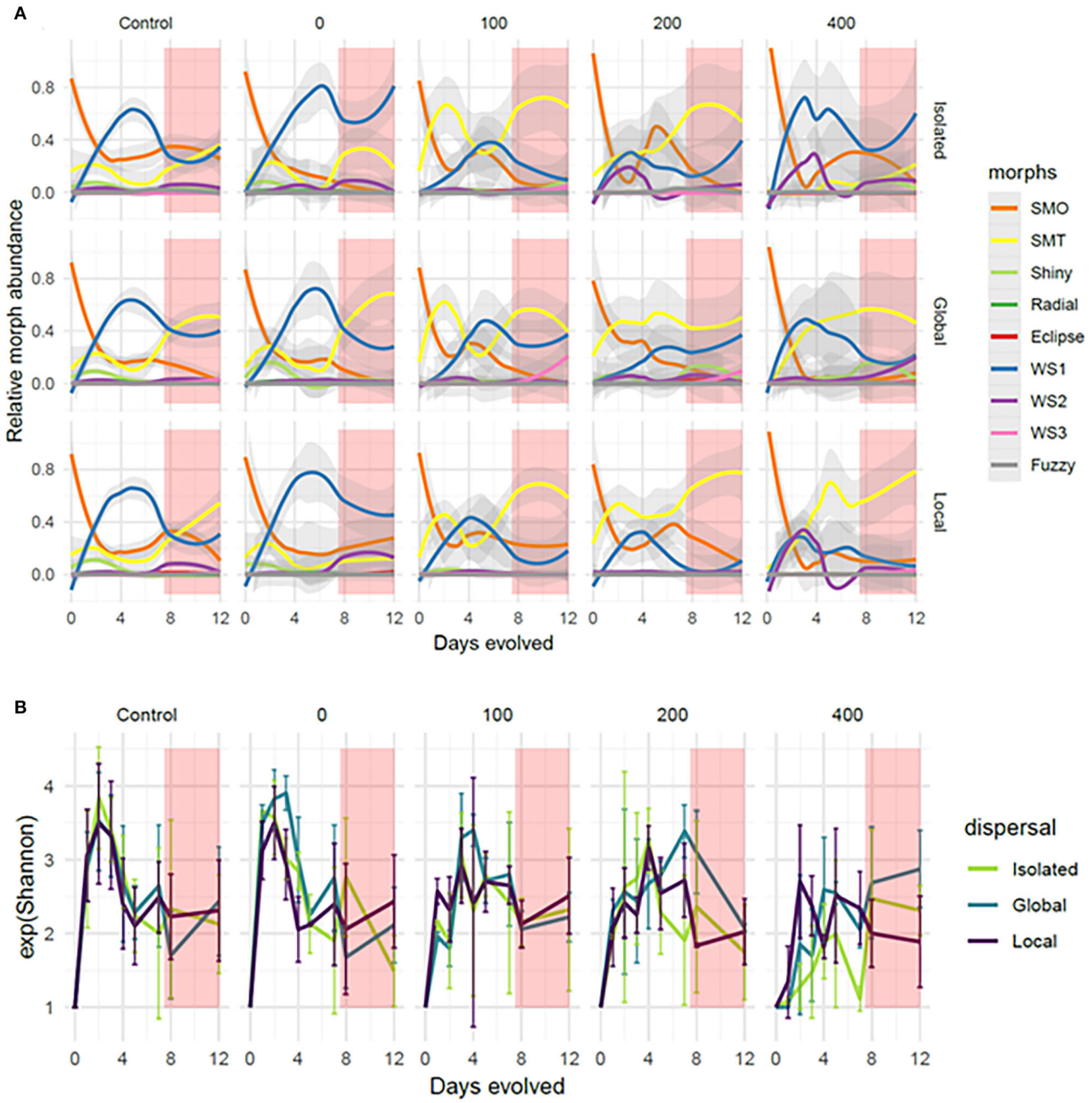


FIGURE 3 | Diversification dynamics of metacommunities throughout the experiment. Time series of morphotype relative abundance **(A)** and alpha diversity measured as Shannon exponent **(B)** in the different treatments. Plots are ranked from left to right according to streptomycin exposure during Phase 1 (values indicated at the top of the figure in μg/mL). "Controls" refer to control metacommunities in which none of the wells received streptomycin. Phase 2 is indicated by a red shading after the 7th transfer. In **(A)**, we show fitted values of "LOESS" smoothing functions interpolating relative abundance over time. In **(B)**, lines represent the average among replicate communities within each dispersal treatment, indicated by colors. Error bars = 1 standard deviation.

communities exposed to streptomycin. In environments supplemented with streptomycin in concentrations of 100 and 200 μg/mL, communities displayed a consistently different diversification pattern where SMT rapidly became dominant, replacing SMO as the dominant morph within the first 2 days, and subsequently co-occurring with SMO and WS1 (and WS2 in highly stressful environments) throughout the rest of Phase 1. Communities exposed to streptomycin in concentrations of 400 μg/mL did not display such a consistent trend in morphological diversification, in part because the dispersal treatment modulated the increase in the relative abundance of SMT (**Figure 3A**): SMT was

abundant in concentrations of 400 μg/mL only in spatially connected communities.

Dispersal clearly influenced diversification dynamics and the generation of alpha diversity during Phase 1 (**Figure 3**). By the end of Phase 1, morphotype diversity was significantly higher in communities connected through dispersal, in both control metacommunities (ANOVA: $F = 3.82$, $p = 0.0337$) and exposed metacommunities (ANOVA: $F = 5.24$, $p = 0.0148$). However, the spatial structure of dispersal did not influence final Phase 1 diversity, as communities linked by global and local dispersal had comparable diversity, in both control metacommunities (Tukey HSD: $p = 0.97$) and exposed metacommunities (Tukey HSD: $p = 0.73$). In exposed metacommunities, streptomycin exposure had a weak but non-significant effect on diversity (ANOVA: $F = 2.78$, $p = 0.068$), while the interaction between streptomycin and dispersal was not significant ($F = 0.75$, $p = 0.62$).

The dispersal and streptomycin treatments influenced both the mean composition of communities and the variance in composition among local communities (**Figure 4**). In control metacommunities, dispersal did not have a statistically significant effect on morphotype composition (pMANOVA: $p = 0.06$), but had a very strong effect on heterogeneity among local communities receiving the same dispersal treatment (**Figure 4A**; PERMDISP: $p < 0.0001$). Local dispersal reduced heterogeneity more than global dispersal. In streptomycin-exposed metacommunities, the antibiotic gradient had a much stronger influence on composition than did dispersal. Indeed, while streptomycin exposure influenced the mean composition of communities (**Figure 4B**; pMANOVA: $p = 0.002$), the main effect of dispersal on composition and the two-way interaction between dispersal and streptomycin concentration were not statistically-significant (pMANOVA: $p = 0.26$ and 0.66, respectively). Nonetheless, as in control metacommunities, dispersal reduced heterogeneity among local communities (PERMDISP: $p = 0.03$). Streptomycin exposure had the opposite effect of increasing heterogeneity among communities at higher doses (**Figure 4B**; PERMDISP: $p = 0.0003$). At the metacommunity-scale, variance in community composition among the four local communities forming a metacommunity was highly reduced by dispersal (**Figure 4C**; ANOVA, main effect of dispersal: $F = 24.95$, $p = 0.001$). This effect however disappeared in exposed metacommunities (**Figure 4C**; ANOVA, main effect of metacommunity-scale streptomycin treatment: $F = 8.96$, $p = 0.02$; two-way interaction effect of streptomycin and dispersal: $F = 5.87$, $p = 0.04$).

In summary, dispersal and streptomycin exposure over Phase 1 jointly affected the diversification of evolving *Pseudomonas* metacommunities, resulting in a gradient of morphotype diversity, composition, and history of stress across treatments, all of which were hypothesized to influence subsequent recovery of abundance and diversity in Phase 2.

Phase 2

In Phase 2, all communities were transferred to a concentration of streptomycin lethal to the ancestral cells (500 μg/mL). In the 24 h following exposure to 500 μg/mL of streptomycin, communities that had never been exposed to streptomycin in

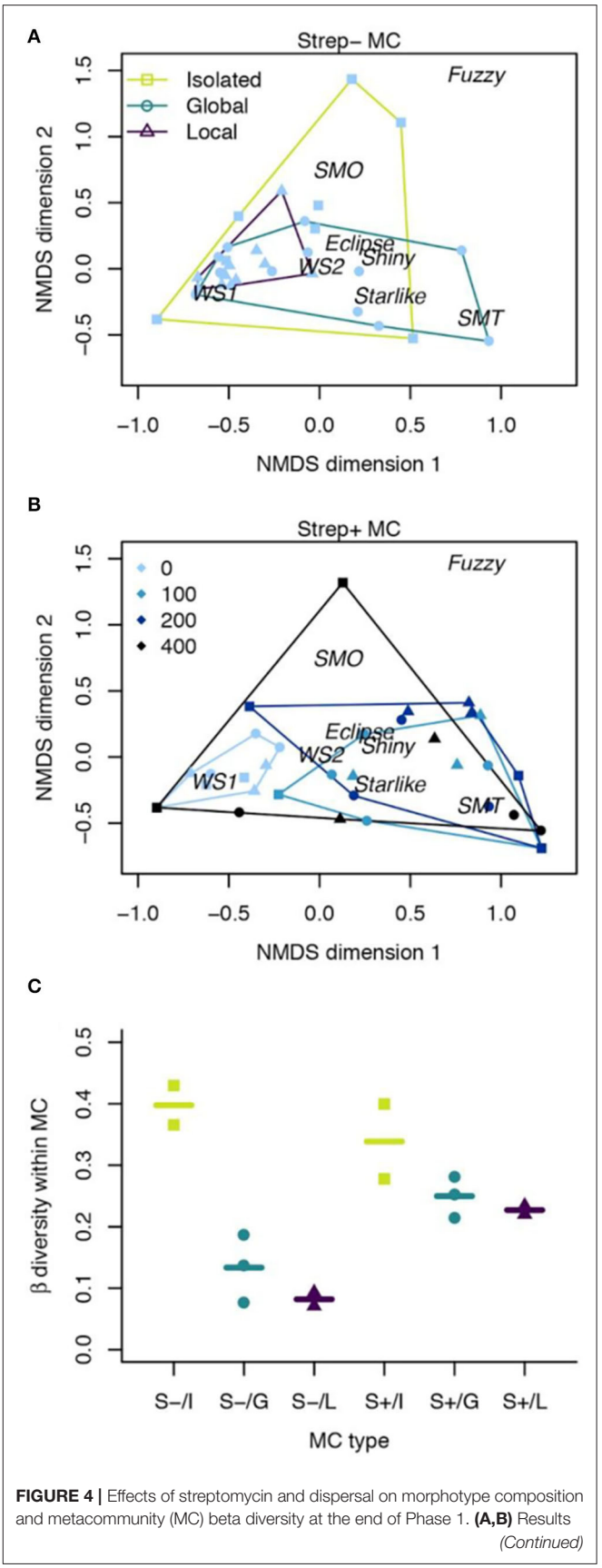


FIGURE 4 | Effects of streptomycin and dispersal on morphotype composition and metacommunity (MC) beta diversity at the end of Phase 1. **(A,B)** Results *(Continued)*

FIGURE 4 | of NMDS ordination of community composition, indicating the location of local communities (points) and morphotypes (words) in multivariate space. For visualization purposes, results are shown separately for control metacommunities **(A)** and streptomycin-exposed metacommunities **(B)**. Symbols denote dispersal treatments while colors indicate streptomycin exposure. Convex hulls regroup communities having received the same dispersal **(A)** or streptomycin **(B)** treatment. Morph acronyms are defined in **Figure 1**. **(C)** Metacommunity-scale beta diversity (mean distance to group centroid of 4 local communities comprising a metacommunity) as a function of metacommunity type and dispersal treatment. Symbols represent metacommunities while horizontal lines correspond to treatment means. S-, control metacommunities. S+, streptomycin-exposed metacommunities. I, Isolated; G, global dispersal; L, local dispersal.

Phase 1 underwent a 5-fold decline in abundance compared with the end of Phase 1 (**Figures 2**, **5A**), confirming that this dose of streptomycin is highly stressful to the majority of cells even after *in vitro* diversification. In contrast, local communities that had been exposed to at least 100 μg/mL of streptomycin during Phase 1 recovered an abundance two to three times higher than naïve communities in the first 24 h of Phase 2 (**Figure 5A**; ANOVA, main effect of streptomycin exposure: $F = 14.8$, $p < 0.0001$). However, dispersal altered this general trend (**Figure 5A**; ANOVA, interaction effect of streptomycin exposure and dispersal: $F = 2.97$, $p = 0.02$), as the beneficial effect of exposure to a high dose of streptomycin in Phase 1 was diminished in isolated communities. The spatial structure of dispersal did not influence these responses (Tukey HSD between global vs. local dispersal: $p > 0.1$ at all Phase 1 streptomycin concentrations).

Contrary to our hypothesis, diversity levels achieved at the end of Phase 1 did not have a significant effect on the abundance recovered by communities at the onset of Phase 2 (**Figure 5B**; linear regression: $R^2 = 0.002$, $p = 0.71$). Community composition at the end of Phase 1 did however have a significant effect on the abundance recovered in the first 24 h of Phase 2 (**Figure 5C**; linear regression: $R^2 = 0.25$, $p = 0.0028$). More specifically, communities where SMT occurred in higher abundances at the end of Phase 1 recovered a significantly higher abundance following the first 24 h of Phase 2 ($p < 0.0001$). This morph eventually came to dominate most communities throughout Phase 2 (**Figure 3A**), while SMO, WS1 and WS2 occurred at lower abundances, and other rare morphotypes that existed in Phase 1 were lost entirely. For example, "radial" smooth morph was only detected in one of 143 communities throughout Phase 2, and "fuzzy spreader" in only five communities. Conversely, one morph ("wrinkly spreader 3") that had never been detected in Phase 1 was detected in 17 of 143 communities throughout Phase 2. However, in contrast to Phase 1 where differences in diversity were noted across treatments, in the first 24 h of Phase 2, communities displayed similar levels of morphological diversity with no significant effect of the treatment they had received during Phase 1 (ANOVA: $F = 1.05$, $p = 0.4215$). Importantly, all communities lost a large fraction of their cells at the onset of Phase 2 regardless of which morphs they contained (even communities dominated by SMT), suggesting that no morph was fully resistant to this dose of antibiotic and that within-morph evolutionary rescue was required for persistence in Phase 2.

Eventually, by the end of Phase 2, all communities recovered similar levels of abundance and diversity, with no significant effect of their history of stress and dispersal on final abundance and diversity (**Figures 2**, **3B**; ANOVA for OD_{590}: $F = 1.07$, $p = 0.3997$; for diversity: $F = 0.91$, $p = 0.5544$). Effects of Phase 1 treatments on community composition had also vanished by the end of Phase 2 (pMANOVA: $p > 0.05$ for all factors).

In sum, evolutionary rescue of multiple morphs occurred in all communities, allowing abundance and diversity to be maintained at a dose of antibiotic lethal to the majority of the ancestral, isomorphic cells. Thus, dispersal and the history of streptomycin exposure influenced the trajectory of evolutionary rescue (i.e., the initial decline in abundance and growth following lethal stress) but not the final outcome of rescue.

DISCUSSION

Since the original formulation of evolutionary rescue theory for isolated populations of single species (Gomulkiewicz and Holt, 1995), extending the theory to conditions closer to those at play in natural communities has been a major challenge in the study of evolutionary rescue (Fussmann and Gonzalez, 2013; Osmond and de Mazancourt, 2013). This challenge is now being addressed through careful experimentation (Low-Décarie et al., 2015; Fugère et al., 2020; Scheuerl et al., 2020). Here we studied evolutionary rescue in metacommunities undergoing adaptive diversification across a gradient of environmental stress. We have shown that the history of exposure to stress, and the presence and spatial structure of dispersal in metacommunities of the rapidly evolving bacterium *P. fluorescens SBW25* can affect adaptation, diversification and evolutionary rescue following severe environmental degradation caused by antibiotic stress.

During the experiment, the isomorphic ancestral cells diversified *in vitro* into a total of nine morphotypes, forming phenotypically diverse communities that recovered both abundance and diversity following exposure to initially lethal levels of stress, demonstrating evolutionary rescue in these metacommunities. Mutations are the source of this phenotypic variation, allowing bacteria to colonize different ecological niches, in particular the surface of the liquid medium (Spiers et al., 2002; McDonald et al., 2009). Despite extensive genetic and phenotypic variation, however, the bacteria still belong to the same species, *P. fluorescens SBW25*. The species concept is blurry in bacteria (Rosselló-Mora and Amann, 2001; Riley and Lizotte-Waniewski, 2009) and challenging to quantify (Staley, 2006), and the case of rapid diversification in *P. fluorescens* is no exception. Nonetheless, asexual growth combined with niche specialization and morphological diversity supports the analogy between the different morphotypes and different species in a community, and ultimately allows us to interpret the recovery of diverse and abundant populations of morphotypes in terms of evolutionary rescue of multiple species forming a metacommunity. Our results show that: (1) dispersal and antibiotic stress jointly influenced diversification dynamics. At the end of this diversification

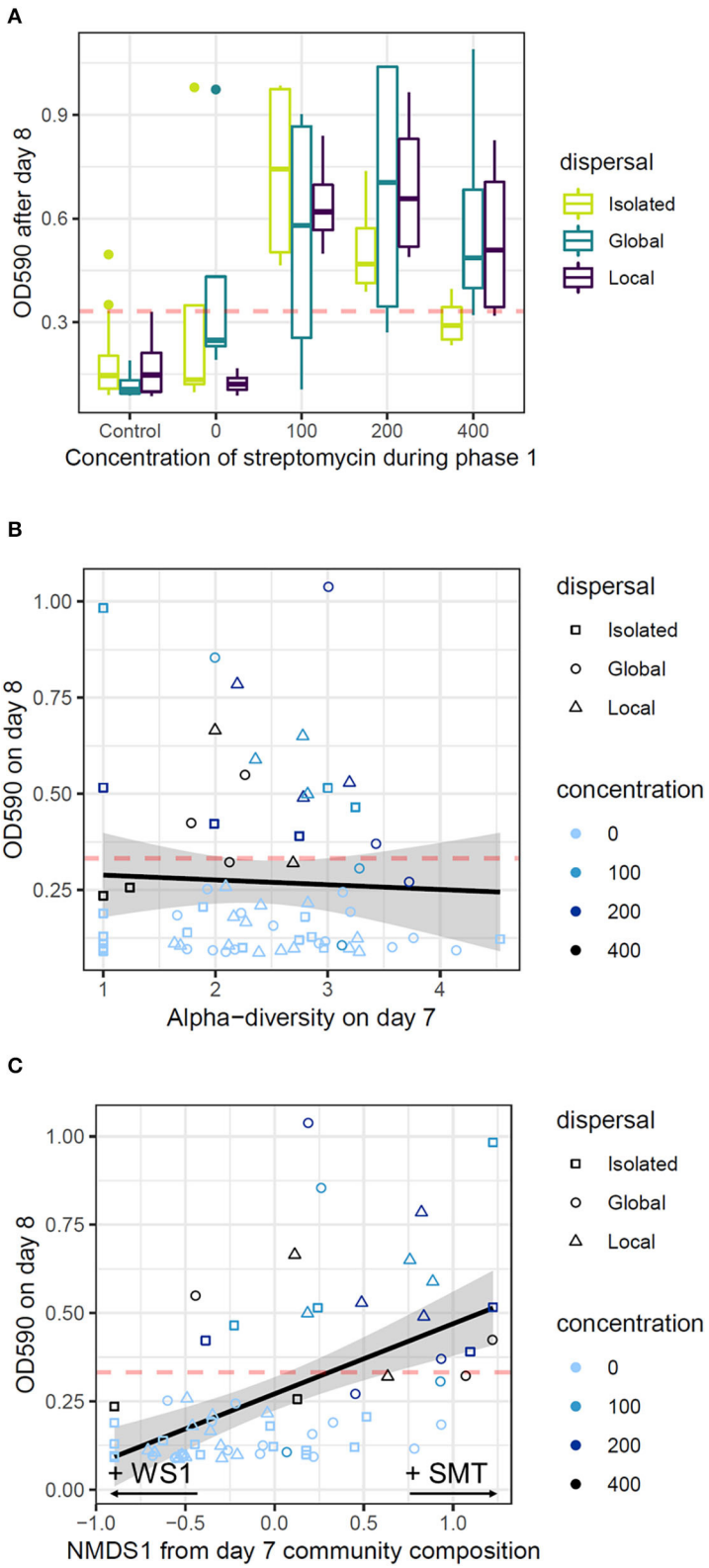


FIGURE 5 | Drivers of initial growth at a lethal dose of streptomycin. Panels represent the OD_{590} of communities after 24 h of Phase 2 as a function of streptomycin concentrations during Phase 1 **(A)**, diversity (Shannon exponent) at the end of Phase 1 **(B)**, and community composition (NMDS 1st axis) at the end of Phase 1 **(C)**. In each panel, the dashed red line represents the 95th percentile of the OD_{590} of control communities, representing the threshold for evolutionary rescue. In **(A)**, boxplots

(Continued)

FIGURE 5 | represent the median and quartiles of the OD_{590} of replicate communities, colored by their mode of dispersal during Phase 1. In **(B,C)**, each dot represents a single local community and the line represents the linear response of OD_{590} on day 8 to diversity and community composition on day 7, respectively. Shapes represent the mode of dispersal, and shades of blue represents the concentrations of streptomycin communities were exposed to during Phase 1 (in μg/mL).

process, variation in community composition, but not diversity, subsequently influenced evolutionary rescue; (2) the history of exposure to stress was a strong predictor of the trajectory of evolutionary rescue, but not the outcome of rescue. Communities with a history of antibiotic exposure had greater fitness at the onset of severe stress than naive communities, even though all communities eventually rescued; and (3) the presence of dispersal, but not its spatial structure, modulated diversification in Phase 1 and evolutionary rescue in Phase 2. We further elaborate on each of these results below.

Effect of Antibiotic Stress and Dispersal on Diversification and Adaptation

During the selection phase, growth, diversity and community composition changed over time but followed different dynamics depending on local environmental conditions, corroborating results from previous studies with this model system (Rainey and Travisano, 1998; Kassen et al., 2000; Massin and Gonzalez, 2006). At the beginning of the selection phase, both growth and diversity were reduced in higher concentrations of streptomycin. This effect on diversity could relate to the nature of the system. Diversification is driven by competition for oxygen in rapidly growing cultures of the aerobic bacteria *P. fluorescens*, but because streptomycin hampers growth, lower densities at sublethal concentrations could lead to greater oxygen availability, reduced competition, and slower diversification rates. For example, SMT rapidly dominates harsh environments in Phase 1 only in spatially connected communities, while its emergence is much slower in highly stressed, isolated communities. By the end of the selection phase, stressed communities evolved and grew to abundances and diversity levels close to that of benign environments, demonstrating a striking adaptation to high doses of streptomycin within just a few days.

Further, we have shown that streptomycin markedly altered community dynamics and dominance patterns among morphotypes. For example, while rare in benign environments, the morph SMT was dominant at intermediate doses of streptomycin (100 or 200 μg/mL) and at the highest dose of streptomycin (400 μg/mL) when communities were also linked by dispersal, potentially indicating a mass effect (Leibold et al., 2004). Other morphotypes such as SMO and WS1, which were dominant in benign environments, occurred in lower abundances in streptomycin-exposed environments, while yet other rare morphs (e.g., fuzzy spreader) went locally extinct after exposure to lethal levels of streptomycin.

These shifts in dominance patterns during the experiment suggest that the potential for resistance evolution is different among morphs. Alternatively, streptomycin might alter the relative competitive abilities of the different morphs as they simultaneously evolve within patches (Rainey and Travisano, 1998) and across the gradient of antibiotic stress between patches (Osmond and de Mazancourt, 2013). Streptomycin normally kills bacteria by binding to the 30S ribosomal subunit, inhibiting mRNA translation and thus protein synthesis (Biswas and Gorini, 1972). Mutations conferring resistance to streptomycin may trade-off with growth potential in benign environments, making resistant individuals less abundant in the absence of streptomycin. Yet another hypothesis for the differences in diversification dynamics concerns the mechanisms by which different morphs emerged in different environments. For example, the transition from SMO-dominated communities to SMT-dominated communities with streptomycin exposure could be the result of both genetic evolution (streptomycin-induced selection on standing variation or novel mutations) and phenotypic plasticity. Antibiotic application is known to alter other phenotypes (e.g., biofilm formation) in populations of the closely related *Pseudomonas aeruginosa* that also acquire antibiotic resistance (Drenkard and Ausubel, 2002). Nonetheless, a strong genetic contribution to phenotypic differentiation has previously been shown for some *P. fluorescens* morphs, where the emergence of a wrinkly spreader morph from a monoclonal isogenic population of smooth morphs was driven by novel mutations and selection (Rainey and Travisano, 1998; Spiers et al., 2002; McDonald et al., 2009). Further analyses would be necessary to distinguish plastic (e.g., expression of cellulose polymer forming biofilm, Spiers et al., 2002) from genetic influences on morphological diversification and antibiotic resistance in our experimental design, bearing in mind that it is also possible, and perhaps more realistic, that both processes could be involved (Chevin et al., 2013; Kovach-Orr and Fussmann, 2013; Lind et al., 2018; Carja and Plotkin, 2019).

At the metacommunity level, dispersal and streptomycin had opposing effects on both alpha and beta diversity generated during the selection phase. While streptomycin greatly lowered local (alpha) diversity, the effect of streptomycin on community composition also translated into greater compositional difference between local communities. This is expected as the antibiotic gradient creates habitat heterogeneity, promoting beta diversity within metacommunities (Veech and Crist, 2007; Matthiessen et al., 2010). However, dispersal countered the effects of streptomycin, as it increased local diversity in harshly stressed (200 or 400 μg/L) and otherwise depauperate communities, while at the same time homogenizing community composition within metacommunities. Even in control metacommunities without an antibiotic gradient, dispersal increased mean local diversity and reduced metacommunity beta diversity. Both of these effects (higher local diversity, lower beta diversity) of the intermediate rate of dispersal that we used (1%) are consistent with the metacommunity theory (Leibold et al., 2004; Howeth and Leibold, 2010) and with experimental evidence (Matthiessen et al., 2010). Despite these strong effects of dispersal on diversification, the spatial structure of dispersal did not have

a consistent effect on diversity; both local and global dispersal resulted in similar patterns of alpha and beta diversity at the end of the selection phase.

Increased diversity through dispersal has the potential to be selected upon in the case of environmental change (Schiffers et al., 2013). We therefore predicted faster or more complete adaptation to the antibiotic stress in metacommunities linked by dispersal. Our results do not support this prediction: neither the presence nor the spatial structure of dispersal influenced community abundance reached by the end of Phase 1. However, the presence of dispersal clearly influenced how fast communities with a history of streptomycin exposure recovered their abundance during the rescue trial of Phase 2.

Drivers of Evolutionary Rescue

We observed repeated and consistent evolutionary rescue in *P. fluorescens* metacommunities exposed to harsh levels of streptomycin. By the end of Phase 2, viable and diverse communities grew in conditions that were lethal to the ancestral, isomorphic cells, which demonstrates evolutionary rescue of multiple morphs. Because the ancestral population lacked variation, this rescue process was ultimately driven by evolution. Adaptive evolution occurred both during Phase 1 as isomorphic bacteria underwent adaptive radiation driven by competition and modulated by antibiotic exposure, and during the rescue trial in Phase 2 when communities adapted quickly to the high dose of antibiotic. Past exposure to streptomycin was the main cause of the difference in the trajectory of recovery following exposure to severe stress, confirming previous findings from experimental studies of evolutionary rescue in microbial systems (Gonzalez and Bell, 2013; Low-Décarie et al., 2015; Bell et al., 2019; Fugère et al., 2020).

Evolutionary rescue can arise from standing variation or from mutations arising early (i.e., first few cell divisions) in the selection treatment. Our design was focused on the study of the net outcome in the race between the decline in abundance and the rate of recovery by rare resistant cells, or individuals, among different morphotypes of *P. fluorescens*. Evolutionary rescue is above all a question of whether the rate of adaptation to environmental change occurs on the same time scale as the demographic decline (Gienapp et al., 2013; Lindsey et al., 2013). If all communities eventually recovered similar levels of abundance and diversity, they did so at varying rates.

Following exposure to harsh dose of streptomycin in Phase 2, communities that had been exposed to at least 100 μg/mL of streptomycin in Phase 1 had greater fitness (i.e., population growth averaged across morphotypes) than communities naive to the antibiotic. This effect likely arose because genetic and plastic changes conferring increased resistance at an intermediate dose of stress also provided a correlated advantage at a higher dose of stress. This result supports the conclusion that historical selection facilitates adaptation to a deteriorating environment (Gonzalez and Bell, 2013; Samani and Bell, 2016).

By the end of Phase 2, all communities recovered similar levels of abundance and diversity, perhaps owing to the evolved diversity that was absent in the ancestral cells. Indeed, communities that were allowed to evolve for 7 days even in benign conditions had a much higher morphological diversity than the ancestral cells, variation upon which natural selection could act in Phase 2. However, we did not find a significant effect of diversity on either the trajectory or the outcome of evolutionary rescue. Instead, our results suggest that community composition, rather than diversity, determined the trajectory of evolutionary rescue. Indeed, the abundance of SMT at the end of the selection phase significantly increased the abundance in communities 24 h after the onset of Phase 2 – although only in communities that had also experienced streptomycin in Phase 1. That is, both historical selection and a high relative abundance of SMT were necessary for a swift recovery in Phase 2 (**Figure 5C**), suggesting that ecological processes (morphotype sorting in favor of the relatively more resistant SMT morph) and evolutionary processes (past adaptation of the SMT morph to streptomycin) both promoted community recovery. Higher abundance of SMT before the rescue trial of Phase 2 was a result of both past exposure to streptomycin and presence of dispersal. Some isolated communities exposed to high doses in Phase 1 had a low relative abundance of SMT, while some control communities with dispersal had a high abundance of SMT – and both these types of communities collapsed to low abundances at the onset of Phase 2. Therefore, our results suggest that community composition influenced the speed of community recovery, as a result of the interplay between past dispersal events and previous exposure to stress. However, as for diversification, the presence of dispersal mattered most, not its spatial structure.

Here, we quantified the outcome of rescue based on the recovery of abundance following exposure to severe stress. By the end of Phase 2, communities recovered abundance and diversity in levels comparable to the end of the selection phase, indicating that evolutionary rescue of multiple morphs occurred in all communities by the end of Phase 2 of the experiment. Abundance or diversity are both aggregate measures of community recovery. Our analysis of community composition showed that communities at the end of Phase 2 were very different in composition compared with Phase 1 communities, indicating a shift to different compositional states despite the recovery of similar levels of abundance and diversity.

CONCLUSIONS AND IMPLICATIONS

Here, we studied the dynamics of evolutionary rescue occurring in evolving communities. We found that the history of stress and dispersal promotes the incidence of evolutionary rescue (Low-Décarie et al., 2015; Bell et al., 2019; Fugère et al., 2020). Our results have management implications, although a few caveats should be acknowledged before extrapolating to natural systems. While dispersal could in theory increase the likelihood of adaptation and subsequent recovery in communities across a fragmented, heterogeneous landscape, other ecological factors such as population size and generation time are crucial to the recovery of populations. Here, the very large populations responsible for striking evolutionary rescue in *P. fluorescens* may be common for microbial species but are rarely so for metazoan species that typically exist at much lower densities or have slower

generation times and lower reproductive rates (vander Wal et al., 2013) although cases of evolutionary rescue in metazoans have been observed (Ozgo, 2014; Reid et al., 2016). This suggests that evolutionary rescue may be less likely in conservation contexts but more likely in agroecosystems or clinical settings where microbes respond to high doses of biocides (Alexander et al., 2014).

We found that dispersal fosters evolutionary rescue following environmental degradation, which corroborates previous findings (Perron et al., 2007; Bell and Gonzalez, 2011; Low-Décarie et al., 2015; Gokhale et al., 2018). For pathogens and invasive species, globalization is increasing the potential for dispersal to contribute to the evolution of resistance across common selective environments, including the widespread application of common antibiotics and pesticides (Thanner et al., 2016; Hudson et al., 2017). For example, streptomycin is used in agriculture worldwide for the control of plant pathogenic bacteria (e.g., in orchards). Resistance is often observed in multiple bacterial pathogens, including *Pseudomonas* sp. in these orchard settings (Vanneste and Voyle, 2002; Sundin and Wang, 2018), although this may not influence the abundance and diversities of major bacteria taxa soil communities or cause convergence in community similarity (McManus, 2014; Walsh et al., 2014). It remains unclear whether the dispersal of micro-organisms between exposed sites may engender resistance across a microbial metacommunity.

Evolutionary rescue theory is also relevant for conservation biology, where the focus is not the eradication of species but their protection and restoration. Widespread habitat loss is decreasing the potential of already vulnerable populations and communities to disperse across habitat patches and entire landscapes to adapt to new disturbances such as climate change (Norberg et al., 2012). Our results suggest that, especially for depauperate communities, isolation of habitat patches or dispersal barriers could hinder the recovery of communities following severe environmental deterioration (Cheptou et al., 2017). Although we used a simple laboratory model system that lacks some of the complex dynamics and interactions that characterize natural ecosystems exposed to human impacts, our results support the conclusions from theory that spatial connectivity through dispersal is an important determinant of eco-evolutionary dynamics and persistence of diversity across changing and degraded landscapes (Crooks and Sanjayan, 2006; Norberg et al., 2012; Thompson and Fronhofer, 2019).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AG designed research. LO'C carried out the experiment. LO'C and VF performed statistical analysis and prepared the figures. All authors contributed significantly to interpreting the results and writing the manuscript.

FUNDING

VF was supported by a postdoctoral fellowship from the Natural Science and Engineering Research Council of Canada (NSERC). AG was supported by Liber Ero Chair in Biodiversity Conservation, and an NSERC Discovery grant.

ACKNOWLEDGMENTS

The authors are grateful to Chloé Debyser, Clara Marino, Sandra Klemet N'Guessan, Ilke Geladi, Charles Bazerghi, and Nathalie Chehab for help with laboratory analyses.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.517434/full#supplementary-material

REFERENCES

Alexander, H. K., Martin, G., Martin, O. Y., and Bonhoeffer, S. (2014). Evolutionary rescue: linking theory for conservation and medicine. *Evol. Appl.* 7, 1161–1179. doi: 10.1111/eva.12221

Bantinaki, E., Kassen, R., Knight, C. G., Robinson, Z., Spiers, A. J., and Rainey, P. B. (2007). Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. III. Mutational origins of wrinkly spreader diversity. *Genetics* 176, 441–453. doi: 10.1534/genetics.106.069906

Barrett, R. D. H., and Bell, G. (2006). The dynamics of diversification in evolving Pseudomonas populations. *Evolution* 60:484. doi: 10.1554/05-673.1

Bell, G. (2017). Evolutionary rescue. *Annu. Rev. Ecol. Evol. Syst.* 48, 605–627. doi: 10.1146/annurev-ecolsys-110316-023011

Bell, G., Fugère, V., Barrett, R., Beisner, B., Cristescu, M., Fussmann, G., et al. (2019). Trophic structure modulates community rescue following acidification. *Proc. R. Soc. B Biol. Sci.* 286:20190856. doi: 10.1098/rspb.2019.0856

Bell, G., and Gonzalez, A. (2009). Evolutionary rescue can prevent extinction following environmental change. *Ecol. Lett.* 12, 942–948. doi: 10.1111/j.1461-0248.2009.01350.x

Bell, G., and Gonzalez, A. (2011). Adaptation and evolutionary rescue in metapopulations experiencing environmental deterioration. *Science* 332, 1327–1330. doi: 10.1126/science.1203105

Biswas, D. K., and Gorini, L. (1972). The attachment site of streptomycin to the 30S Ribosomal subunit. *Proc. Natl. Acad. Sci. U.S.A.* 69, 2141–2144. doi: 10.1073/pnas.69.8.2141

Bürger, R., and Lynch, M. (1995). Evolution and extinction in a changing environment: a quantitative-genetic analysis. *Evolution* 49, 151–163. doi: 10.1111/j.1558-5646.1995.tb05967.x

Carja, O. and Plotkin, J. B. (2019). Evolutionary Rescue Through Partly Heritable Phenotypic Variability. *Genetics* 211, 977–988. doi: 10.1534/genetics.118.301758

Carlson, S. M., Cunningham, C. J., and Westley, P. A. H. (2014). Evolutionary rescue in a changing world. *Trends Ecol. Evol.* 29, 521–530. doi: 10.1016/j.tree.2014.06.005

Cheptou, P.-O., Hargreaves, A. L., Bonte, D., and Jacquemyn, H. (2017). Adaptation to fragmentation: evolutionary dynamics driven by human influences. *Philos. Trans. R. Soc. B Biol. Sci.* 372:20160037. doi: 10.1098/rstb.2016.0037

Chevin, L. M., Gallet, R., Gomulkiewicz, R., Holt, R. D., and Fellous, S. (2013). Phenotypic plasticity in evolutionary rescue experiments. *Philos. Trans. R. Soc. B Biol. Sci.* 368. doi: 10.1098/rstb.2012.0089

Crooks, K. R., and Sanjayan, M. (2006). *Connectivity Conservation*. Cambridge: Cambridge University Press. 14. doi: 10.1017/CBO9780511754821

Drenkard, E., and Ausubel, F. M. (2002). Pseudomonas biofilm formation and antibiotic resistance are linked to phenotypic variation. *Nature* 416, 740–743. doi: 10.1038/416740a

Fugère, V., Hébert, M. P., da Costa, N. B., Xu, C. C. Y., Barrett, R. D. H., Beisner, B. E., et al. (2020). Community rescue in experimental phytoplankton communities facing severe herbicide pollution. *Nat. Ecol. Evol.* 4, 578–588. doi: 10.1038/s41559-020-1134-5

Fussmann, G. F., and Gonzalez, A. (2013). Evolutionary rescue can maintain an oscillating community undergoing environmental change. *Interface Focus* 3:20130036. doi: 10.1098/rsfs.2013.0036

Gienapp, P., Lof, M., Reed, T. E., McNamara, J., Verhulst, S., and Visser, M. E. (2013). Predicting demographically sustainable rates of adaptation: can great tit breeding time keep pace with climate change? *Philos. Trans. R. Soc. B Biol. Sci.* 368:20120289. doi: 10.1098/rstb.2012.0289

Gokhale, S., Conwill, A., Ranjan, T., and Gore, J. (2018). Migration alters oscillatory dynamics and promotes survival in connected bacterial populations. *Nat. Commun.* 9:5273. doi: 10.1038/s41467-018-07703-y

Gomulkiewicz, R., and Holt, R. D. (1995). When does evolution by natural selection prevent extinction? *Evolution* 49, 201–207. doi: 10.1111/j.1558-5646.1995.tb05971.x

Gonzalez, A., and Bell, G. (2013). Evolutionary rescue and adaptation to abrupt environmental change depends upon the history of stress. *Philos. Trans. R. Soc. B Biol. Sci.* 368:20120079. doi: 10.1098/rstb.2012.0079

Gonzalez, A., Ronce, O., Ferriere, R., and Hochberg, M. E. (2013). Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. *Philos. Trans. R. Soc. B Biol. Sci.* 368:20120404. doi: 10.1098/rstb.2012.0404

Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., et al. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Sci. Adv.* 1:e1500052. doi: 10.1126/sciadv.1500052

Hao, Y.-Q., Brockhurst, M. A., Petchey, O. L., and Zhang, Q.-G. (2015). Evolutionary rescue can be impeded by temporary environmental amelioration. *Ecol. Lett.* 18, 892–898. doi: 10.1111/ele.12465

Hodgson, D. J., Rainey, P. B., and Buckling, A. (2002). Mechanisms linking diversity, productivity and invasibility in experimental bacterial communities. *Proc. R. Soc. B Biol. Sci.* 269, 2277–2283. doi: 10.1098/rspb.2002.2146

Howeth, J. G., and Leibold, M. A. (2010). Species dispersal rates alter diversity and ecosystem stability in pond metacommunities. *Ecology* 91, 2727–2741. doi: 10.1890/09-1004.1

Hudson, J. A., Frewer, L. J., Jones, G., Brereton, P. A., Whittingham, M. J., and Stewart, G. (2017). The agri-food chain and antimicrobial resistance: a review. *Trends Food Sci. Technol.* 69, 131–147. doi: 10.1016/j.tifs.2017.09.007

Hufbauer, R. A., Szucs, 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. U.S.A.* 112, 10557–10562. doi: 10.1073/pnas.1504732112

Jost, L. (2006). Entropy and diversity. *Oikos* 113, 363–375. doi: 10.1111/j.2006.0030-1299.14714.x

Kassen, R., Buckling, A., Bell, G., and Ralney, P. B. (2000). Diversity peaks at intermediate productivity in a laboratory microcosm. *Nature* 406, 508–512. doi: 10.1038/35020060

Kovach-Orr, C., and Fussmann, G. F. (2013). Evolutionary and plastic rescue in multitrophic model communities. *Philos. Trans. R. Soc. B Biol. Sci.* 368:20120084. doi: 10.1098/rstb.2012.0084

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. doi: 10.1111/j.1461-0248.2004.00608.x

Lind, P. A., Libby, E., Herzog, J., and Rainey, P. B. (2018). Predicting mutational routes to new adaptive phenotypes. *Elife 8:e38822*. doi: 10.7554/eLife.38822.027

Lindsey, H. A., Gallie, J., Taylor, S., and Kerr, B. (2013). Evolutionary rescue from extinction is contingent on a lower rate of environmental change. *Nature* 494, 463–467. doi: 10.1038/nature11879

Low-Décarie, E., Kolber, M., Homme, P., Lofano, A., Dumbrell, A., Gonzalez, A., et al. (2015). Community rescue in experimental metacommunities. *Proc. Natl. Acad. Sci. U.S.A.* 112, 14307–14312. doi: 10.1073/pnas.1513125112

MacLean, R. C., and Bell, G. (2002). Experimental adaptive radiation in *Pseudomonas*. *Am. Nat.* 160, 569–581. doi: 10.1086/342816

Massin, N., and Gonzalez, A. (2006). Adaptive radiation in a fluctuating environment: disturbance affects the evolution of diversity in a bacterial microcosm. *Evol. Ecol. Res.* 8, 471–481. Available online at: http://www.evolutionary-ecology.com/issues/v08n03/hhar1917.pdf

Matthiessen, B., Mielke, E., and Sommer, U. (2010). Dispersal decreases diversity in heterogeneous metacommunities by enhancing regional competition. *Ecology* 91, 2022–2033. doi: 10.1890/09-1395.1

McDonald, M. J., Gehrig, S. M., Meintjes, P. L., Zhang, X. X., and Rainey, P. B. (2009). Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. IV. Genetic constraints guide evolutionary trajectories in a parallel adaptive radiation. *Genetics* 183, 1041–1053. doi: 10.1534/genetics.109.107110

McManus, P. S. (2014). Does a drop in the bucket make a splash? Assessing the impact of antibiotic use on plants. *Curr. Opin. Microbiol.* 19, 76–82. doi: 10.1016/j.mib.2014.05.013

Norberg, J., Urban, M. C., Vellend, M., Klausmeier, C. A., and Loeuille, N. (2012). Eco-evolutionary responses of biodiversity to climate change. *Nat. Clim. Chang.* 2, 747–751. doi: 10.1038/nclimate1588

Osmond, M. M., and de Mazancourt, C. (2013). How competition affects evolutionary rescue. *Philos. Trans. R. Soc. B Biol. Sci.* 368:20120085. doi: 10.1098/rstb.2012.0085

Ozgo, M. (2014). Rapid evolution and the potential for evolutionary rescue in land snails. *J. Molluscan Stud.* 80, 286–290. doi: 10.1093/mollus/eyu029

Pereira, H. M., Leadley, P. W., Proenca, V., Alkemade, R., Scharlemann, J. P. W., Fernandez-Manjarres, J. F., et al. (2010). Scenarios for global biodiversity in the 21st century: supporting information. *Science* 330, 1496–1502. doi: 10.1126/science.1196624

Perron, G. G., Gonzalez, A., and Buckling, A. (2007). Source-sink dynamics shape the evolution of antibiotic resistance and its pleiotropic fitness cost. *Proc. R. Soc. B Biol. Sci.* 274, 2351–2356. doi: 10.1098/rspb.2007.0640

Perron, G. G., Zasloff, M., and Bell, G. (2006). Experimental evolution of resistance to an antimicrobial peptide. *Proc. R. Soc. B Biol. Sci.* 273, 251–256. doi: 10.1098/rspb.2005.3301

R Core Team (2017). R: *A Language and Environment for Statistical Computing*. Vienna: R foundation for statistical computing. Available online at: http://www.R-project.org.

Rainey, P. B., and Bailey, M. J. (1996). Physical and genetic map of the Pseudomonas fluorescens SBW25 chromosome. *Mol. Microbiol.* 19, 521–533. doi: 10.1046/j.1365-2958.1996.391926.x

Rainey, P. B., and Travisano, M. (1998). Adaptive radiation in a heterogeneous environment. *Nature* 394, 69–72. doi: 10.1038/27900

Ramsayer, J., Kaltz, O., and Hochberg, M. E. (2013). Evolutionary rescue in populations of *Pseudomonas fluorescens* across an antibiotic gradient. *Evol. Appl.* 6, 608–616. doi: 10.1111/eva.12046

Reid, N. M., Proestou, D. A., Clark, B. W., Warren, W. C., Colbourne, J. K., Shaw, J. R., et al. (2016). The genomic landscape of rapid repeated evolutionary adaptation to toxic pollution in wild fish. *Science* 354, 1305–1308. doi: 10.1126/science.aah4993

Riley, M. A., and Lizotte-Waniewski, M. (2009). Population genomics and the bacterial species concept. *Methods Mol. Biol.* 532, 367–377. doi: 10.1007/978-1-60327-853-9_21

Rosselló-Mora, R., and Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiol. Rev.* 25, 39–67. doi: 10.1016/S0168-6445(00)00040-1

Samani, P., and Bell, G. (2016). The ghosts of selection past reduces the probability of plastic rescue but increases the likelihood of evolutionary rescue to novel stressors in experimental populations of wild yeast. *Ecol. Lett.* 19, 289–298. doi: 10.1111/ele.12566

Scheuerl, T., Hopkins, M., Nowell, R. W., Rivett, D. W., Barraclough, T. G., and Bell, T. (2020). Bacterial adaptation is constrained in complex communities. *Nat. Commun.* 11:754. doi: 10.1038/s41467-020-14570-z

Schiffers, K., Bourne, E. C., Lavergne, S., Thuiller, W., and Travis, J. M. J. (2013). Limited evolutionary rescue of locally adapted populations facing climate change. *Philos. Trans. R. Soc. B Biol. Sci.* 368:20120083. doi: 10.1098/rstb.2012.0083

Spiers, A. J., Kahn, S. G., Bohannon, J., Travisano, M., and Rainey, P. B. (2002). Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. I. Genetic and phenotypic bases of wrinkly spreader fitness. *Genetics* 161, 33–46. Available online at: https://www.genetics.org/content/161/1/33.article-info

Staley, J. T. (2006). The bacterial species dilemma and the genomic-phylogenetic species concept. *Philos. Trans. R. Soc. B Biol. Sci.* 361, 1899–1909. doi: 10.1098/rstb.2006.1914

Sundin, G. W., and Wang, N. (2018). Antibiotic resistance in plant-pathogenic bacteria. *Annu. Rev. Phytopathol.* 56, 161–180. doi: 10.1146/annurev-phyto-080417-045946

Thanner, S., Drissner, D., and Walsh, F. (2016). Antimicrobial resistance in agriculture. *MBio* 7, 1–7. doi: 10.1128/mBio.02227-15

Thompson, P. L., and Fronhofer, E. A. (2019). The conflict between adaptation and dispersal for maintaining biodiversity in changing environments. *Proc. Natl. Acad. Sci. U.S.A.* 116, 21061–21067. doi: 10.1073/pnas.1911796116

vander Wal, E., Garant, D., Festa-Bianchet, M., and Pelletier, F. (2013). Evolutionary rescue in vertebrates: evidence, applications and uncertainty. *Philos. Trans. R. Soc. B Biol. Sci.* 368:20120090. doi: 10.1098/rstb.2012.0090

Vanneste, J. L., and Voyle, M. D. (2002). Characterisation of transposon, genes and mutations which confer streptomycin resistance in bacterial strains isolated from New Zealand orchards. *Acta Horticulturae* 590, 494–495. doi: 10.17660/ActaHortic.2002.590.76

Veech, J. A., and Crist, T. O. (2007). Habitat and climate heterogeneity maintain beta-diversity of birds among landscapes within ecoregions. *Glob. Ecol. Biogeogr.* 16, 650–656. doi: 10.1111/j.1466-8238.2007.00315.x

Venail, P., MacLean, R. C., Bouvier, T., Brockhurst, M. A., Hochberg, M. E., and Mouquet, N. (2008). Diversity and productivity peak at intermediate dispersal rate in evolving metacommunities. *Nature* 452, 210–214. doi: 10.1038/nature06554

Venail, P., Maclean, R. C., Meynard, C. N., and Mouquet, N. (2010). Dispersal scales up the biodiversity-productivity relationship in an experimental source-sink metacommunity. *Proc. R. Soc. B Biol. Sci.* 277, 2339–2345. doi: 10.1098/rspb.2009.2104

Walsh, F., Smith, D. P., Owens, S. M., Duffy, B., and Frey, J. E. (2014). Restricted streptomycin use in apple orchards did not adversely alter the soil bacteria communities. *Front. Microbiol.* 4:383. doi: 10.3389/fmicb.2013.00383

World Health Organization (2015). *WHO Model List of Essential Medicines - 19th List*. Available online at: http://www.who.int/medicines/publications/pharmacopoeia (accessed April 2015).

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 18 January 2021
doi: 10.3389/fmicb.2020.582655

Geographical Variability of Mineral Elements and Stability of Restrictive Mineral Elements in Terrestrial Cyanobacteria Across Gradients of Climate, Soil, and Atmospheric Wet Deposition Mineral Concentration

Weibo Wang[1,2]*, *Hua Li*[3], *René Guénon*[4], *Yuyi Yang*[1,2], *Xiao Shu*[1], *Xiaoli Cheng*[1] and *Quanfa Zhang*[1,2]*

[1] CAS Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, China, [2] Center of Plant Ecology, Core Botanical Gardens, Chinese Academy of Sciences, Wuhan, China, [3] CAS Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China, [4] EPHOR, Institut Agro, Angers, France

OPEN ACCESS

Edited by:
Cendrine Mony,
University of Rennes 1, France

Reviewed by:
Xiaogang Li,
Nanjing Forestry University, China
Shuo Jiao,
Northwest A and F University, China
Toshio Sakamoto,
Kanazawa University, Japan

***Correspondence:**
Weibo Wang
wangweibo@wbgcas.cn
Quanfa Zhang
qzhang@wbgcas.cn

Specialty section:
This article was submitted to Terrestrial Microbiology, a section of the journal Frontiers in Microbiology

Received: 13 July 2020
Accepted: 18 December 2020
Published: 18 January 2021

Citation:
Wang W, Li H, Guénon R, Yang Y, Shu X, Cheng X and Zhang Q (2021) Geographical Variability of Mineral Elements and Stability of Restrictive Mineral Elements in Terrestrial Cyanobacteria Across Gradients of Climate, Soil, and Atmospheric Wet Deposition Mineral Concentration. Front. Microbiol. 11:582655. doi: 10.3389/fmicb.2020.582655

Terrestrial cyanobacteria *Nostoc commune* is an ideal species to study the geographical variation of mineral elements of soil cyanobacteria at the species level. Here, we first address the following questions: (1) from where are these mineral elements, (2) are there geographical variations for these mineral elements, and if so, (3) which environmental factors drive the geographical variation of these mineral elements? Second, we tested whether the soil cyanobacterial mineral elements followed the "restrictive element stability hypothesis" of higher plants. Finally, we explored the effect of mineral geographic variation on ecological adaptation of soil cyanobacteria. We collected *N. commune* samples across gradients of climate, soil, and atmospheric wet deposition mineral concentration in mainland China. We measured fifteen minerals, including five macroelements (N, Ca, K, Fe, P), five microelements (Mn, Zn, Cu, Co, Se), and five heavy metals (Pb, Cr, As, Cd, Hg). We found that five elements (P, Cu, Zn, Co, Pb) had significant geographical variation. They increased as the distance from the equator increased and decreased as the distance from the prime meridian increased. Mean annual precipitation and mean annual temperature explained most of the variation. We did not find any significant correlations between the mineral element contents in *N. commune* and the minerals in soil and rainfall, except for P. There was no significant correlation between the variation coefficients of different elements and their actual detected contents and their potential physiological required contents. The statistical results of our experiment did not support the "restrictive element stability hypothesis." We speculated that net accumulation of mineral elements in cyanobacterial cells and extracellular polysaccharides (EPS) might play an important role for terrestrial cyanobacteria in the adaptation to dry and cold conditions.

Keywords: terrestrial cyanobacteria, geographic variation, mineral elements, climate, soil nutrients, atmospheric wet deposition, restrictive element stability hypothesis, environmental adaptation

INTRODUCTION

Recently, significant progress has been achieved in terrestrial microbial biogeography (Fierer and Jackson, 2006; Ranjard et al., 2010, 2013). However, compared to aquatic microbial biogeography (Quigg et al., 2003; Garcia et al., 2016; Godwin and Cotner, 2018) and the biogeography of large plants and animals (both aquatic and terrestrial) (Humphries and Parenti, 1999; Dormann et al., 2007), studies of terrestrial microbial biogeography remain relatively rare. For aquatic microorganisms and higher plants and animals, the geographical variation of their community structure and the variation of their biochemical composition and mineral elements (stoichiometric characteristics) can be studied, both at the community level (Han et al., 2005, 2011; Yvon-Durocher et al., 2015) and the species level (Zhou et al., 2013; Garcia et al., 2016; Brady and Seth, 2017; Godwin and Cotner, 2018). For example, biogeography and variability of mineral elements in higher plant leaves have been well-studied and the "restrictive element stability hypothesis" has been posed (Han et al., 2005, 2011). Studies on terrestrial microbial geography mainly focus on the geographical variation of microbial community structure (Garcia-Pichel et al., 2013), while geographical variations in its biochemical composition and mineral elements (stoichiometric characteristics) are rarely reported.

The main limiting factor for the study of geographical variations in terrestrial microbial biochemical composition and mineral elements is the availability of adequate microbial biomass. Microbial biomass in soil at both the community and species levels has been difficult to determine. The separation of soil microorganisms from soil particles and organic matter is difficult. Even if a small amount of biomass can be obtained, its content is too small to carry out detailed biochemical composition analysis or mineral element analysis. On the other hand, the biochemical composition and mineral elements of microbial biomass from artificial propagation may change relative to naturally growing microorganisms due to the difference in its environment (Dawson, 1919).

Nostoc commune, a form species, is one of the few soil microorganisms that can gain a large amount of biomass in its natural state. Along with two other form species, *Nostoc flagelliforme* and *Nostoc sphaeroids*, it is usually considered as belonging to the same genetic species (Wright et al., 2001). *N. commune* live mainly on the soil surface and are geographically distributed from polar valleys to tropical regions (Dodds et al., 1995). It is a filamentous cyanobacteria, which not only falls within prokaryotes, but also has chlorophyll and can carry out photosynthesis (Dodds et al., 1995). In the natural state, the filaments of *N. commune* and their secreted extracellular polysaccharides (EPS) can form a colony structure with large biomass, and is a traditional food in China, East Asia, and some African countries (Briones-Nagata et al., 2007) (**Supplementary Figure 1**).

China has large gradients of vegetation and has north-south and east-west gradients in climate, soil substrate materials, and atmospheric wet deposition mineral concentrations (Han et al., 2011; Zhu et al., 2016a,b). *N. commune* can be found in almost all areas of China and achieves a large biomass, so it is a great candidate for the study of soil microbial geography and of biochemical composition and mineral elements (stoichiometric characteristics) at the species level.

In the present study, we focused on 15 mineral elements of *N. commune* (**Figure 1**) and investigated the geographical variation of mineral elements of soil microorganisms at the species level in mainland China. First, we addressed the following questions: (1) from where are these mineral elements, (2) are there geographical variations for these mineral elements in *N. commune* like those of higher plants or aquatic algae, and (3) which environmental factors dominate the geographical variation of these mineral elements? Second, we tested whether the soil cyanobacterial mineral elements followed the higher plants "restrictive element stability hypothesis" (Han et al., 2011), which implies that variability is lowest for elements that are required in the highest concentrations. We divided the 15 mineral elements according to their physiological necessity: macroelements, microelements, and heavy metals (**Supplementary Figure 1**). Finally, we explored the effect of geographic variation of mineral element content on the ecological adaptation of soil cyanobacteria.

MATERIALS AND METHODS

Sample Collection and Pretreatment

We collected *N. commune* samples from 30 different counties, distributed in 16 provinces or autonomous regions in mainland China (**Supplementary Figure 2**, **Supplementary Table 1**). To minimize the influence of seasonal aspect on the variation of sample characteristics, all the samples were collected in the late spring in 2011 and 2012. In each sampling area, 5–10 samples from different microclimates and soil textures were collected to make one composite sample to minimize the heterogeneity of the microenvironment. For each composite sample, more than one kilogram of dry matter was collected to minimize the influence of life-history differences of the samples. We obtained a total of 33 composite samples for our study. All fresh samples were washed with distilled water, air dried, and stored in the desiccators for further analyses.

Biochemical Composition and Mineral Content Analysis

We measured crude protein and ash according to the methods prescribed by the Chinese Standard Agency for food as described by Hao et al. (2011) (**Supplementary Table 2**). More specifically, crude protein was estimated using the Kjeldahl method and ash content was expressed as the percentage of residue remaining after dry oxidation at $550 \pm 25^{\circ}C$ (Hori et al., 1990). Total organic carbon (TOC) was measured by the potassium dichromate method (**Supplementary Table 2**).

The total nitrogen (N) in the *N. commune* was measured using the semi-micro Kelvin method (**Supplementary Table 3**). The total phosphorus (P) was determined by molybdenum blue colorimetry (**Supplementary Table 3**). Potassium (K), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), cobalt (Co), selenium (Se), calcium (Ca), lead (Pb), cadmium (Cd), and chromium (Cr) were measured following the

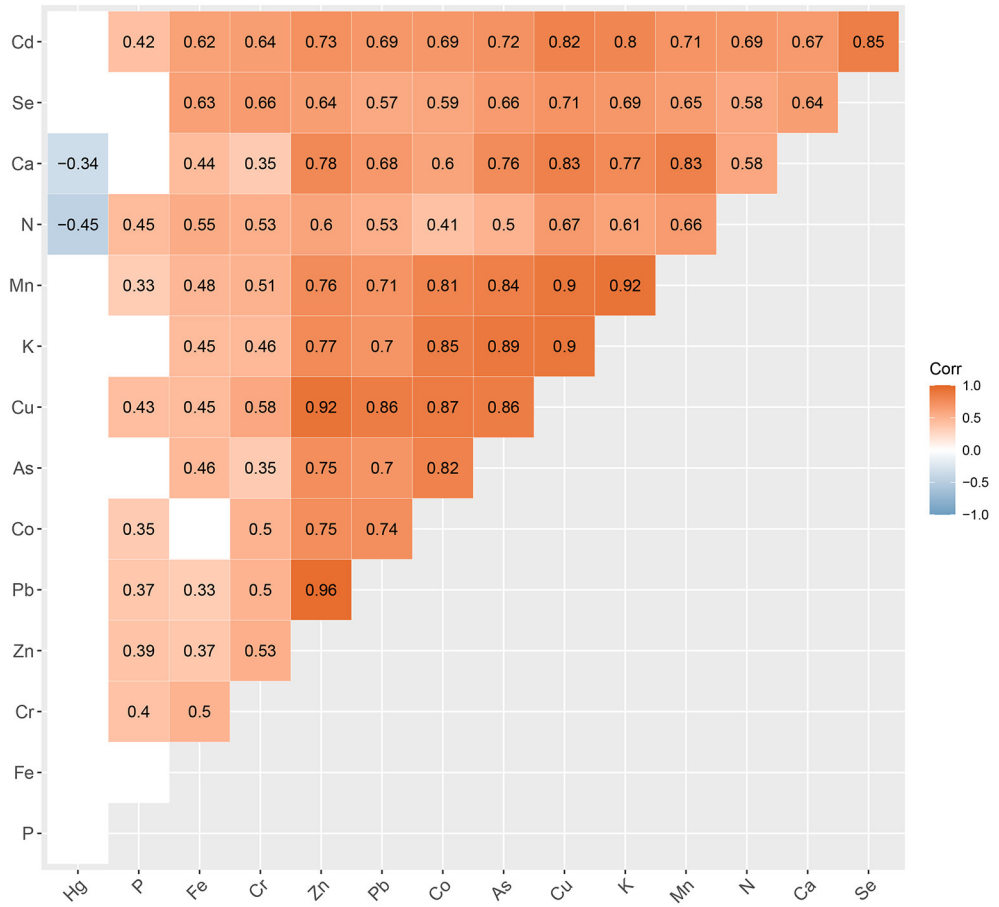


FIGURE 1 | Correlation heat map of the 15 elements analyzed in our study. Levels of statistical significance $p < 0.05$ are not shown.

procedure previously described by Tamasi et al. (2013) with flame atomic absorption spectrophotometers Perkin-Elmer 5000 and Perkin-Elmer 300 (Perkin-Elmer, Monza, Italy) (**Supplementary Table 3**). Mercury (Hg) concentrations were analyzed following the U.S. EPA Method 1630, including pre-digestion with 0.5% (v/v) 0.2 N bromine monochloride, reduction with hydroxylamine hydrochloride, further reduction with tin chloride, purging of Hg onto gold traps, and quantification of Hg by cold vapor atomic fluorescence spectrometry (CVAFS, Tekran Model 2,500 Hg Analyzer, Knoxville, USA) (Rothenberg et al., 2012). For quantification of arsenic content, dry matter samples were predigested with a mixture of nitric acid and perchloric acid. Arsenic content in the resulting solution was determined by atomic absorption spectrophotometry (Spectr AA220, Varian Medical Systems, Inc., USA) (de Freitas-Silva et al., 2016).

Climate Variables, Soil Data, and Atmospheric Deposition Data

In this study, we collected eleven climatic variables (**Supplementary Table 4**) to analyze the climatic controls on the spatial patterns of the 15 mineral elements and biochemical composition of *N. commune*: mean annual temperature (MAT; °C), mean annual precipitation (MAP; mm), mean annual solar radiation (MAR; MJ m^{-2} day^{-1}), mean maximum annual temperature (MATmax; °C), mean minimum annual temperature (MATmin; °C), mean annual precipitation days (MAPD, d), relative humidity (RH, %), frost free days (FFD, d), mean annual evaporation (MAE, mm), drought index (DI), and UV radiation (MJ m^{-2} $year^{-1}$). Climate data for the period 1981–2010 were compiled from the Chinese National Meteorological Information Center.

Mean soil mineral elements concentrations in China were obtained from the national soil survey (http://vdb3.soil.csdb.cn/) (**Supplementary Table 5**). Mean plant leaf mineral elements concentrations in China were from Han et al. (2011) (**Supplementary Table 5**). Soil TOC, N, P, C/N, N/P, and K data for each sample area were obtained from the national soil survey (http://vdb3.soil.csdb.cn/), and other soil minerals were from the soil pollution condition investigation communiqué (Environmental Protection Department of and The Ministry of Land and Resources of the People' s Republic of China, 2014; Chen et al., 2015) (**Supplementary Table 6**).

Mean atmospheric total deposition data for mineral concentrations were obtained from Pan and Wang (2014) (**Supplementary Table 7**). The mean atmospheric wet deposition

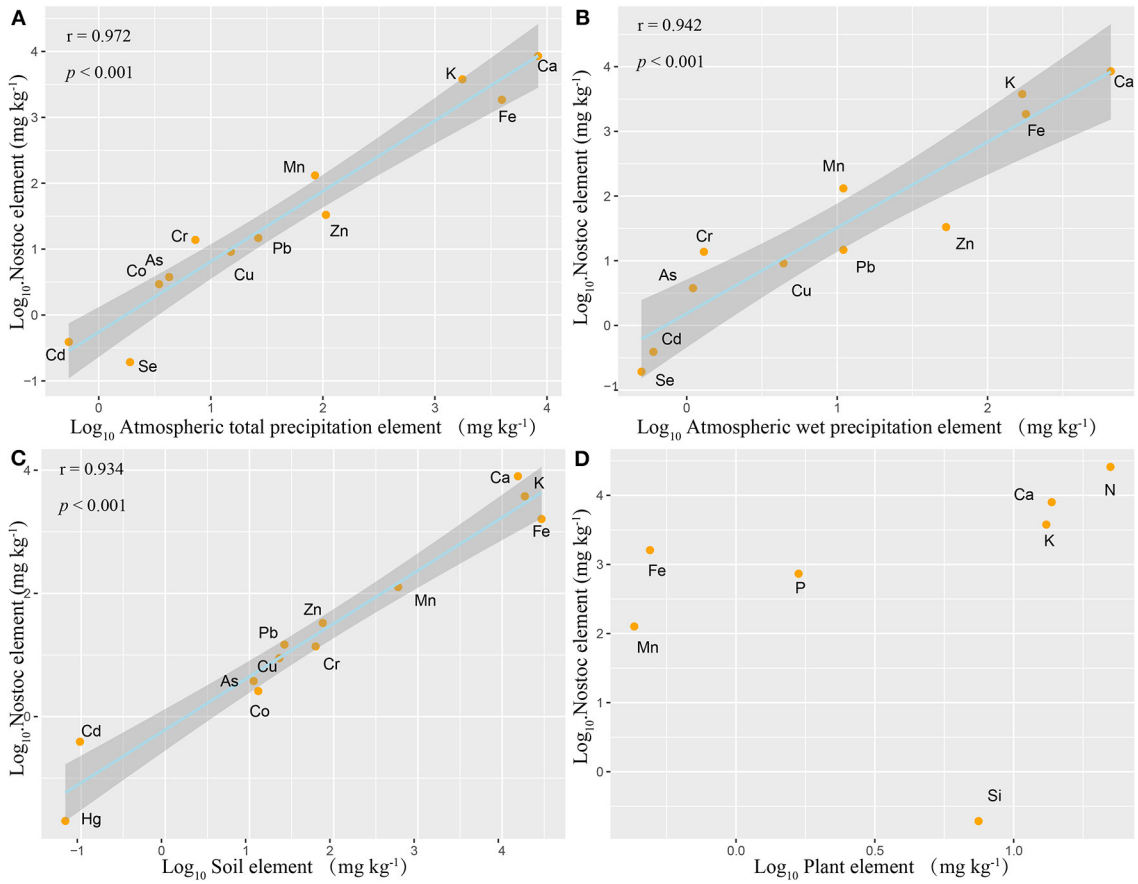


FIGURE 2 | Relationships between mean *N. commune* and plant leaf mineral concentration and atmospheric deposition and soil mineral concentration. **(A)** Nostoc vs. Atmospheric total deposition, $Log_{10}(y) = 1.069\ Log_{10}(x) - 0.258$ ($r^2 = 0.972$, $p < 0.001$); **(B)** Nostoc vs. Atmospheric wet deposition, $Log_{10}(y) = 0.669\ Log_{10}(x) - 0.007$ ($r^2 = 0.942$, $p < 0.001$); **(C)** Nostoc vs. Soil, $Log_{10}(y) = 0.861\ Log_{10}(x) - 0.213$ ($r^2 = 0.934$, $p < 0.001$); **(D)** Nostoc vs. Plant leaf. Ninety fine percentage of confidence bands for all fitted curves are also shown.

data for mineral concentrations were obtained from observation by Michaelis (1997). Part of the chemical characteristics of wet deposition (Ca, K) for our sample areas were obtained from Xie and Xue (2012) (**Supplementary Table 8**). The other wet deposition minerals (N, P, Cr, Pb, Cd), pH, and total salinity were estimated using the Kriging extrapolation method by the software ArcGIS 10, and the original data were from the Chinese Ecosystem Research Network (CERA) (Zhu et al., 2016a,b) (**Supplementary Table 8**).

Data Analysis

All *N. commune* mineral concentrations were log_{10}-transformed before analyses to improve the normality of the data. As the protein concentration is relatively stable, elemental concentrations relative to protein were used as an index of stoichiometry. The preliminary statistical analyses were first performed to screen the environmental factors. Those factors (MAPD, RH, FFD, MAE, and DI) that had high collinearity (VIF > 10) with other environmental factors and those (altitude; UV radiation; soil TOC, C/N, and N/P) that had no significant correlations with the mineral elements of *Nostoc commune* were removed from the next statistical analyses. To explore the possible geographic variation of Nostoc minerals, we performed Pearson correlation and Spearman's rank correlation between Nostoc minerals and geographic characteristics (distance from the equator and distance from the prime meridian).

Linear regressions and stepwise multiple regressions were used to determine the most influential climate variables among the five climate variables (MAP, MAT, MAR, MAT_{max}, MAT_{min}). Pearson correlation and Spearman's rank correlation were performed between Nostoc minerals and MAP and MAT. To explore the possible effects of soil and atmospheric wet deposition, Spearman's rank correlation was performed between Nostoc minerals and the corresponding soil mineral contents and the mineral concentrations of atmospheric wet deposition.

We conducted redundancy analysis (RDA) to study the relationship between elements of significant geographic variation and their related environment variables. Variation partitioning was performed to explain the importance of climate variables, soil variables and atmospheric wet

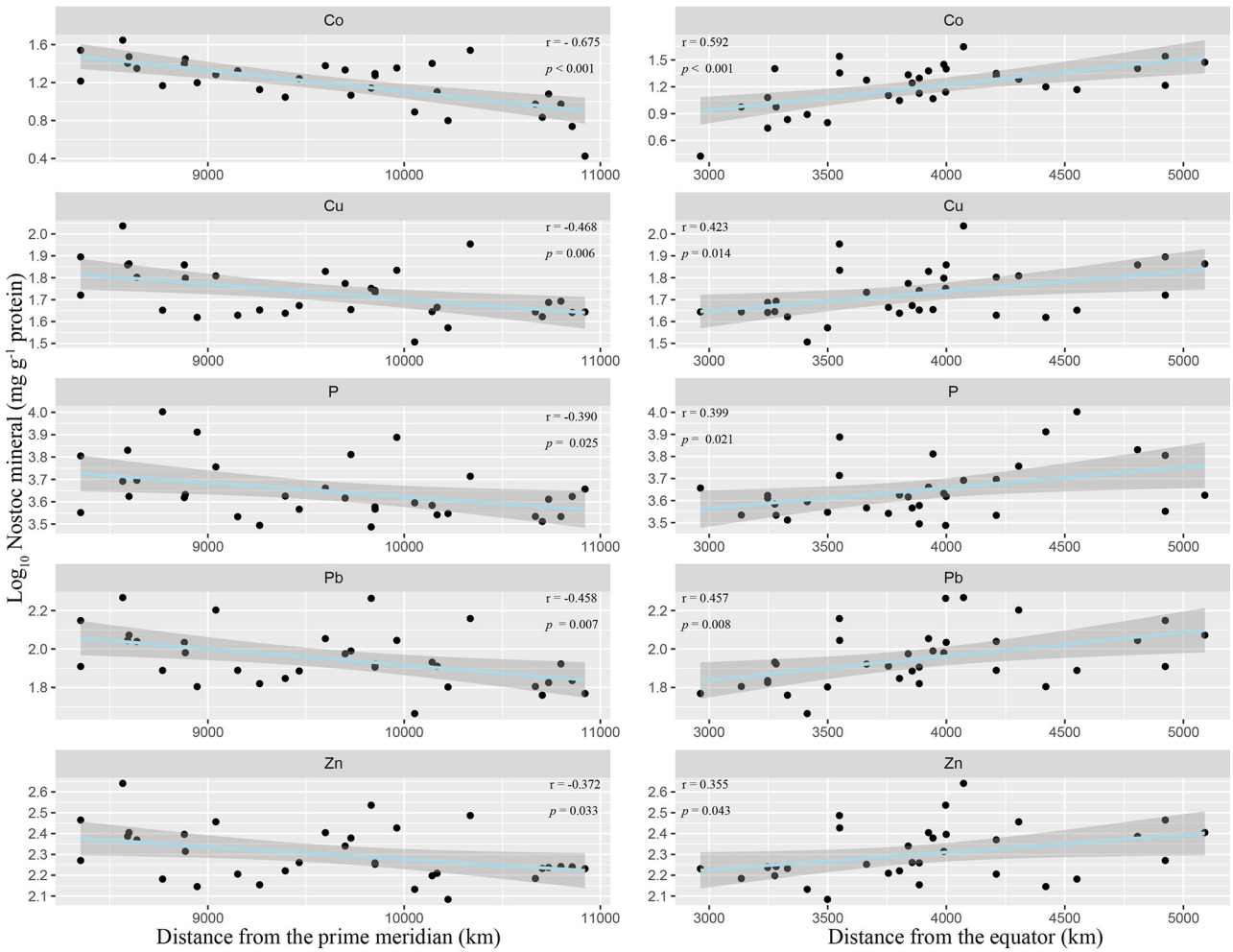


FIGURE 3 | Geographic patterns of *N. commune* minerals in China. Five minerals show significant latitudinal (distance from the equator at certain longitude) and longitudinal (distance from the prime meridian at certain latitude) linear correlations ($p < 0.05$).

deposition variables. Meanwhile, for each mineral element which had significant geographic variation, stepwise multiple regressions were implemented to identify the most influential environmental variables.

All analyses were performed with statistical software IBM SPSS 23 (IBM Corp, Armonk) and R 4.0.2 (R Development Core Team, 2020) using the "vegan" and "ggplot2" packages and custom scripts.

RESULTS

Statistics and Mineral Source Analysis

The 15 mineral elements in this study include 5 macroelements (N, Ca, K, Fe, P), 5 microelements (Mn, Zn, Cu, Co, Se), and 5 heavy metals (Pb, Cr, As, Cd, Hg) (**Supplementary Figure 3**). The mean values of 15 mineral elements varied greatly, from 0.02 mg kg^{-1} dry weight of Hg to 261.79 g kg^{-1} dry weight of N (**Supplementary Table 9**).

The content of some heavy metals was much higher than that of trace elements, such as the contents of Pb (15.66 mg kg^{-1}) and Cr (14.74 mg kg^{-1}) were higher than Cu (9.12 mg kg^{-1}), as (4.01 mg kg^{-1}) was higher than Co (2.94 mg kg^{-1}), and Cd (0.40 mg kg^{-1}) was higher than Se (0.20 mg kg^{-1}) (**Supplementary Figure 3**, **Supplementary Table 9**). There were significant positive correlations between the contents of some mineral elements, especially the divalent cations Zn, Cu, and Pb, and their correlation coefficients were all over 0.80 (Pb & Zn, $r = 0.93$; Pb & Cu, $r = 0.88$; Zn & Cu, $r = 0.90$) (**Figure 1**).

The element ratio of *N. commune* was similar to the compositions of atmospheric total deposition minerals ($r^2 = 0.97$, $p < 0.001$) and wet deposition minerals ($r^2 = 0.94$, $p < 0.001$) (**Figures 2A,B**), and is similar to the composition of soil minerals ($r^2 = 0.96$, $p < 0.001$) (**Figure 2C**). However, the element ratio of *N. commune* is completely different than that of higher plants (**Figure 2D**), and the element ratio of higher plants and the soil in which they grow is also different.

TABLE 1 | Correlations between Nostoc biochemicals and minerals with climate variables.

Biochemicals and minerals (mg g^{-1}protein)	MAP (mm)				MAT (°C)			
	Pearson correlation		Spearman's rank correlation		Pearson correlation		Spearman's rank correlation	
	r	*p*	*ρ*	*p*	*r*	*p*	*ρ*	*p*
Ash	−0.332	0.068	−0.380	0.035*	−0.380	0.035*	−0.435	0.014*
TOC	−0.063	0.728	−0.241	0.176	−0.025	0.892	−0.219	0.221
N	−0.001	0.997	0.003	0.985	0.076	0.673	0.015	0.932
Ca	0.036	0.841	−0.019	0.918	−0.103	0.569	−0.099	0.584
K	−0.353	0.044*	−0.361	0.039*	−0.311	0.078	−0.354	0.043*
Fe	0.180	0.316	0.154	0.392	0.227	0.205	0.232	0.194
P	−0.405	0.019*	−0.429	0.013*	−0.380	0.029*	−0.393	0024*
Mn	−0.277	0.118	−0.341	0.052	−0.319	0.070	−0.371	0.034*
Zn	−0.383	0.028*	−0.347	0.048*	−0.364	0.037*	−0.393	0.024*
Cu	−0.449	0.009**	−0.424	0.014*	−0.482	0.005**	−0.471	0.006**
Co	−0.636	0.000***	−0.584	0.000***	−0.605	0.000***	−0.617	0.000***
Se	−0.017	0.923	−0.118	0.513	−0.200	0.265	−0.239	0.181
Pb	−0.474	0.005**	−0.423	0.014*	−0.418	0.015*	−0.431	0.012*
Cr	−0.327	0.063	−0.274	0.123	−0.252	0.157	−0.171	0.341
As	−0.296	0.094	−0.286	0.107	−0.326	0.064	−0.262	0.140
Cd	−0.200	0.265	−0.149	0.408	−0.221	0.217	−0.142	0.430
Hg	0.017	0.923	−0.175	0.329	−0.023	0.898	−0.154	0.392

Levels of statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Biogeographic Patterns of Nostoc Minerals and Environmental Influence

Among the 15 elements, 5 elements (P, Cu, Zn, Co, Pb) showed significant geographical variation (**Figure 3**, **Supplementary Table 10**). They increased with the increase of the distance from the equator and decreased with the increase of the distance from the prime meridian (**Supplementary Table 10**). What is noteworthy is that the increase of these elements was not accompanied by the decrease of other elements. Meanwhile, the ash content showed similar geographical variation (**Supplementary Table 10**), and the five elements (K, Cu, Zn, Co, Pb) were positively correlated with the ash content (**Supplementary Figure 4**).

Six minerals (P, K, Cu, Zn, Co, Pb) were significantly and negatively correlated with MAP (**Table 1**, **Supplementary Table 11**, **Supplementary Figure 5**), and 5 (P, Cu, Zn, Co, Pb) of these were also significantly and negatively correlated with MAT (**Table 1**, **Supplementary Table 11**). In addition to MAP and MAT, we also introduced MAR, MAT_{max}, and MAT_{min} climate variables to conduct a stepwise regression analysis for these 6 elements (**Supplementary Table 12**). The results showed that for most elements (P, K, Zn, Co, Pb), MAP could explain all the variations, and other climate factors were removed in the stepwise regression process. But for Cu, MAT can explain all the variations, and other climate factors were removed in the stepwise regression process.

Spearman rank's correlation were conducted for the content of different mineral elements in *N. commune* and their corresponding soil elements, and the results showed that only the content of P was significantly and positively correlated with the content of soil P, while the other elements were not significantly correlated or were negatively correlated (**Table 2**). Among the 15 elements analyzed, the contents of Co and Pb were significantly and positively correlated with soil pH (**Supplementary Table 13**).

We also conducted Spearman rank's correlation for the content of different mineral elements in *N. commune* and their corresponding atmospheric wet deposition elements. We found that no element was significantly and positively correlated with the content of the corresponding wet deposition elements (**Table 2**). In addition, no significantly and positively correlations were found between the content of mineral elements and the total salinity of atmospheric wet deposition. However, among the 15 elements analyzed, 3 elements (Zn, Cu, Pb) were positively correlated with rainfall pH (**Supplementary Table 13**).

RDA analysis was used to study the relationships between 6 elements (P, K, Cu, Zn, Co, Pb) and their associated environmental factors, and we found that MAP and MAP were the most important environmental factors (**Figure 4A**). The variance partitioning showed that environmental factors can explain 22.8% of the variation, the variation is mainly explained by climate factors (8.5%), the interactive effect of climate and soil (9.8%), and the interactive effect of climate, soil and atmospheric wet precipitation (4.5%) (**Figure 4B**). Stepwise regression analysis was then conducted (**Table 3**), and the results showed that MAT explained all the variations of the four elements P, Zn, Co, and Pb, while MAT explained all the variations for Cu.

TABLE 2 | Spearman's rank correlations (ρ) between Nostoc minerals and soil minerals and atmospheric wet precipitation minerals.

N = 33	Soil minerals		Atmospheric wet precipitation minerals	
	ρ	*p*	*ρ*	*p*
N	−0.074	0.683	0.182	0.311
Ca	NA	NA	−0.057	0.753
K	0.281	0.147	0.131	0.468
P	0.351	0.045*	−0.339	0.054
Zn	−0.409	0.018*	NA	NA
Cu	−0.534	0.001**	NA	NA
Pb	−0.378	0.030*	−0.521	0.002**
Cr	−0.020	0.914	−0.164	0.361
As	0.084	0.641	NA	NA
Cd	−0.029	0.871	−0.132	0.463
Hg	−0.160	0.373	NA	NA

*Nostoc minerals (mg g^{-1} protein), soil minerals (mg g^{-1}), and atmospheric wet precipitation minerals [N, Ca, K, P (μmol l^{-1}), Pb, Cr, Cd (mg m^{-2})] are log_{10}-tranformed before analysis. Levels of statistical significance: *p < 0.05, **p <0.01.*

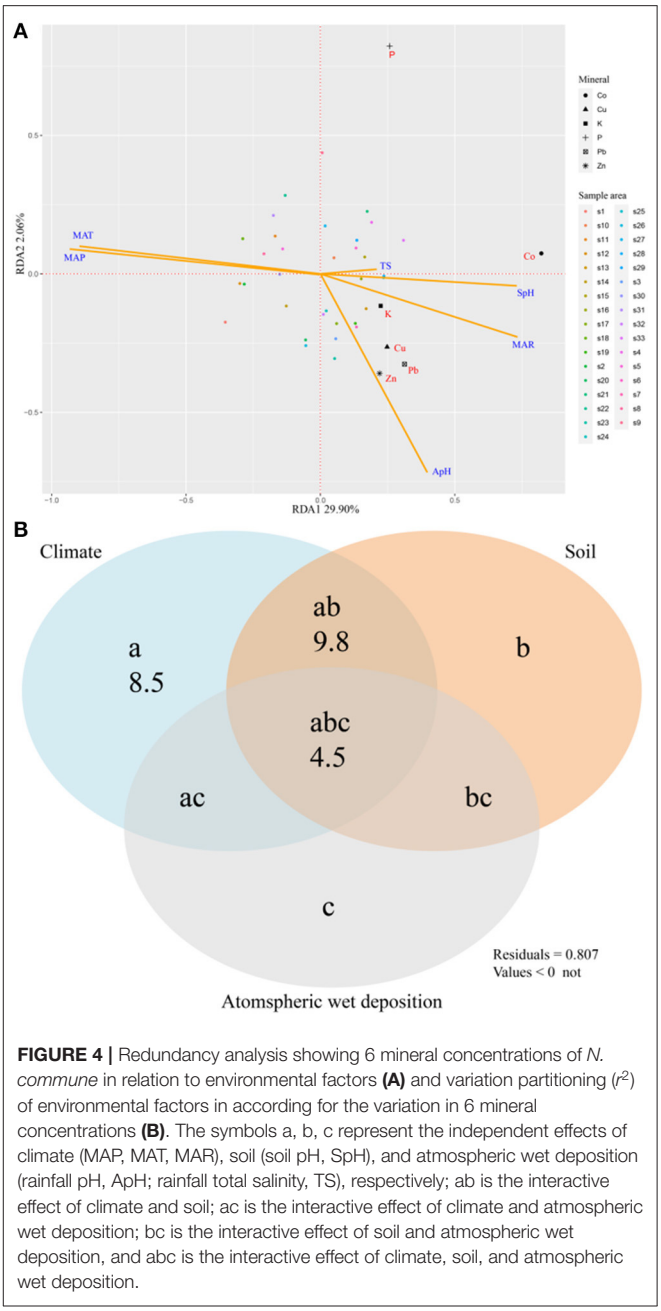


FIGURE 4 | Redundancy analysis showing 6 mineral concentrations of *N. commune* in relation to environmental factors **(A)** and variation partitioning (r^2) of environmental factors in according for the variation in 6 mineral concentrations **(B)**. The symbols a, b, c represent the independent effects of climate (MAP, MAT, MAR), soil (soil pH, SpH), and atmospheric wet deposition (rainfall pH, ApH; rainfall total salinity, TS), respectively; ab is the interactive effect of climate and soil; ac is the interactive effect of climate and atmospheric wet deposition; bc is the interactive effect of soil and atmospheric wet deposition, and abc is the interactive effect of climate, soil, and atmospheric wet deposition.

Stability of Limiting Mineral Elements

In this study, the element ratio of cyanobacteria BG11 medium (**Supplementary Table 14**) was selected as the nutrient requirement ratio, macroelements were generally considered as limiting elements, trace elements were considered to be non-limiting elements, and heavy metals were considered to be non-essential elements. The coefficient of variation was used to represent the relative variation (stability) of elements. We found that the element ratio of the samples and nutrient requirement of *N. commune* was roughly the same ($r^2 = 0.93$, $p < 0.001$, **Figure 5B**). However, the variation coefficients of different elements were not significantly correlated with their measured contents [**Figure 5C** (heavy metals included); **Figure 5D** (heavy metals not included)], and they were not significantly correlated with required nutrient contents (**Figure 5A**).

DISCUSSION

Absorption (Intracellular) and Adsorption (Extracellular) of Mineral Elements in Cyanobacteria

The mineral element content in cyanobacteria comes from two sources, the physiological absorption/secretion process and the physiochemical adsorption and desorption process (Volesky and Holan, 1995) (**Figure 6**). The physiological absorption and secretion process are an active, energy-consuming metabolic process that mainly occurs in the cell membrane and requires that the cyanobacteria have metabolic activity (Gadd, 1990). Some of the physiologically absorbed mineral elements participate in metabolic activities as physiologically essential elements, and some are stored in the cells for reserve (luxury consumption) (Brown and Shilton, 2014). When environmental conditions change, cyanobacterial cells can also secrete mineral elements to maintain osmotic balance or expel toxic and harmful elements (Kaplan, 2013).

Cyanobacteria physiochemical adsorption and desorption is a passive process that mainly occurs in extracellular polymers (EPS), runs by ion exchange or by binding positive metals with negative charged carboxylic groups in EPS (Davis et al., 2003), and does not require the metabolic activity of cyanobacterial cells (Davis et al., 2003). It can also be carried out in dead algal colonies. Mineral physiochemical desorption occurs when the pH value of the water decreases and ion exchange drive this process (Blanco et al., 1998). Mineral elements passively adsorbed by algae can be further actively absorbed by cyanobacterial cells, and

TABLE 3 | Linear regressions of *N. commune* minerals vs. climate, soil, and atmospheric wet deposition (AWP) characteristics.

	Climate		AWP	Soil		
	MAP	MAT	TS	pH	pH	Stepwise multiple Regression (SMR)
***N* = 33**	**1 Model (Adj. r^2)**	**2 Model (Adj. r^2)**	**3 Model (Adj. r^2)**	**4 Model (Adj. r^2)**	**5 Model (Adj. r^2)**	**6 Model (Adj. r^2) (predictive variable)**
P	0.137	0.117	0.110			0.137 (MAP)
K	0.097					
Zn	0.119	0.104				0.119 (MAP)
Cu	0.176	0.207				0.207 (MAT)
Co	0.384	0.346		0.263		0.384 (MAP)
Pb	0.200	0.148		0.105		0.200 (MAP)

MAP (mean annual precipitation, mm) enters model 6 (stepwise multiple regression, SMR) before other characteristics, indicating that MAP is more powerful in explaining variations in the Nostoc mineral than is rainfall TS (total salinity), rainfall pH, soil pH. All $p < 0.05$ in models are shown in the table.

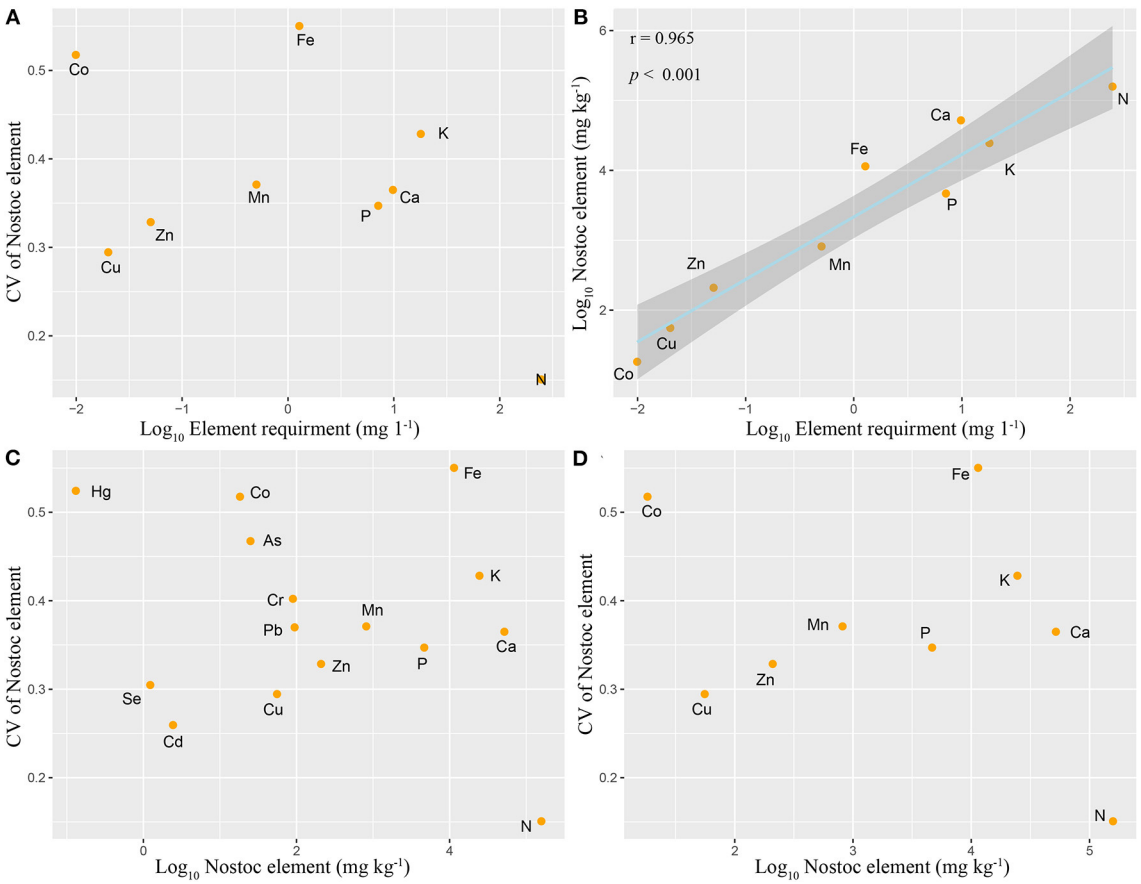


FIGURE 5 | Relationships between *N. commune* elemental requirement, mean *N. commune* elemental concentration, and its coefficient of variation (CV). **(A)** CV vs. Elemental requirement; **(B)** Nostoc element (heavy metals not included) vs. Elemental requirement, $Log_{10}(y) = 0.893\ Log_{10}(x) + 3.333$ ($r^2 = 0.965$, $p < 0.001$); **(C)** CV vs. Nostoc element (heavy metals included); **(D)** CV vs. Nostoc element (heavy metals not included). Ninety fine percentage of confidence bands for all fitted curves are also shown.

the mineral elements actively secreted by algal cells can be passive re-adsorbed in EPS (Fiore and Trevors, 1994; Kaplan, 2013).

Source Analysis of Mineral Elements in *N. commune*

N. commune has no root, stem, or leaf differentiation and no specialized absorption tissue, so it is not possible for them to directly absorb various mineral elements from the soil subsurface (Dodds et al., 1995). The element source may come from rainfall (atmospheric wet deposition), or soil surface runoff induced by rainfall, in which case the element composition is affected by rainfall as well as soil composition. We found that the element ratio of Nostoc elements was remarkably similar to that of atmospheric deposition (total deposition and wet deposition)

and soil elements, which confirmed that the mineral elements mainly came from rainfall or the soil. However, the elements ratio did not change during physiological activities, as some minerals were enriched and some were diluted. This conservation of the elemental ratio was different from higher plants, whose elemental ratio is significantly altered according to the soil in which they grow. There may be two reasons for this difference. First, the content of physiochemical and passively adsorbed mineral elements far exceeds physiological demand. The ratio of mineral elements of *N. commune* measured in this study is mainly from the ratio of passively adsorbed mineral elements. Secondly, both the ratio of bioaccumulated mineral elements (physiologically required and stored) and passively adsorbed mineral elements are similar to atmospheric rainfall and soil surface runoff, which is the result of long-term evolution of cyanobacteria. Our results showed that the ratio of essential elements in cyanobacteria (BG11 medium) was the same as the elemental ratio of rainfall ($r^2 = 0.78$, $p = 0.002$). Therefore, our study was more inclined to support the second reason.

Geographical Variation of Elements and Environmental Determinants

Among the 15 elements investigated, 5 mineral elements showed significant geographical variation, while the others showed no significant geographical variation. Our results also showed that *N. commune* had a preference for the absorption and adsorption of different mineral elements. These preferences may be related to physiological needs (regional environmental adaptation) of *N. commune*, or the absorption and adsorption process and mechanisms for different mineral elements. For example, P was usually absorbed as a luxury (Brown and Shilton, 2014), while some divalent cation such as Cu, Pb, and Zn are the main elements of passive adsorption (Davis et al., 2003), and Co plays an important role in biological nitrogen fixation of cyanobacteria (De Philippis et al., 2001).

Our results showed that there was no significant positive correlation between the contents of these elements in *N. commune* (except P, who showed a positive correlation with soil P) and their contents in the natural environment (including rainfall and soil), which indicated that passive adsorption was not the main factor determining its geographical variation. However, the contents of 2 and 3 of these 5 elements in *N. commune* were positively correlated with the pH values of rainfall and soil, respectively, indicating that passive desorption may have a certain impact on the geographical variation of mineral elements, because acidic conditions can result in competition between free metal ions and H^+ for the same uptake sites, leading to an increase in cell desorption (Fiore and Trevors, 1994).

Among all the environmental factors, climatic factors can explain the variation of the geographically variable mineral elements (P, Cu, Zn, Co, Pb), which decreased with decreases in temperature and rainfall. This geographical variation may be related to the adaptation of *N. commune* to drought and low temperature conditions, which is regulated by active physiological demand. In addition to the above five mineral elements with significant geographical variation, the content of K in *N. commune* was also significantly correlated with MAP and MAT. It increased with increased drought and decreased temperature. As K is often a limiting element in the environment, its increase (luxury absorption) under drought or low temperature stress may also be actively regulated by physiological demand (Qiu et al., 2004).

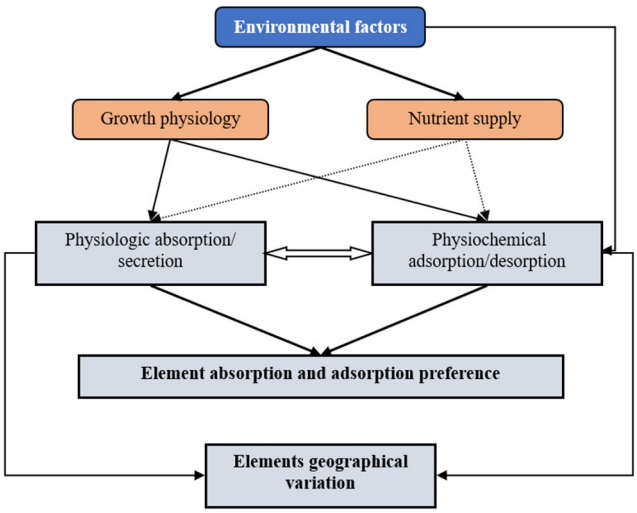


FIGURE 6 | The schematic diagram showing environmental factors control the mineral patterns of *N. commune*. Environmental factors (especially precipitation and temperature) shape the element absorption and adsorption preference and the elements geographical variation of *N. commune*. Environmental factors (especially precipitation and temperature) can indirectly influence physiologic absorption/secretion and physiochemical adsorption/desorption by regulating growth physiology of *N. commune*, and can also directly influence the physiochemical adsorption/desorption of *N. commune*.

Our results supported the "growth rate hypothesis" (Allen and Gillooly, 2009; Garcia et al., 2016), which implied that all the mineral geographic variation is adjusted by the physiological demand. Due to the mismatching of mineral concentrations of Nostoc with their environments, the results did not support the "environmental nutrient supply hypothesis" (Reich and Oleksyn, 2004). Meanwhile, for *N. commune*, passive desorption determined by environment pH supplied another path for the regulation of its stoichiometry (**Figure 6**).

Element Physiological Requirement and Stability of Limiting Elements

We found that the elemental ratio of *N. commune* was basically the same as that of the BG11 medium, indicating that under natural condictions, the physiological requirements of *N. commune* were basically the same as those of other cyanobacteria. There was no particularity for *N. commune*. Therefore, the results of this study can be expanded to other cyanobacteria or even other soil microorganisms.

Depending on the "restrictive element stability hypothesis," macroelements are usually limiting elements, while microelements are non-limiting elements. Variation of elements

is inversely proportional to the element content, that is to say, the variation of largely required elements is small (Han et al., 2011). Our study not only investigated the variability of macroelements and microelements, but also investigated the variability of heavy metals. According to the hypothesis inference, the variability of heavy metals should be the highest because there is no physiological need for them, and the variability of microelements and macroelements was minimal. However, the statistical results of our experiment do not support the stability of limiting elements hypothesis. We showed that there was no significant correlation between the variation coefficients of different elements and their actual detected contents and their potential physiological required contents. We speculate that active luxury absorption and passive adsorption may be the primary reasons for the departure from the "restrictive element stability hypothesis" (Patova et al., 2000; Patova and Sivkov, 2003).

The Adaptability of *N. commune* to Regional Environment and the Response to Global Change

Higher plants have a relatively narrow geographical range compared to cyanobacteria, which has a higher dispersal ability and longer evolutionary age that contribute to cyanobacteria attaining a larger range (Slatyer et al., 2013). Cyanobacteria has evolved relatively perfect environmental adaptability, so that individual species have a wider geographic distribution. The evolution of Nostoc species has endured more extreme and complex climate changes than today and have survived successfully (Sand-Jensen and Jespersen, 2012). They not only have high genetic diversity (OTU > 97% related to the definition of species) (Walter et al., 2017), but also have a variety of physiological regulatory mechanisms (i.e., secretion of UV shielding pigment, secretion of large amounts of EPS, change of pigment composition ratio, and activation of antioxidant enzyme system) (Wang et al., 2013). Cyanobacteria, especially soil cyanobacteria *N. commune*, have enough tolerance capacity to be adapted to the current or future global changes.

Here, we found that the total carbon content did not increase significantly with increased drought and decreased temperature, which was different from previous observations in experimental control studies (Tamaru et al., 2005; Kehr and Dittmann, 2015; Chrismas et al., 2016). Therefore, we concluded that the increase of polysaccharides may be a short-term physiological adaptation, which overturns our traditional understanding. With increased drought and decreased temperature, there was a net increase of some mineral elements that led to an increase in ash content. Under drought and in low temperatures, *N. commune* can reduce the osmotic potential by increasing the mineral elements or the ash. Then it can rapidly absorb water when rainfall occurs, and rapidly grow and propagate under limited water resources. Therefore, the increase in mineral elements or ash may signal that *N. commune* has an adaptation mechanism for drought and low temperature environments, but this mechanism has not yet been reported.

CONCLUSIONS

In this study, *N. commune* samples were collected across gradients of climate, soil, and atmospheric wet deposition mineral concentration in mainland China and fifteen minerals, including five macroelements (N, Ca, K, Fe, P), five microelements (Mn, Zn, Cu, Co, Se), and five heavy metals (Pb, Cr, As, Cd, Hg), were measured. Among the 15 elements investigated, 5 mineral elements showed significant geographical variation, while the others showed no significant geographical variation. Climatic factors could explain the variation of the geographically variable mineral elements (P, Cu, Zn, Co, Pb), which decreased with decreases in temperature and rainfall. Because there was no significant correlation between the variation coefficients of different elements and their actual detected contents and their potential physiological required contents, the soil cyanobacterial mineral elements did not follow the "restrictive element stability hypothesis" of higher plants.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors contributed intellectual input to this study and manuscript preparation. WW and QZ conceived the idea and designed the study. HL and XS conducted mineral analysis and collected the data with help from RG, YY, and XC. WW wrote the paper with input from all authors.

FUNDING

This study was supported financially by the National Science Foundation of China (31870452, 31700337, and 31720103905).

ACKNOWLEDGMENTS

We thank 3 reviewers and Prof. Kerong Zhang for his comments and Dr. Russell Doughty for his language editing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.582655/full#supplementary-material

REFERENCES

Allen, A. P., and Gillooly, J. F. (2009). Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. *Ecol. Lett.* 12, 369–384. doi: 10.1111/j.1461-0248.2009.01302.x

Blanco, A., Llama, M. J., Sanz, B., and Serra, J. L. (1998). Reutilization of nonviable biomass of *Phormidium laminosum* for metal biosorption. *Biotechnol. Appl. Biochem.* 27, 167–174.

Brady, R. C., and Seth, G. J. (2017). The effect of iron limitation on cyanobacteria major nutrient and trace element stoichiometry. *Limnol. Oceanogr.* 62, 846–858. doi: 10.1002/lno.10484

Briones-Nagata, M. P., Martinez-Goss, M. R., and Hori, K. (2007). A comparison of the morpho-cytology and chemical composition of the two forms of the cyanobacterium, *Nostoc commune* Vauch., from the Philippines and Japan. *J. Appl. Phycol.* 19, 675–683. doi: 10.1007/s10811-007-9240-1

Brown, N., and Shilton, A. (2014). Luxury uptake of phosphorus by microalgae in waste stabilisation ponds: current understanding and future direction. *Rev. Environ. Sci. Bio/Technol.* 13, 321–328. doi: 10.1007/s11157-014-9337-3

Chen, H., Teng, Y., Lu, S., Wang, Y., and Wang, J. (2015). Contamination features and health risk of soil heavy metals in China. *Sci. Total Environ.* 512–513, 143–153. doi: 10.1016/j.scitotenv.2015.01.025

Chrismas, N. A. M., Barker, G., Anesio, A. M., and Sánchez-Baracaldo, P. (2016). Genomic mechanisms for cold tolerance and production of exopolysaccharides in the Arctic cyanobacterium *Phormidesmis priestleyi* BC1401. *BMC Genomics* 17:533. doi: 10.1186/s12864-016-2846-4

Davis, T. A., Volesky, B., and Mucci, A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res.* 37, 4311–4328. doi: 10.1016/S0043-1354(03)00293-8

Dawson, A. I. (1919). Bacterial variations induced by changes in the composition of culture media. *J. Bacteriol.* 4, 133–148. doi: 10.1128/JB.4.2.133-148.1919

de Freitas-Silva, L., de Araújo, T. O., da Silva, L. C., de Oliveira, J. A., and de Araujo, J. M. (2016). Arsenic accumulation in Brassicaceae seedlings and its effects on growth and plant anatomy. *Ecotoxicol. Environ. Saf.* 124, 1–9. doi: 10.1016/j.ecoenv.2015.09.028

De Philippis, R., Sili, C., Paperi, R., and Vincenzini, M. (2001). Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. *J. Appl. Phycol.* 13, 293–299. doi: 10.1023/A:1017590425924

Dodds, W. K., Gudder, D. A., and Mollenhauer, D. (1995). The ecology of Nostoc. *J. Phycol.* 31, 2–18. doi: 10.1111/j.0022-3646.1995.00002.x

Dormann, C. F., Schweiger, O., Augenstein, I., Bailey, D., Billeter, R., Blust, G. D., et al. (2007). Effects of landscape structure and land-use intensity on similarity of plant and animal communities. *Glob. Ecol. Biogeogr.* 16, 774–787. doi: 10.1111/j.1466-8238.2007.00344.x

Fierer, N., and Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. U.S.A.* 103, 626–631. doi: 10.1073/pnas.0507535103

Fiore, M. F., and Trevors, J. T. (1994). Cell composition and metal tolerance in cyanobacteria. *Biometals* 7, 83–103. doi: 10.1007/BF00140478

Gadd, G. M. (1990). Heavy metal accumulation by bacteria and other microorganisms. *Experientia* 46, 834–840. doi: 10.1007/BF01935534

Garcia, N. S., Bonachela, J. A., and Martiny, A. C. (2016). Interactions between growth-dependent changes in cell size, nutrient supply and cellular elemental stoichiometry of marine Synechococcus. *ISME J.* 10, 2715–2724. doi: 10.1038/ismej.2016.50

Garcia-Pichel, F., Loza, V., Marusenko, Y., Mateo, P., and Potrafka, R. M. (2013). Temperature drives the continental-scale distribution of key microbes in topsoil communities. *Science* 340, 1574–1577. doi: 10.1126/science.1236404

Godwin, C. M., and Cotner, J. B. (2018). What intrinsic and extrinsic factors explain the stoichiometric diversity of aquatic heterotrophic bacteria? *ISME J.* 12, 598–609. doi: 10.1038/ismej.2017.195

Han, W., Fang, J., Guo, D., and Zhang, Y. (2005). Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *N. Phytol.* 168, 377–385. doi: 10.1111/j.1469-8137.2005.01530.x

Han, W. X., Fang, J. Y., Reich, P. B., Woodward, F. I., and Wang, Z. H. (2011). Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecol. Lett.* 14, 788–796. doi: 10.1111/j.1461-0248.2011.01641.x

Hao, Z. J., Li, D. H., Li, Y. H., Wang, Z. C., Xiao, Y., Wang, G. H., et al. (2011). Nostoc sphaeroides Kützing, an excellent candidate producer for CELSS. *Adv. Space Res.* 2011, 48, 1565–1571. doi: 10.1016/j.asr.2011.06.035

Hori, K., Ueno-Mori, T., and Okita, T. (1990). Chemical composition, in vitroprotein digestibility and *in vitro* available iron of blue-green alga, Nostoc commune. *Plant Foods Hum. Nutr.* 40, 223–229.

Humphries, C. J., and Parenti, L. R. (1999). *Cladistic Biogeography, Interpreting Patterns of Plant and Animal Distributions*. 2nd ed. Oxford: Oxford University Press.

Kaplan, D. (2013). "Absorption and adsorption of heavy metals by microalgae," in *Handbook of Microalgal Culture: Applied Phycology and Biotechnology,* eds A. Richmond and Q. Hu (Chichester: Blackwell Publishing), 439–447 doi: 10.1002/9781118567166.ch32

Kehr, J. C., and Dittmann, E. (2015). Biosynthesis and function of extracellular glycans in cyanobacteria. *Life* 5, 164–180. doi: 10.3390/life5010164

Michaelis, W. (1997). "Trace Elements in Rainwater: Concentrations and wet deposition," in *Air Pollution*, ed W. Michaelis (Berlin: Springer-Verlag), 66. doi: 10.1007/978-3-642-60456-0_7

Pan, Y., and Wang, Y. (2014). Atmospheric wet and dry deposition of trace elements at ten sites in northern China. *Atmos. Chem. Phys. Discuss.* 14, 951–972 doi: 10.5194/acpd-14-20647-2014

Patova, E. N., and Sivkov, M. D. (2003). Accumulation of heavy metals by *Nostoc commune* Vauch. ex Bornet et Flahault (Cyanoprokaryota) in terrestrial tundra ecosystems of the Russian Arctic. *Algol. Stud.* 109, 469–473. doi: 10.1127/1864-1318/2003/0109-0469

Patova, E. N., Sivkov, M. D., and Getzen, M. V. (2000). The accumulation of heavy metals by terrestrial nitrogen-fixing alga *Nostoc commune* Vauch. in the East European Tundra. *Int. J. Algae* 2, 11–18. doi: 10.1615/InterJAlgae.v2.i3.20

Qiu, B., Zhang, A., Zhou, W., Wei, J., Dong, H., and Liu, Z. (2004). Effects of potassium on the photosynthetic recovery of the terrestrial cyanobacterium, *Nostoc Flagelliforme* (*Cyanophyceae*) during rehydration. *J. Phycol.* 40, 323–332. doi: 10.1111/j.1529-8817.2004.03152.x

Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho, T. Y., Reinfelder, J. R., et al. (2003). The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature* 425, 291–294. doi: 10.1038/nature01953

Ranjard, L., Dequiedt, S., Chemidlin Prévost-Bour,é, N., Thioulouse, J., Saby, N. P. A., Lelievre, M., et al. (2013). Turnover of soil bacterial diversity driven by wide-scale environmental heterogeneity. *Nat. Commun.* 4:1434. doi: 10.1038/ncomms2431

Ranjard, L., Dequiedt, S., Jolivet, C., Saby, N. P. A., Thioulouse, J., Harmand, J., et al. (2010). Biogeography of soil microbial communities: a review and a description of the ongoing french national initiative. *Agron. Sustain. Dev.* 30, 359–365. doi: 10.1051/agro/2009033

Reich, P. B., and Oleksyn, J. (2004). Global patterns of plant leaf N and P in relation to temperature and latitude. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11001–11006. doi: 10.1073/pnas.0403588101

Rothenberg, S. E., Feng, X., Zhou, W., Tu, M., Jin, B., and You, J. (2012). Environment and genotype controls on mercury accumulation in rice (*oryza sativa l.*) cultivated along a contamination gradient in Guizhou, China. *Sci. Total Environ.* 426, 272–280. doi: 10.1016/j.scitotenv.2012.03.024

Sand-Jensen, K., and Jespersen, T. S. (2012). Tolerance of the widespread cyanobacterium *Nostoc commune* to extreme temperature variations (−269 to 105°C), pH and salt stress. *Oecologia* 169, 331–339. doi: 10.1007/s00442-011-2200-0

Slatyer, R. A., Hirst, M., and Sexton, J. P. (2013). Niche breadth predicts geographical range size: a general ecological pattern. *Ecol. Lett.* 16, 1104–1114. doi: 10.1111/ele.12140

Tamaru, Y., Takani, Y., Yoshida, T., and Sakamoto, T. (2005). Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. *Appl. Environ. Microbiol.* 71, 7327–7333. doi: 10.1128/AEM.71.11.7327-7333.2005

Tamasi, G., Owens, N. F., Cascella, F., Cerqua, M., and Cini, R. (2013). A case study of selected volatile phenols from brettanomyces and micronutrients Mn, Fe, Cu, Zn in Chianti Red Wines. *J. Food Res.* 2, 31–40. doi: 10.5539/jfr.v2n1p31

Volesky, B., and Holan, Z. R. (1995). Biosorption of heavy metals. *Biotechnol. Prog.* 11, 235–250. doi: 10.1021/bp00033a001

Walter, J. M., Coutinho, F. H., Dutilh, B. E., Swings, J., Thompson, F. L., and Thompson, C. C. (2017). Ecogenomics and taxonomy of cyanobacteria phylum. *Front. Microbiol.* 8:2132. doi: 10.3389/fmicb.2017.02132

Wang, W., Wang, Y., Shu, X., and Zhang, Q. (2013). Physiological responses of soil crust-forming cyanobacteria to diurnal temperature variation. *J. Basic Microbiol.* 53, 72–80. doi: 10.1002/jobm.201100510

Wright, D., Prickett, T., Helm, R. F., and Potts, M. (2001). Form species *Nostoc commune* (*Cyanobacteria*). *Int. J. Syst. Evol. Microbiol.* 51, 1839–1852. doi: 10.1099/00207713-51-5-1839

Xie, N., and Xue, L. Y. (2012). The spatial sifferences of chemical characteristics of precipitation in seven regions of China. *Hubei Agric. Sci.* 51, 2971–2975. doi: 10.14088/j.cnki.issn0439-8114.2012.14.014

Yvon-Durocher, G., Dossena, M., Trimmer, M., Woodward, G., and Allen, A. P. (2015). Temperature and the biogeography of algal stoichiometry. *Glob. Ecol. Biogeogr.* 24, 562–570. doi: 10.1111/geb.12280

Zhou, W., Wang, Z., Davy, A. J., and Liu, G. (2013). Geographic variation and local adaptation in *Oryza rufipogon* across its climatic range in China. *J. Ecol.* 101, 1498–1508. doi: 10.1111/1365-2745.12143

Zhu, J. X., Wang, Q. F., He, N. P., Smith, M. D., Elser, J. J., Du, J. Q., et al. (2016a). Imbalanced atmospheric nitrogen and phosphorus depositions in China: implications for nutrient limitation. *J. Geophys. Res. Biogeosci.* 121, 1605–1616. doi: 10.1002/2016JG003393

Zhu, J. X., Wang, Q. F., Yu, H. L., Li, M. L., and He, N. P. (2016b). Heavy metals deposition through rainfall in Chinese natural terrestrial ecosystems: evidences from national-scale network monitoring. *Chemosphere*. 164, 128–133. doi: 10.1016/j.chemosphere.2016.08.105

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 26 March 2021
doi: 10.3389/fmicb.2021.618742

Comparative Study of the Rhizosphere and Root Endosphere Microbiomes of Cholistan Desert Plants

Salma Mukhtar, Samina Mehnaz and Kauser Abdulla Malik**

KAM School of Life Sciences, Forman Christian College (A Chartered University), Lahore, Pakistan

Microbial communities associated with the rhizosphere and roots of desert halophytes play an important role in plants' growth and development. Very limited information has been available on the microbial diversity of arid environments of Pakistan. Hence in the current study, the microbial diversity of rhizosphere and root endosphere of desert halophytes, *Zygophyllum simplex, Haloxylon salicoricum, Aerva javanica*, and *Capparis decidua* was evaluated. The rhizosphere and root endosphere samples of desert halophytes collected from the three geographic sites of Cholistan desert, Punjab, Pakistan were analyzed by using 16S rRNA based Illumina sequencing. The results showed that Proteobacteria were more abundant in the rhizospheric soils while Actinobacteria were more dominant in the root endosphere of halophytes. Bacteroidetes, Firmicutes, and Deinococcus-Thermus were identified from all rhizospheric soils and roots across the three sites, with variable percentage. *Bacillus, Kocuria, Pseudomonas, Halomonas*, and *Flavobacterium* were commonly identified from the rhizosphere and root endosphere of halophytes across all the three sites. At the genus level, microbial diversity from *Haloxylon* showed the greatest variations between the rhizosphere and root endosphere from the site 2. This study revealed that microbial diversity analysis can be used to study how changes in abiotic factors such as soil moisture content and salinity affect the microbial communities associated with the rhizospheric soils and root endosphere of halophytes across the three sites. This study will also help in the discovery of potential inoculants for crops growing in arid and semi-arid regions of Pakistan.

Keywords: halophytes, root endosphere, Illumina sequencing, 16S rRNA gene, halophilic bacteria

OPEN ACCESS

Edited by:
Philippe Vandenkoornhuyse, University of Rennes 1, France

Reviewed by:
Ajar Nath Yadav, Eternal University, India
Jie Hu, University of Rennes 1, France

****Correspondence:***
Kauser Abdulla Malik
kausermalik@fccollege.edu.pk
Salma Mukhtar
salmamukhtar85@gmail.com

Specialty section:
This article was submitted to Terrestrial Microbiology, a section of the journal Frontiers in Microbiology

Received: *18 October 2020*
Accepted: *04 March 2021*
Published: *26 March 2021*

Citation:
Mukhtar S, Mehnaz S and Malik KA (2021) Comparative Study of the Rhizosphere and Root Endosphere Microbiomes of Cholistan Desert Plants. Front. Microbiol. 12:618742. doi: 10.3389/fmicb.2021.618742

INTRODUCTION

Soil salinity is considered as one of the major abiotic pressures that affects more than 840 million hectares of agricultural area worldwide. Soil salinity continuously increase in arid and semi-arid regions globally. Salinization reduces plant growth and adversely affects the crops yield and productivity (Rengasamy, 2006; Etesami and Beattie, 2018). Halophytes growing in arid and saline environments play a vital role in the maintenance of soil composition by nutrient mineralization and cycling, sequestration of carbon and improving the micro-environments (English and Colmer, 2011). These plants have great potential to preserve ecosystems. They can be used for production of biofuel and fiber and as fodder crops (Dagla and Shekhawat, 2005; Chaudhary et al., 2015).

Plant microbiome serves as a "second genome" to the plant and plays an important role in growth and productivity of halophytes. Rhizosphere and root endosphere microbiomes from extreme environments such as arid, saline, hot, cold, and acidic help the plants to grow in these environments (Oren, 2013; Santhanam et al., 2017; Mukhtar et al., 2018). Recent studies about rhizosphere microbiome of desert halophytes revealed a high portion of halophilic bacteria as compared to rhizosphere microbiome of salinity sensitive plants (Marasco et al., 2016; Tian and Zhang, 2017). A number of halophilic bacterial genera including *Bacillus, Halomonas, Halobacillus, Oceanobacillus, Marinobacter, Marinococcus*, and *Nesterenkonia* have been identified from the rhizosphere and roots of halophytes and xerophytes (Ramadoss et al., 2013; Zhao et al., 2016; Etesami and Maheshwari, 2018; Mukhtar et al., 2019).

It is well documented that halophilic and halotolerant PGPRs (plant growth promoting bacteria) enhance growth of halophytes (Yuan et al., 2016; Mukhtar et al., 2017a). These bacteria have the ability to improve plant growth and ultimately increase crop yield by nitrogen fixation, solubilization of minerals, production of phytohormones and siderophores, under salinity and drought conditions (Hussain et al., 2015; Zhou et al., 2017; Etesami and Beattie, 2018). PGPRs also provide plant protection against fungal and bacterial diseases by production of a variety of antifungal and antibacterial compounds (Bulgarelli et al., 2013). Halophiles are able to produce different industrially important enzymes such as protease, lipase, amylase, laccase, xylanase, and cellulase with polyextremophilic properties (Kumar et al., 2012; Mukhtar et al., 2019). Halophilic enzymes have potential for different industrial applications including textile, paper and pulp, pharmaceutical, baking, and detergent industries (Liszka et al., 2012; Mukhtar et al., 2019). Halophilic bacteria can also be used for bioremediation of a variety of hazardous compounds in saline and arid environments (Liszka et al., 2012; Castillo-Carvajal et al., 2014; Tian and Gao, 2014).

A number of studies have already reported desert halophytes and xerophytes, such as Cactus, Agave, *Halocnemum, Halostachys*, *Lycium, Salicornia*, and *Kalidium* associated microbial diversity by using high throughput sequencing approaches (Coleman-Derr et al., 2016; Fonseca-Garcia et al., 2016; Yuan et al., 2016; Tian and Zhang, 2017). However, the microbial communities associated with the desert halophytes growing in Cholistan, Pakistan, an extremely dry and saline environment, have not been previously explored. Authors evaluated the rhizosphere and root endosphere microbiomes of four desert halophytes *Zygophyllum simplex, Haloxylon salicoricum, Aerva javanica*, and *Capparis decidua,* collected from three sites by using culture-independent (Illumina sequencing) approaches. The main objectives of the present study were: (1) to compare the microbial communities associated with the rhizosphere and root endosphere of each halophyte, individually, among the three geographic sites and (2) to compare the microbial diversity from the rhizosphere and root endosphere of four halophyte species so as to understand the differences among the plant species and across the three geographic sites.

MATERIALS AND METHODS

Soil Sampling

Cholistan is a hot arid sandy desert covering an area of 26,000 km^2 and is locally known as Rohi. It is situated in the South-West of Punjab province (Pakistan) and has an average annual rainfall from 128 to 178 mm (**Supplementary Figure 1**). Geographically, it is located 27°42′ and 29°45′ North, 69°52′ and 75°24′ East. Water is available at 25–90 m depth and is too brackish. Drought in this region is quite common, sometimes extending from 2 to 3 years, causing a lot of harms (Chaudhry and Nasim, 1995). Vegetation in this area includes a variety of grasses (*Panicum, Cenchrus, Aristida*, and *Lasiurus*), herbs (*Suaeda, Chenopodium*, *Aerva, Zygophyllum*, and *Dipterygium*), and shrubs (*Haloxylon*, *Justicia, Capparis*). Authors have surveyed an area of approximately 71 km near the Lal Suhanra National Park, Cholistan, and selected three geographic sites (approximately 23 km far from each other) for sampling of halophytes according to land use and vegetation cover (**Supplementary Figure 2**). These sampling sites were considered as the most drought affected regions. Drought tolerant halophytes, including *Zygophyllum simplex, Haloxylon salicoricum, Aerva javanica*, and *Capparis decidua,* dominant across all three sites, were collected. The sampling area was selected according to land use and vegetation cover. The whole plants were excavated with surrounding soil in blocks (15–20 cm in depth, 20 cm in width, and 20 cm in length). Four replicates for each plant and soil sample were collected from each site (Koranda et al., 2011; Mukhtar et al., 2018). Plant and soil samples were taken to the laboratory from the collection site in an ice box and stored at −80°C for microbial diversity analysis.

Soil Physicochemical Characteristics

About 350 grams of soil were dried and sieved for estimation of physicochemical properties. The pH of soil was measured by preparing a mixture of 1:2.5 (w/v) soil to water. Electrical conductivity (dS/m) was calculated according to Adviento-Borbe et al. (2006). Soil moisture and texture class were estimated by using the method from Anderson and Ingram (1993). Total organic carbon (C_{org}) was determined by Walkley and Black (1934) method (1934) and total organic nitrogen was calculated using Kjeldahl method. Available phosphorous (P) was estimated by using a calorimetric method with sodium bicarbonate, ammonium molybdate, and ascorbic acid (Olsen et al., 1954). Potassium (K), calcium (Ca), and magnesium (Mg) were detected by atomic absorption spectrometry. Carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) ions were determined by using Vogel (1978) method (1978).

DNA Extraction, Amplification of 16S rRNA Gene and Illumina MiSeq Sequencing

About 100 g of rhizospheric soil was mixed thoroughly and filtered with 2 mm sieve. The total DNA from soil samples (0.5 g) were extracted using FastDNA Spin Kit for Soil, according to manufacturer's instructions. For DNA extraction from root

endosphere, roots were surface sterilized according to Mukhtar et al. (2019) and FastDNA Spin Kit specific for plant tissues was used. The quality of DNA was determined by using 0.9% agarose gel and quantity was measured by using Nanodrop (NanoDrop 200c Thermo Fisher Scientific, Waltham, MA, United States).

In total, 96 DNA samples; 12 rhizosphere and 12 root samples of each plant collected from the three geographic sites were used for amplification of 16S rRNA gene and Illumina (MiSeq) sequencing. The V3–V4 region of the 16S rRNA gene was amplified by using primers (Bakt_341F: CCTACGGGNGGCWGCAG and Bakt_805R: GACTACHVGGGTATCTAATCC), which were linked with unique identifier and adapter sequences (**Supplementary Table 1**). The detailed PCR conditions for amplicon sequencing were the same as described by Herlemann et al. (2011). Amplified PCR products were purified with Agencourt AMPure beads (Beckman Coulter, Brea, CA, United States). Finally, about 10 ng of DNA from each sample was sequenced on the Illumina MiSeq platform by Macrogen (Geumcheon-gu, Seoul, South Korea).

Bioinformatics and Statistical Analyses

Sequences were processed and sorted using the default parameters in QIIME 1.3 (Caporaso et al., 2010). An offset of 10 nucleotides was set in order to remove the first 10 bases of each sequence, and high quality sequences with an average length of 350 bases were selected. Chimera Slayer software was used to check chimeric sequences (DeSantis et al., 2006). *De novo* OTU (operational taxonomic unit) picking was done to generate OTU files using UCLUST that is a default parameter of QIIME, with 97% sequence similarity and RDP classifier was used to assign taxonomy (Wang et al., 2007). For different taxonomic levels, such as phylum, class, order, family, and genus, Good's coverage was calculated by using 97% similarity cutoff (Good, 1953).

Alpha and beta diversity were determined using QIIME alpha_rerefaction.py and beta_diversity_through_plots.py commands, respectively. Alpha diversity was calculated at a sequence depth of 82,152 reads per soil sample, as alpha diversity indices are correlated with the number of sequences by using the Kruskal–Wallis test. The selected maximum sampling depth corresponded to minimum number of reads obtained from any of the remaining sequenced samples. Beta diversity was analyzed by using non-metric multidimensional scaling analysis (NMDS) in "Mass" and "vegan" packages. A matrix was calculated using the weighted and unweighted UniFrac distances among samples at a sequence depth of 82,152 reads per soil sample (Lozupone et al., 2011). Distances were calculated using the "envfit" function of the package "vegan" for Bray-Curtis. To further explore the relationship between bacterial communities and soil properties, redundancy analysis (RDA) and mental test were performed to study the relationship between the most abundant bacteria and soil properties. To explain the differences in the composition of taxa inside the data matrix community, a heatmap (relative abundance matrix) was generated at class level using XLSTAT 7.0 software (Fahmy, 2003). Number of OTUs was calculated by using Venn diagrams analysis and distances were calculated using the "vegdist" function of the "vegan R package." Variation partitioning was performed on the basis of plant species, soil physicochemical characteristics, and geographical distance on composition of rhizosphere and root endosphere microbiomes across the three sites.

To detect the taxonomic classifications that were significantly abundant in rhizospheric soils and root samples, Wilkcoxon's non-parametric rank-sum test and LDA using the LEfSe (LDA Effective Size) program was used (Segata et al., 2011). One-way ANOVA was applied to analyze differences among rhizospheric soils and root samples. Environmental fitting analysis using "envfit" function in vegan R package was used to find out which soil physicochemical factors strongly associated with rhizosphere and root endosphere microbiomes. To predict the functional profile of bacteria identified from the rhizosphere and root endosphere of halophytes, Tax4Fun software (R package) was used (Abhauer et al., 2015). Variation partitioning analysis was performed to study the impact of the relative influences of plant species, soil physicochemical characteristics, and geographical distance on composition of rhizosphere and root endosphere microbiomes. Retrieved 16S rRNA sequences datasets were deposited in NCBI GenBank under SRA accession numbers SRR9588854 to SRR9588861.

RESULTS

Correlations Between Physicochemical Characteristics of Soil and Microbial Community Structure

Soil pH ranged from 7.02 to 7.69 with the maximum value in *Haloxylon* soils collected from the site 2 and the minimum in *Zygophyllum* soils collected from the site (**Table 1**), electrical conductance ($EC_{1:1}$) ranged from 3.79 to 5.51 dS/m with the maximum value in *Haloxylon* soils collected from the site 2 and minimum in *Aerva* soils collected from the site 3 (**Table 1**). The moisture content ranged from 20.25 to 25.49%. The soil samples collected from site 2 were more dried as compared to soil samples from the geographic sites 1 and 3. Soil temperature ranged from 38.79 to 42.54°C (**Table 1**). The average organic matter ranged from 12.55 to 18.37 $g.kg^{-1}$ with the highest values in *Capparis* rhizospheric soils collected from site 2 and the lowest in *Haloxylon* rhizospheric soils collected from site 1. The average values for P, K, Ca, and Mg contents were 6.78, 0.55, 182.25, and 82.16 $mg.kg^{-1}$, respectively. The values of nitrate ions were higher in *Capparis* rhizospheric soils as compared to other plants soils from all sites. Carbonate and bicarbonate ions were higher in *Haloxylon* and *Aerva* rhizospheric soils, as compared to other plants soils (**Table 1**).

Differences in community structure among different rhizospheric soil and root samples across the three geographic sites were explained by redundancy analysis (RDA) (**Figure 1**). It was observed that microbial communities in the rhizosphere and roots of *Haloxylon* and *Zygophyllum* showed greatest variations as compared to microbial communities identified

TABLE 1 | Physical and chemical properties of rhizospheric soil samples of desert halophytes.

Soil properties	Site 1				Site 2				Site 3			
	Zygophyllum	*Haloxylon*	*Aerva*	*Capparis*	*Zygophyllum*	*Haloxylon*	*Aerva*	*Capparis*	*Zygophyllum*	*Haloxylon*	*Aerva*	*Capparis*
pH	7.02^{a}	7.26^{a}	7.21^{a}	7.51^{b}	7.53^{b}	7.69^{a}	7.29^{a}	7.11^{a}	7.11^{a}	7.57^{b}	7.19^{a}	7.47^{b}
$EC_{1:1}$ (dS/m)	4.14^{a}	4.77^{b}	3.85^{a}	4.17^{ab}	4.75^{b}	5.51^{c}	4.59^{b}	4.21^{ab}	3.79^{a}	4.27^{ab}	3.59^{a}	4.47^{b}
Moisture (%)	22.15^{a}	20.78^{a}	22.23^{a}	25.49^{b}	24.34^{ab}	20.25^{ab}	21.57^{a}	23.55^{a}	23.24^{a}	21.26^{a}	22.67^{a}	25.29^{b}
Temperature (°C)	40.21^{a}	42.54^{b}	41.71^{ab}	41.83^{ab}	40.87^{ab}	41.11^{ab}	41.49^{b}	40.98^{ab}	38.79^{a}	39.15^{a}	39.27^{a}	39.61^{a}
Texture class	Sandy soil	Sandy soil	Sandy soil	Sandy soil	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
OM ($g.Kg^{-1}$)	16.31^{ab}	12.55^{a}	15.61^{ab}	17.37^{b}	15.53^{ab}	17.61^{b}	14.89^{ab}	18.35^{b}	16.49^{ab}	11.71^{a}	16.53^{ab}	17.91^{b}
P ($mg.kg^{-1}$)	7.05^{ab}	7.66^{b}	6.29^{a}	6.14^{a}	6.97^{ab}	7.69^{b}	6.45^{a}	6.11^{a}	7.77^{b}	6.81^{ab}	6.19^{a}	6.33^{a}
K ($mg.kg^{-1}$)	0.47^{a}	0.51^{a}	0.59^{b}	0.65^{b}	0.44^{a}	0.59^{b}	0.45^{a}	0.61^{b}	0.49^{a}	0.63^{b}	0.61^{b}	0.60^{b}
Ca ($mg.kg^{-1}$)	131.41^{a}	187.45^{b}	205.49^{b}	179.32^{b}	157.11^{a}	207.53^{b}	211.12^{b}	191.49^{b}	149.21^{a}	199.57^{b}	209.55^{b}	157.73^{a}
Mg ($mg.kg^{-1}$)	64.47^{a}	97.05^{b}	71.22^{a}	103.4^{b}	67.73^{a}	89.29^{ab}	93.13^{ab}	69.37^{a}	65.47^{a}	91.44^{ab}	109.52^{b}	93.67^{ab}
NO_3^- ($mg.kg^{-1}$)	9.56^{a}	10.13^{a}	9.17^{a}	12.97^{b}	10.09^{a}	10.33^{a}	9.99^{a}	13.25^{b}	9.97^{a}	10.17^{a}	10.37^{a}	12.65^{b}
CO_3^{2-} ($mg.kg^{-1}$)	17.56^{a}	24.29^{b}	22.19^{b}	15.53^{a}	18.19^{a}	23.53^{b}	19.93^{ab}	17.79^{a}	18.56^{a}	23.21^{b}	22.78^{b}	19.17^{a}
HCO_3^- ($mg.kg^{-1}$)	1.51^{a}	2.61^{b}	2.75^{b}	1.93^{ab}	1.93^{ab}	2.77^{b}	2.45^{b}	1.49^{a}	1.59^{a}	2.59^{b}	2.67^{b}	1.95^{ab}

EC, Electrical conductivity; OM, Organic matter; P, Phosphorous; K, Potassium; Ca, Calcium; Mg, Magnesium; NO_3^-, Nitrate; CO_3^{2-}, Carbonate ion; HCO_3^-, Bicarbonate ion; Letters represent statistically different values at 5% level.

from the rhizosphere and roots of *Aerva* and *Capparis,* from the geographic site 2 (**Figure 1**). Some bacterial genera were more abundant than others at each sampling site. Soil physicochemical characteristics, especially salinity and moisture content affect the microbial diversity in each plant across the three sites.

General Characteristics of 16S rRNA Based Illumina Sequencing

In this study, a total of 697743 sequences were obtained from the rhizospheric soil samples and 647802 sequences from the root endosphere of halophytes, before the removal of mitochondrial and plastid contaminants while 638292 sequences were obtained from the rhizospheric soil samples and 530006 sequences from the root endosphere of halophytes, after the removal of mitochondrial and plastid contaminants (**Supplementary Table 2**).

About 67–83% reads were assigned at the phylum level, 59–81% to class level, 34–51% to the family, and 31–49% were assigned to the genus level, from the geographic site 1 (**Supplementary Figure 3**). About 71–81% reads were assigned at the phylum level, 51–72% to the class level, 34–51% to the family, and 27–50% were assigned to the genus level, from the geographic site 2 (**Supplementary Figure 4**). About 69–81% reads were assigned at the phylum level, 57–71% to the class level, 29–55% to the family, and 26–45% were assigned to the genus level from the geographic site 3 (**Supplementary Figure 5**).

Microbial Diversity Comparisons at Global OTU Level

The results for observed OTUs for microbial communities identified from the rhizosphere and root endosphere of *Haloxylon* were highly variable as compared to soil and root samples of other plants, collected across all sites (**Figure 2A** and **Supplementary Table 3**). Within plant species, alpha diversity was highest in the rhizosphere and root endosphere of *Haloxylon* from the geographic site 2. Overall, microbial communities from the rhizosphere of all plants showed higher diversity as compared to the root endosphere and microbial diversity differed significantly among plant species and across the three sites (**Figure 2B** and **Supplementary Table 4**).

Beta diversity was calculated by using weighted and unweighted UniFrac distances for non-metric multidimensional scaling plots. Microbial communities associated with the rhizosphere and root endosphere were clustered significantly different among the plant species and across the sites (**Figures 3A,B**). In case of site 1, microbial diversity from the rhizosphere samples was clustered differently from the root endosphere samples, within and between plant species (**Figures 3C,D**). The microbial communities identified from the site 2 showed higher variations among plant-type and sample-type as compared to microbial communities identified from the site 1 and site 3 (**Figures 3E–H**, respectively). Overall, the NMDS analysis showed that the rhizosphere and root endosphere microbiomes of *Haloxylon* exhibited the highest variations from the site 2.

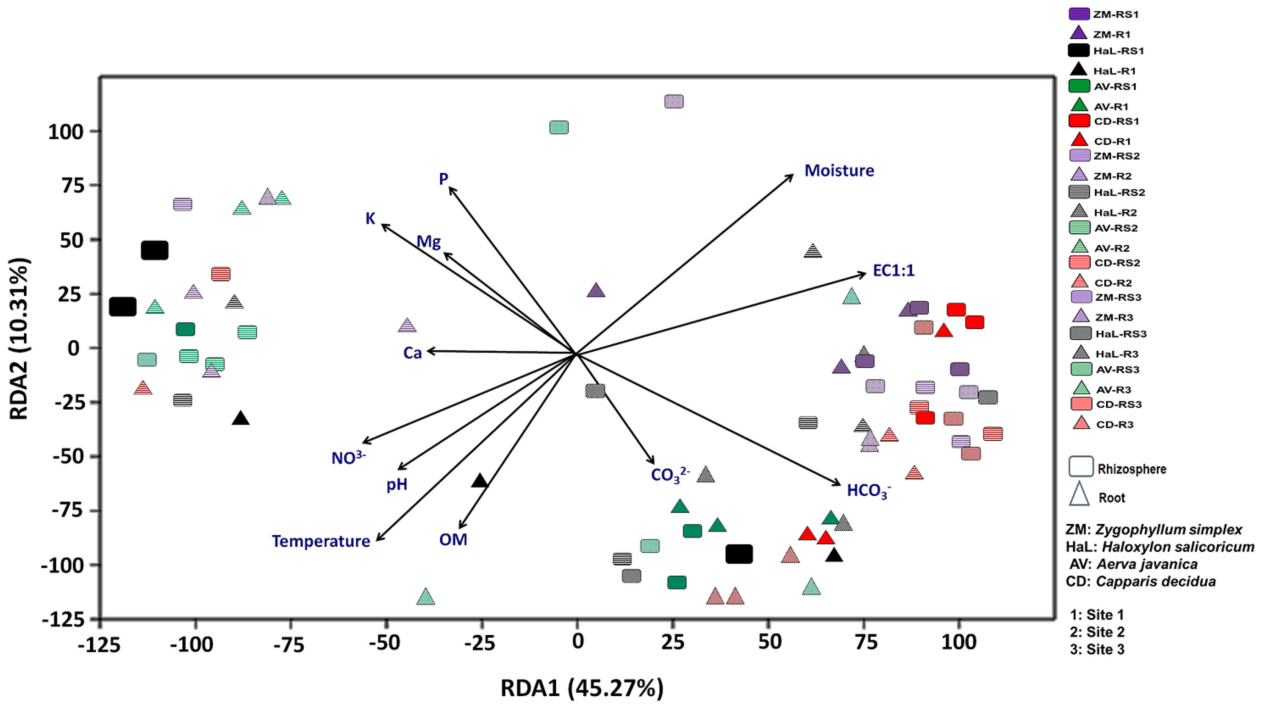


FIGURE 1 | Environmental fitting analysis of rhizosphere and root endosphere microbiomes across the three sites by using redundancy analysis (RDA) plot.

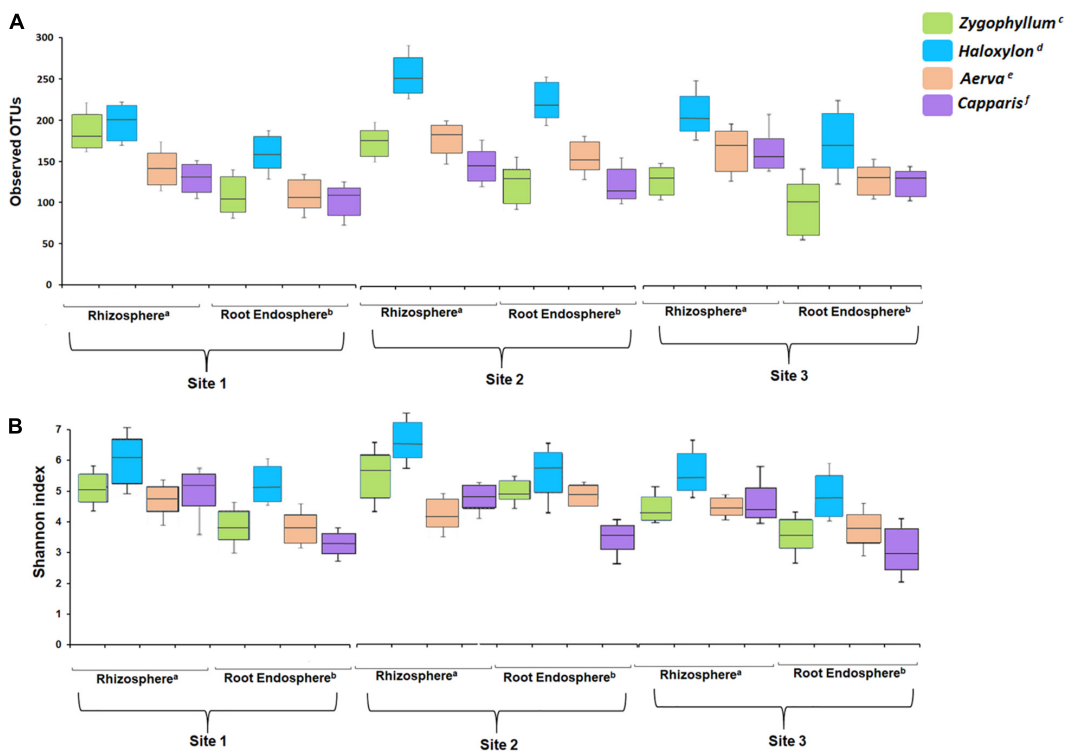
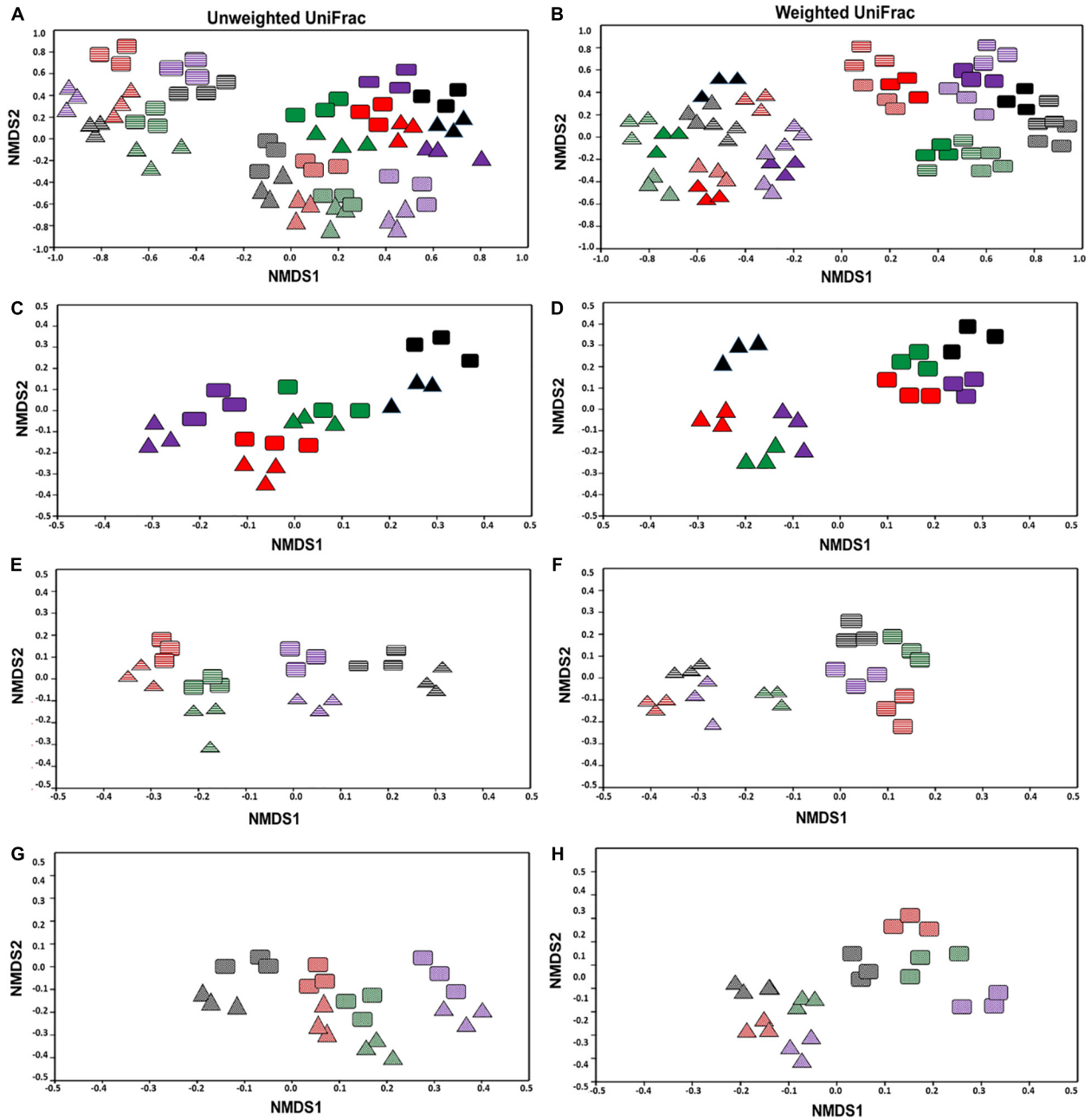


FIGURE 2 | Alpha diversity analysis. **(A)** Number of observed OTUs and **(B)** Shannon index. All the soil and root samples showed significant difference after a Kruskal–Wallis test with a confidence level of 99% ($p \leq 0.05$) in each index. Superscripts (a and b) indicate significant differences in the rhizosphere and root endosphere among plant species, while superscripts (c–f) indicate significant differences between sample types associated with a plant species.



Sampling site	Sample type	Plant species			
		Zygophyllum	*Haloxylon*	*Aerva*	*Capparis*
Site 1	Rhizosphere				
	Root endosphere				
Site 2	Rhizosphere				
	Root endosphere				
Site 3	Rhizosphere				
	Root endosphere				

FIGURE 3 | Beta diversity index of the bacterial communities in the rhizosphere and root endosphere of desert halophytes. Beta diversity was calculated by using weighted and unweighted UniFrac distances for non-metric multidimensional scaling plots **(A,B)** samples collected from all the sites, **(C,D)** samples collected from only site 1, **(E,F)** samples collected from only site 2, and **(G,H)** samples collected from only site 3 using unweighted **(A,C,E,G)** and weighted **(B,D,F,H)** UniFrac distances.

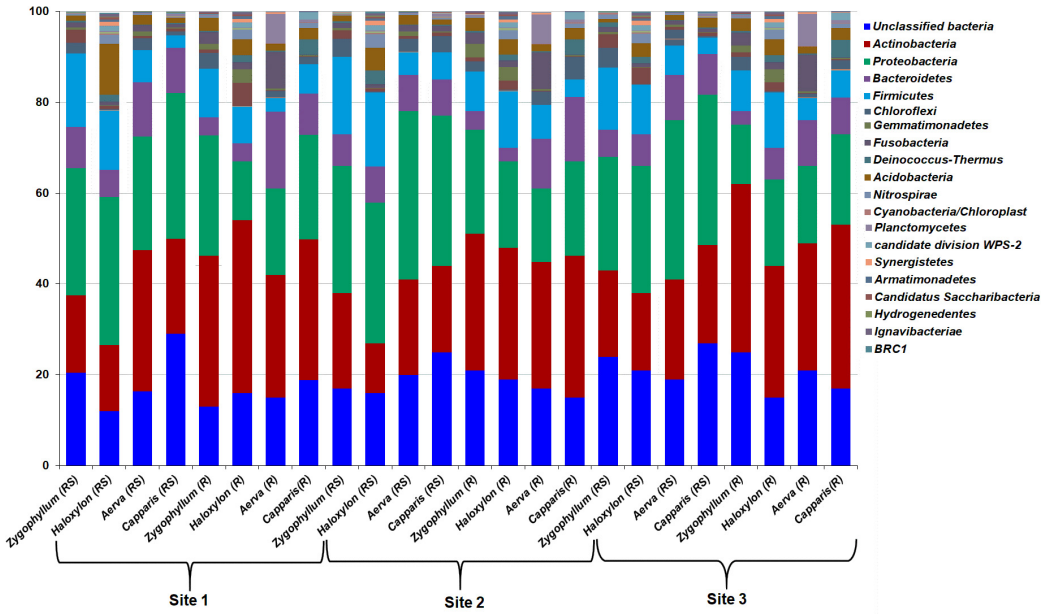


FIGURE 4 | Relative abundance of bacterial phyla from the rhizosphere (RS) and root endosphere (R) of desert halophytes collected from three sites of Cholistan.

Microbial Diversity Comparisons at the Phylum and Class Level

The OTUs from all the soil and root samples were assigned to 19 bacterial phyla. Microbial diversity at the phylum level showed significant differences among rhizosphere and root endosphere of halophytes across the three geographic sites (**Figure 4**). *Proteobacteria* (25.31–37.13%) was the most abundant bacterial phylum identified from the rhizospheric soil samples of all plants while *Actinobacteria* (27.23–38.07%) were dominant in root endosphere of all plants, across the three sites. Members of *Firmicutes* (8.51–17.19%) were dominant in the rhizosphere and roots of *Zygophyllum* and *Haloxylon* whereas in case of *Aerva* and *Capparis, Bacteroidetes* (8.25–13.12%) showed abundance across the three sites. Sequences related to Chloroflexi, Gemmatimonadetes, Fusobacteria, Deinococcus-Thermus, Acidobacteria, and Nitrospirae, were relatively less abundant, however, detected from all the rhizospheric soils with a significant difference in abundance across the three sites (**Figure 4**). At the phylum level, microbial communities associated with the rhizosphere and root endosphere of *Haloxylon* showed the highest variations as compared to microbial communities, identified from the rhizosphere and root endosphere of other desert halophytes, across the site 2. Our results also indicated that *Actinobacteria* significantly correlated with the roots and *Proteobacteria* significantly correlated with the rhizosphere of halophytes, whereas Nitrospirae and Synergistetes correlated least with the rhizosphere of halophytes, across the three sites.

The relative abundance of bacterial classes from the rhizosphere and roots of desert halophytes collected from the three geographic sites were compared in the form of a heatmap (**Supplementary Figure 6**). Bacteroidia, Alphaproteobacteria, Bacilli, Betaproteobacteria, Sphingobacteria, Deltaproteobacteria, Gammaproteobacteria, Planctomycetia, Clostridia, and Halobacteria, were found to be abundant classes identified from all the rhizosphere and root samples of halophytes, collected from the site 1 (**Supplementary Figure 6A**). Bacilli, Alphaproteobacteria, Betaproteobacteria, Bacteroidia, Deltaproteobacteria, Flavobacteriia, Planctomycetia, Sphingobacteria, Acidobacteria_Gp7, Acidobacteria_Gp9, Nitrospira, and Halobacteria were dominating classes detected from all the rhizosphere and root samples of halophytes, collected from the site 2 (**Supplementary Figure 6B**). Bacilli, Alphaproteobacteria, Bacteroidia, Gammaproteobacteria, Deltaproteobacteria, Flavobacteriia, Planctomycetia, Acidobacteria_Gp9, Chlamydiia, Sphingobacteria, Nitrososphaeria, Acidobacteria_Gp7, Deinococci, Holophagae, and Halobacteria were dominating classes detected from all the rhizosphere and root samples of halophytes, collected from the site 3 (**Supplementary Figure 6C**). The cluster structure showed a different distribution pattern in rhizospheric soils as compared to root endosphere of halophytes, across the three sites. At class level also, the highest variations were observed between the rhizosphere and root endosphere of *Haloxylon,* collected from the geographic site 2.

Microbial Diversity Comparisons of Rhizosphere and Root Endosphere Across Three Geographic Sites at Genus Level

The phylotypes identified from the rhizosphere and root endosphere of halophytes were compared across the three geographic sites. Venn diagram analysis revealed that a total

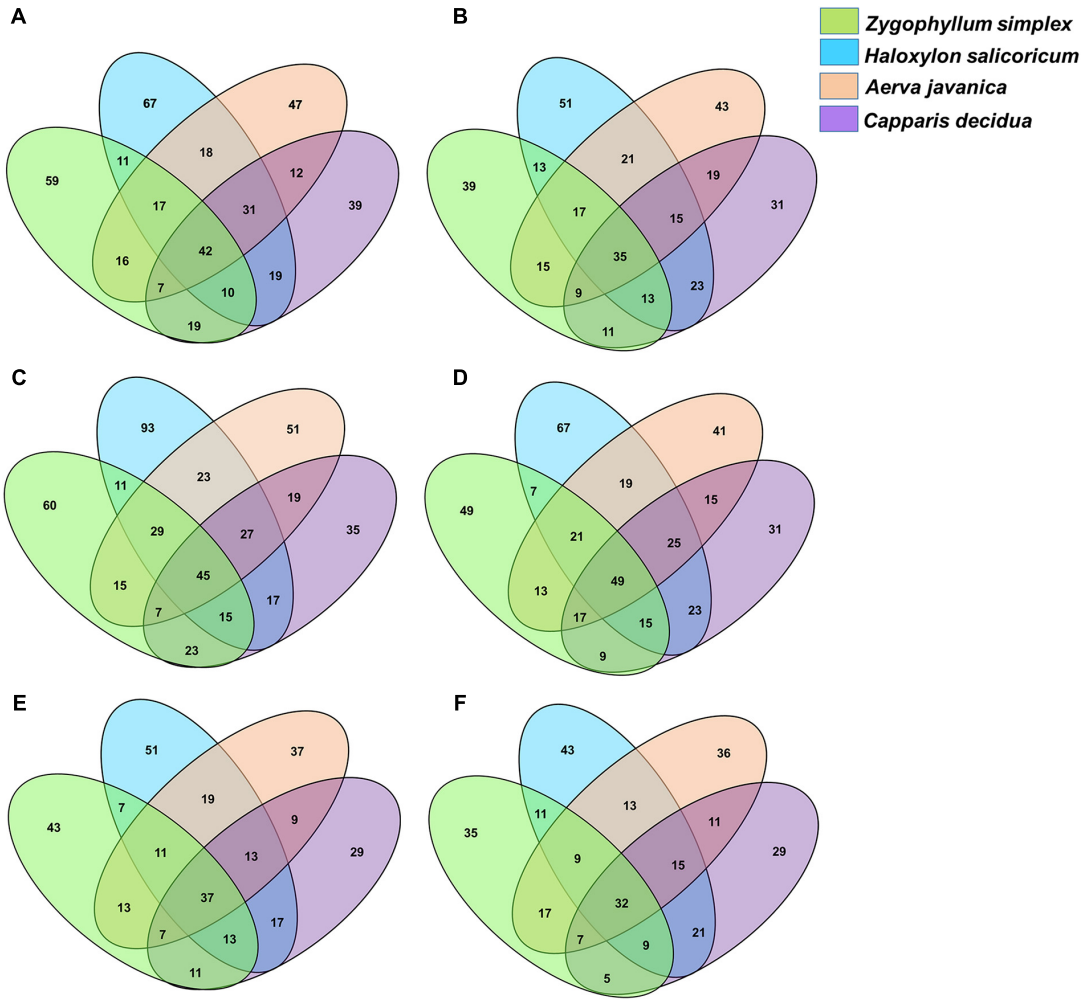


FIGURE 5 | Venn diagram showing the numbers of bacterial genera identified from the rhizosphere and the root endosphere of desert halophytes. **(A,B)** Samples collected from the site 1, **(C,D)** Samples collected from the site 2, and **(E,F)** Samples collected from the site 3.

of 765 phylotypes from the rhizosphere and 682 phylotypes from the root endosphere of halophytes have been identified from the site 1 (**Figures 5A,B**), 869 phylotypes from the rhizosphere and 790 phylotypes from the root endosphere of halophytes from the site 2 (**Figures 5C,D**), and 592 phylotypes from the rhizosphere and 547 phylotypes from the root endosphere of halophytes have been identified from the site 3 (**Figures 5E,F**).

Overall, maximum microbial diversity was detected from the rhizosphere and root endosphere of all plants collected from the geographic site 2. A total of 45 phylotypes were commonly identified from all the rhizospheric soils and 60, 93, 51, and 35 phylotypes were exclusively identified from the rhizosphere of *Zygophyllum, Haloxylon, Aerva*, and *Capparis,* respectively (**Figure 5C**). From the root endosphere of halophytes, 49 phylotypes were commonly identified and 49, 67, 41, and 31 phylotypes were exclusive to the root endosphere associated microbial diversity of *Zygophyllum, Haloxylon, Aerva*, and *Capparis,* respectively (**Figure 5D**).

The results of LEfSe analysis showed the differences among the dominant bacterial genera from the rhizosphere and root endosphere of halophytes across all the sampling sites (**Figure 6** and **Supplementary Figure 7**). The top five bacterial genera based on LDA scores were compared among the sample-type, plant species and across the sites. Bacterial genera, including *Bacillus, Kocuria, Pseudomonas, Halomonas*, and *Flavobacterium* were commonly identified from the rhizosphere and root endosphere of halophytes across all the geographic sites (**Figure 6**, **Supplementary Figure 7** and **Supplementary Tables 5–7**). All the plant species showed great variations from one plant to another, however, within sample-type (the rhizosphere and root endosphere), there was no significant variation. Bacterial genera *Virgibacillus, Oceanobacillus,* and *Planococcus* (Firmicutes); *Aeromonas, Marinobacter, Enterobacter*, and *Citrobacter* (Proteobacteria); *Kocuria, Solirubrobacter, Rubrobacter, Microbacterium*, and *Arthrobacter* (Actinobacteria); were exclusively identified from the rhizosphere and root endosphere of *Zygophyllum*

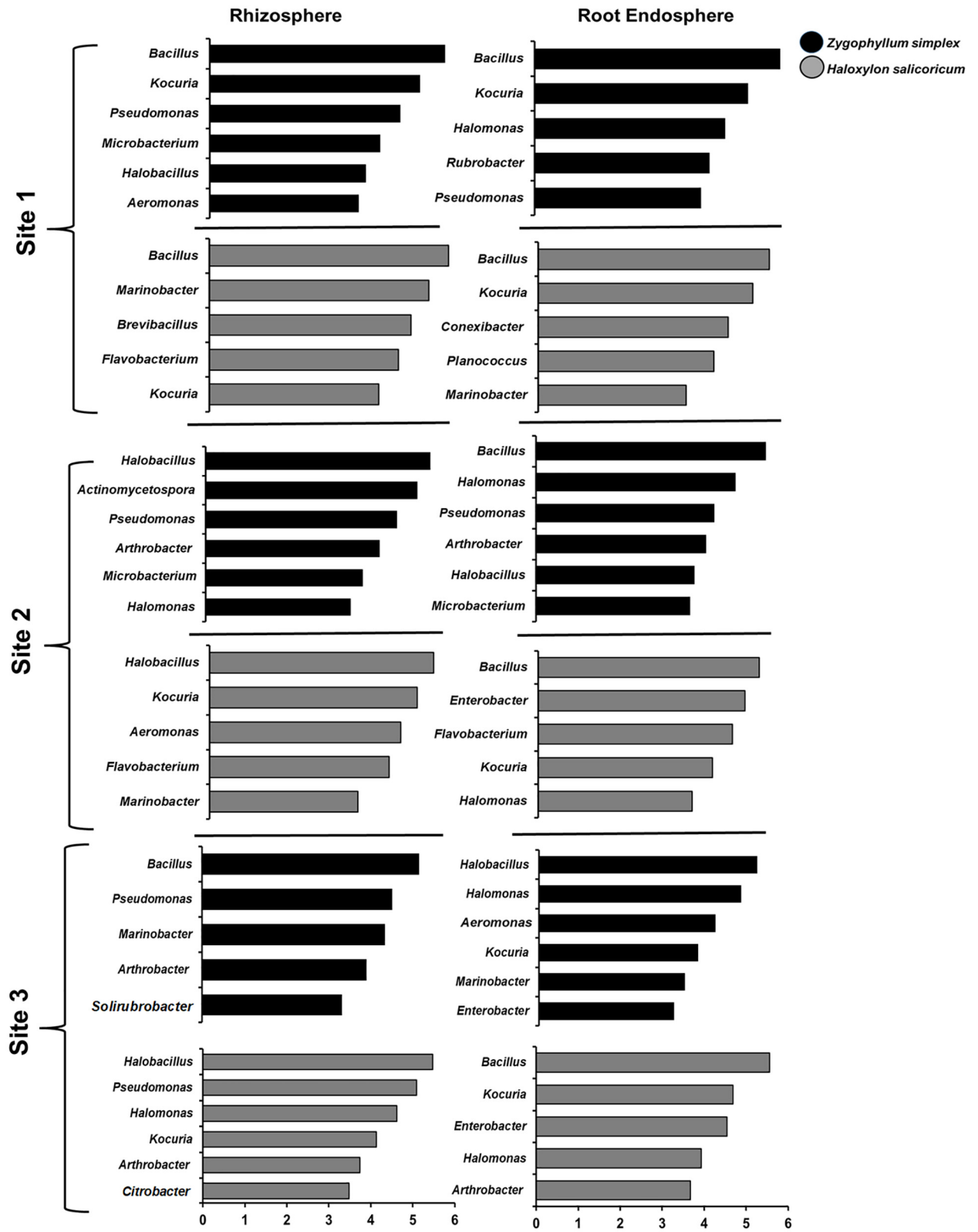


FIGURE 6 | The results of LEfSe analysis showed the identified bacterial genera that were significantly abundant in the rhizosphere and root endosphere of halophytes. Variations in the bacterial genera detected across the rhizosphere and the root endosphere of *Zygophyllum* and *Haloxylon* from three sampling sites. Bacterial genera with the LDA score of more than 3.5 are shown.

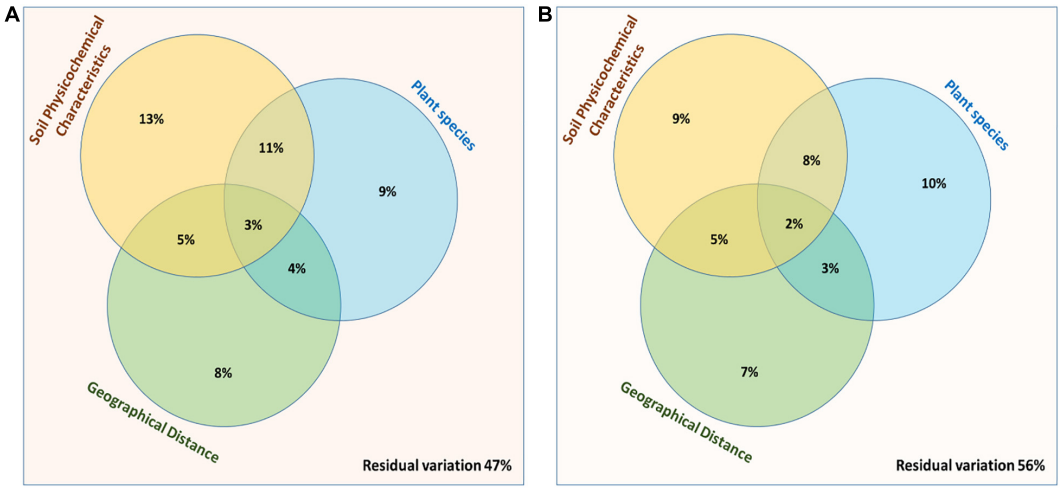


FIGURE 7 | Partitioning between the biological variations in the bacterial community structure. Three explanatory matrices were used here, containing variables pertaining to plant species, soil physicochemical characteristics and geographical distance on composition of **(A)** Rhizosphere and **(B)** Root endosphere microbiomes.

and *Haloxylon* while *Burkholderia, Serratia*, and *Klebsiella* (Proteobacteria), and *Polaribacter* (Bacteroidetes) were identified only from the rhizosphere and root endosphere of *Aerva* and *Capparis*. At the genus level, *Haloxylon* showed the greatest variations between the rhizosphere and root endosphere from the site 2 (**Figure 6** and **Supplementary Table 6**). These results also confirmed the abundance of bacterial phyla Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes, as the top bacterial genera from all the soil and root samples across all sampling sites.

Factors Influencing Bacterial Community Structure and Distribution

A multivariate variation partitioning approach was used to study the effects of soil physicochemical characteristics and the geographical distance on the overall variation of rhizosphere and root endosphere microbiomes based on the most abundant OTUs. A combination of environmental parameters; plant species, soil physicochemical characteristics, and geographical distance could explain 53% of their overall biological variations while 47% variations were unexplained in case of rhizosphere microbiomes (**Figure 7A**) while from the root endosphere microbiomes, different factors could explain only 44% of their overall biological variations while 56% variations were unexplained (**Figure 7B**).

Functional Profile of Bacterial Communities

The results for the prediction of functional profiles of bacterial genera with specific plant growth promoting traits showed that nitrogen fixers were more abundant in the root endosphere of all the halophytes across the geographic sites 1 and 2 (**Figures 8A,B**). Mineral (Phosphate, potassium, and zinc) solubilizers and IAA (indole-3-acetic acid) producers were more dominant in the rhizosphere of *Zygophyllum, Haloxylon*, and *Aerva* as compared to root endosphere across all sites. Bacterial strains with biocontrol activity were found to be most abundant in the rhizosphere of *Haloxylon* and *Capparis,* from the site 2 (**Figures 8B,C**). Microbes involved in bioremediation of toxic compounds were more abundant in the rhizosphere and root endosphere of *Haloxylon, Aerva*, and *Capparis,* across all sites. More than 83% bacterial strains identified in this study were halophilic and alkaliphilic in nature (**Figures 8A,B**).

DISCUSSION

Bacterial strains isolated from the extreme environments have special physiological and genetic characteristics to survive under such conditions. Cholistan desert is considered as naturally occurring extreme environment with drought, salinity and high temperature as the main abiotic factors. In this study, microbial diversity was compared from the rhizosphere and root endosphere of desert halophytes such as *Zygophyllum simplex, Haloxylon salicoricum, Aerva javanica*, and *Capparis decidua*, collected from three geographic sites of Cholistan desert, Pakistan by using 16S rRNA based Illumina sequencing. The main objective of this study was to evaluate the impact of plant species, sample-type (rhizospheric and root endosphere) and geographic site, on the composition of microbial communities associated with desert halophytes. A number of studies have been previously reported on microbial diversity analyses from halophytes and xerophytes, such as microbial communities associated with the root endosphere of *Halocnemum, Halostachys*, *Lycium,* and *Salicornia,* using high throughput sequencing approaches (Coleman-Derr et al., 2016; Yuan et al., 2016; Tian and Zhang, 2017; Fitzpatrick et al., 2018). Here, we have reported the microbial diversity associated with the rhizosphere and root endosphere of halophytes, collected from Cholistan, Pakistan, by using culture-independent approaches.

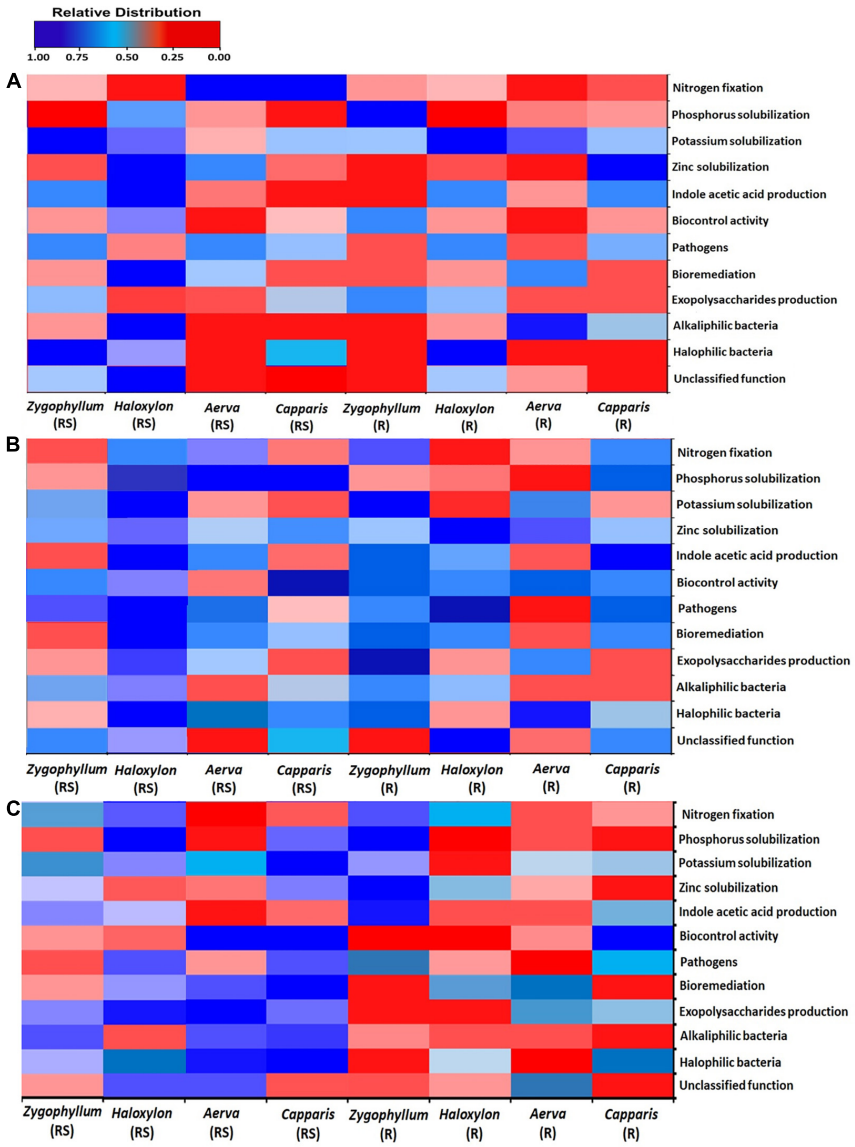


FIGURE 8 | Bacterial linked to different functional categories in the rhizosphere (RS) and root endosphere (R) of desert halophytes collected from the three sites; **(A)** Site 1, **(B)** Site 2, and **(C)** Site 3.

The rhizosphere and root endosphere associated microbial communities of a plant are affected by plant type, plant compartment and environmental factors such as geographic location, soil pH, salinity, moisture content, soil leaching, erosion, and loss of certain nutrients (Pan et al., 2014; Fang et al., 2016; Mukhtar et al., 2018). Microbial diversity associated with the rhizosphere and root endosphere of *Zygophyllum* and *Haloxylon* showed a significant difference as compared to other halophytes, across all geographic sites. The results of sequence analysis of the 16S rRNA gene described that overall maximum microbial diversity identified from the geographic site 2 with more than 98.57% of retrieved sequences assigned to the domain Bacteria and 4.43% sequences related to unclassified microorganisms. Previous studies on Cacti and *Agave* species also reported the similar pattern of microbial diversity (Coleman-Derr et al., 2016; Fonseca-Garcia et al., 2016). At the phylum level, microbial communities identified from the rhizosphere of halophytes showed more diversity as compared to those associated with the root endosphere, across all the three sites. Among the plant species, *Haloxylon* showed overall more microbial diversity as compared to other halophytes, used in this study. Bacterial phyla Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Chloroflexi, Planctomycetes, and Fusobacteria were commonly identified from all rhizosphere and root samples. Some previous studies showed that the abundance of certain phyla, such as Actinobacteria, Proteobacteria, Firmicutes, and CRC1 increased in the rhizosphere of desert halophytes

such as *Halostachys caspica*, and *Salicornia alterniflora,* with the increase in soil salinity and moisture content (Marasco et al., 2016). Increase in soil salinity also negatively affect the abundance of certain phyla, such as Chloroflexi, Acidobacteria, Nitrospirae, and Synergistetes (Foesel et al., 2014; Yan et al., 2015; Mukhtar et al., 2018).

Overall, Actinobacteria were found to be more abundant in the root endosphere of halophytes than the rhizosphere across all the geographic sites, but within plant species, *Zygophyllum* and *Haloxylon* showed more abundance of Actinobacteria across the geographic site 1 and 3. The results of 16S rRNA based metagenomic analysis showed that bacterial genera *Kocuria, Brevibacterium, Micrococcus, Streptomyces, Solirubrobacter*, and *Nocardia* (Actinobacteria) were identified from the root endosphere of all plants. Previous studies also showed that Actinobacteria are dominant in the salinity and drought affected soils. These bacteria have the potential ability to promote plant growth, to produce a large number of antimicrobial compounds and to degrade a variety of toxic organic compounds from the polluted saline environments (Dupont et al., 2014; Deng et al., 2015; Zhang et al., 2015). Bacterial genera *Asanoa, Actinoplanes, Marmoricola*, and *Microlunatus* were detected only from the rhizosphere of *Aerva* and *Capparis* from the geographic site 1 and 2. These strains have previously been isolated from the rhizosphere of halophytes such as *Phragmites australis, Salsola stocksii*, and *Atriplex amnicola*. These can be used for biofuel production and play an important role in carbon cycling of soil ecosystems (Borruso et al., 2014; Krivushin et al., 2015; Mukhtar et al., 2018).

This study showed that the sequences related to *Proteobacteria* (*Alphaproteobacteria, Betaproteobacteria*, and *Gammaproteobacteria*) were detected from the rhizosphere and root endosphere microbiomes of all the plants but with more abundance in the rhizosphere samples. Bacterial genera *Pseudomonas, Halomonas, Enterobacter, Burkholderia, Azospirillum, Marinobacter*, and *Geobacter* were dominant as compared to other strains in all the rhizosphere samples of all the plants, as compared to root samples across the site 1 and 3 while *Serratia, Azotobacter*, and *Xanthomonas* were identified only from the rhizosphere of *Aerva* and *Capparis,* collected from the geographic site 2. Previous studies also reported the isolation and identification of these Protobacterial genera from a number of saline and arid environments (Jiang et al., 2014; Mukhtar et al., 2018, 2020). These bacteria can be used as biofertilizers, as biocontrol agents and for bioremediation of a variety of hazardous compounds from polluted saline and arid environments (Mukhtar et al., 2017a).

Members of Firmicutes were more abundant in the rhizosphere and root endosphere of *Zygophyllum* and *Haloxylon* as compared to *Capparis* and *Aerva* across all the three sites. As expected from the previous studies, these results also confirmed the correlation of increase in soil salinity and abundance of Firmicutes. *Bacillus, Halobacillus, Virgibacillus, Oceanobacillus, Marinococcus, Planococcus*, and *Exiguobacterium* were dominant genera detected in all the rhizosphere and root endosphere of halophytes. Within plant species, *Haloxylon* showed the maximum diversity of Firmicutes at the genus level between the rhizosphere and root endosphere from the geographic site 2. *Bacillus* strains have plant growth promoting and biocontrol abilities and can be used as potential biofertilizers (Krid et al., 2010; Mukhtar et al., 2017a). *Bacillus* like bacteria isolated and characterized from saline environments have novel halophilic enzymes which can be used for a number of industries and bioremediation of organic pollutants in arid and saline environments (Oren, 2015; Liu et al., 2017; Mukhtar et al., 2017b, 2019).

Dominant genera from the phylum *Bacteroidetes* identified in this study were *Flavobacterium, Salinibacter, Sphingobacterium*, and *Cytophaga*. These genera were detected from the rhizosphere and root endosphere of all the plants across the three geographic sites. *Polaribacter, Chlorobium*, and *Chitinivibrio* were exclusively identified from the rhizosphere and root endosphere of *Aerva* and *Capparis*. Members of Bacteroidetes are usually found to be dominant in the rhizosphere of plants growing under salinity and drought stress conditions (Coleman-Derr et al., 2016; Gibtan et al., 2017). *Gp4, Gp6, Gp7, Gp10,* and *Gp21* (Acidobacteria) were detected in this study. Acidobacteria were more abundant in the rhizosphere as compared to root endosphere of all the halophytes across the site 1 and 3. A number of previous studies also reported the abundance of Acidobacteria in the rhizosphere of halophytes and other hypersaline environments (Zhang et al., 2015; Mukhtar et al., 2016). Sequences related to bacterial phyla Fusobacteria, Gemmatimonadetes, Chloroflexi, Synergistetes, Planctomycetes, Deinococcus-Thermus, Armatimonadetes, and Nitrospirae were identified in the rhizosphere of all the halophytes but they were relatively less abundant across the sites 1 and 3 as compared to the site 2. These phyla have already been detected in various extreme environments such as rhizosphere of halophytes and xerophytes, marine water, rock sediments, anaerobic sludge, and hot spring water (Iverson et al., 2012; Zenga et al., 2014).

CONCLUSION

This study described the microbial diversity analysis of the rhizosphere and root endosphere of desert halophytes, collected from the three geographic sites of Cholistan, Pakistan by using 16S rRNA based Illumina sequencing. The present study reveals that plant species, plant compartments and a change in soil physicochemical characteristics affect the composition of rhizosphere and root endophytic microbial communities. Phylogenetic profiling showed that bacterial phyla Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes, and Fusobacteria were commonly identified from all the rhizosphere and root samples with significant variations between the rhizosphere and root endosphere, plant species across the three sites. Microbial diversity associated with the rhizosphere and root endosphere of *Haloxylon* collected from the geographic site 2 showed the greatest variations at the phylum and genus level. From the rhizosphere and the roots of each plant, certain bacterial genera were more dominant. More than 80% bacterial genera identified in this study have the potential ability to promote plant growth under salinity and drought stress environments and could be used as potential candidates for biofertilizers in salt affected agricultural lands.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, SRR9588854-SRR9588861.

AUTHOR CONTRIBUTIONS

SMu: conducted the experiment, analyzed the data, and prepared the manuscript. SMe: guided in experiment plan and edited the manuscript. KM: supervised the research and edited the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We are highly thankful to Higher Education Commission [Project # HEC (FD/2012/1843)] for research grant. We would like to express our gratitude to Dr. Samia Ahmad (Michigan State University) for her help in data analysis and Mr. Mukhtar Ahmad (Assistant Professor), Dyal Singh College, Lahore, for assistance in statistical analyses.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.618742/full#supplementary-material

REFERENCES

Abhauer, K. P., Wemheuer, B., Daniel, R., and Meinicke, P. (2015). Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics.* 31, 2882–2884. doi: 10.1093/bioinformatics/btv287

Adviento-Borbe, M. A., Doran, J. W., Drijber, R. A., and Dobermann, A. (2006). Soil electrical conductivity and water content affect nitrous oxide and carbon dioxide emissions in intensively managed soils. *J. Environ. Qual.* 35, 1999–2010. doi: 10.2134/jeq2006.0109

Anderson, J. M., and Ingram, J. S. (1993). *Tropical Soil Biology and Fertility: A Handbook of Methods*, 2nd Edn. Wallingford: CAB International, 93–94.

Borruso, L., Bacci, G., Mengoni, A., De Philippis, R., and Brusetti, L. (2014). Rhizosphere effect and salinity competing to shape microbial communities in *Phragmites australis* (Cav.) Trin. exSteud. *FEMS Microbiol. Lett.* 359, 193–200. doi: 10.1111/1574-6968.12565

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Themaat, E., and Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Ann. Rev. Plant Biol.* 64, 807–838. doi: 10.1146/annurev-arplant-050312-120106

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., et al. (2010). QIIME allows analysis of high throughput community sequencing data. *Nat. Meth.* 7, 335–336.

Castillo-Carvajal, L. C., Sanz-Martín, J. L., and Barragán-Huerta, B. E. (2014). Biodegradation of organic pollutants in saline wastewater by halophilic microorganisms: a review. *Environ. Sci. Pollut. Res. Int.* 21, 9578–9588. doi: 10.1007/s11356-014-3036-z

Chaudhary, D. R., Gautam, R. K., Yousuf, B., Mishra, A., and Jha, B. (2015). Nutrients, microbial community structure and functional gene abundance of rhizosphere and bulk soils of halophytes. *Appl. Soil Ecol.* 91, 16–26. doi: 10.1016/j.apsoil.2015.02.003

Chaudhry, M. S., and Nasim, F. H. (1995). Combating desertification in Cholistan desert. *Sci Tech Islamic World.* 13, 75–85.

Coleman-Derr, D., Desgarennes, D., Fonseca-Garcia, C., Gross, S., Clingenpeel, S., Woyke, T., et al. (2016). Plant compartment and biogeography affect microbiome composition in cultivated and native *Agave species*. *New Phytol.* 209, 798–811. doi: 10.1111/nph.13697

Dagla, H. R., and Shekhawat, N. S. (2005). In vitro multiplication of Haloxylon recurvum (Moq.)- aplant for saline soil reclamation. *J. Plant Biol.* 7, 155–160.

Deng, S., Chang, X., Zhang, Y., Ren, L., Jiang, F., Qu, Z., et al. (2015). Nocardioides antarcticus sp. nov., isolated from marine sediment of *Ardley cove*. *Int. J. Syst. Evol. Microbiol.* 65, 2615–2621. doi: 10.1099/ijs.0.000309

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., et al. (2006). Green genes, a chimera checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* 72, 5069–5072. doi: 10.1128/aem.03006-05

Dupont, C. L., Larsson, J., Yooseph, S., Ininbergs, K., Goll, J., et al. (2014). Functional tradeoffs underpin salinity-driven divergence in microbial community composition. *PLoS One.* 9:e89549. doi: 10.1371/journal.pone.0089549

English, J. P., and Colmer, T. D. (2011). Salinity and waterlogging tolerances in three stem- succulent halophytes (*Tecticornia species*) from the margins of ephemeral salt lakes. *Plant Soil.* 348, 379–396. doi: 10.1007/s11104-011-0924-6

Etesami, H., and Beattie, G. A. (2018). "Plant-microbe interactions in adaptation of agricultural crops to abiotic stress conditions," in *Probiotics and Plant Health*, eds V. Kumar, M. Kumar, S. Sharma, and R. Prasad (Singapore: Springer), 163–200.

Etesami, H., and Maheshwari, D. K. (2018). Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. *Ecotoxicol. Environ. Saf.* 156, 225–246. doi: 10.1016/j.ecoenv.2018.03.013

Fahmy, T. (2003). *XLSTAT-Pro 7.0 (XLSTAT), Addinsoft*. Paris: XLSTAT.

Fang, T. T., Pan, R. S., Jiang, J., He, F., and Wang, H. (2016). Effect of salinity on community structure and naphthalene dioxygenase gene diversity of a halophilic bacterial consortium. *Front. Env. Sci. Eng.* 10:16. doi: 10.1007/s11783-016-0888-0

Fitzpatrick, C. R., Copeland, J., Wang, P. W., Guttman, D. S., Kotanen, P. M., and Johnson, M. T. J. (2018). Assembly and ecological function of the root microbiome across angiosperm plant species. *Proc. Natl. Acad. Sci.* 115, E1157–E1165.

Foesel, B. U., Nägele, V., and Naether, A. (2014). Determinants of acidobacteria activity inferred from the relative abundances of 16S rRNA transcripts in german grassland and forest soils. *Environ. Microbiol.* 16, 658–675. doi: 10.1111/1462-2920.12162

Fonseca-Garcia, C., Coleman-Derr, D., Garrido, E., Visel, A., Tringe, S. G., and Partida-Martínez, L. P. (2016). The cacti microbiome: interplay between habitat-filtering and host-specificity. *Front. Microbiol.* 7:150. doi: 10.3389/fmicb.2016.00150

Gibtan, A., Park, K., Woo, M., Shin, J. K., Lee, D. W., et al. (2017). Diversity of extremely halophilic archaeal and bacterial communities from commercial salts. *Front. Microbiol.* 8:799. doi: 10.3389/fmicb.2017.00799

Good, I. J. (1953). The population frequencies of species and the estimation of population parameters. *Biometrica.* 40, 237–264. doi: 10.1093/biomet/40.3-4.237

Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., et al. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J.* 5, 1571–1579. doi: 10.1038/ismej.2011.41

Hussain, A., Arshad, M., Zahir, Z. A., and Asghar, M. (2015). Prospects of zinc solubilizing bacteria for enhancing growth of maize. *Pak J Agri Sci.* 52, 915–922.

Iverson, V., Morris, R. M., Frazar, C. D., Berthiaume, C. T., Morales, R. L., and Armbrust, E. V. (2012). Untangling genomes from metagenomes: revealing an uncultured class of marine Euryarchaeota. *Science* 335, 587–590. doi: 10.1126/science.1212665

Jiang, J. Q., Pan, Y. Y., Hu, S. X., Zhang, X. X., Hu, B. Z., et al. (2014). Halomonas songnenensis sp nov., a moderately *Halophilic bacterium* isolated from saline and alkaline soils. *Int. J. Syst. Evol. Microbiol.* 64, 1662–1669. doi: 10.1099/ijs.0.056499-0

Koranda, M., Schnecker, J., Kaiser, C., Fuchslueger, L., Kitzler, B., Stange, C. F., et al. (2011). Microbial processes and community composition in the rhizosphere of European beech - the influence of plant C exudates. *Soil Biol. Biochem.* 43, 551–558. doi: 10.1016/j.soilbio.2010.11.022

Krid, S., Rhouma, A., Mogou, I., Quesada, J. M., Nesme, X., and Gargouri, A. (2010). *Pseudomonas savastonoi* endophytic bacteria in olive tree knots and antagonistic potential of strains of Pseudomonas fluorescens and *Bacillus subtilis*. *J. Plant Pathol.* 92, 335–341.

Krivushin, K., Kondrashov, F., Shmakova, L., Tutukina, M., Petrovskaya, L., and Rivkinaa, E. (2015). Two metagenomes from late pleistocene northeast siberian permafrost. *Genome. Announc.* 3, 1380–1400.

Kumar, S., Karan, R., Kapoor, S., Singh, S. P., and Khare, S. K. (2012). Screening and isolation of *Halophilic bacteria* producing industrially important enzymes. *Braz. J. Microbiol.* 43, 1595–1603. doi: 10.1590/s1517-83822012000400044

Liszka, M., Clark, M., Schneider, E., and Clark, D. S. (2012). Nature versus nurture: developing enzymes that function under extreme conditions. *Ann. Rev. Chem. Biomol. Eng.* 3, 77–102. doi: 10.1146/annurev-chembioeng-061010-114239

Liu, M., Cui, Y., Chen, Y., Lin, X., Huang, H., and Bao, S. (2017). Diversity of Bacillus-like bacterial community in the sediments of the Bamenwan mangrove wetland in Hainan, China. *Can. J. Microbiol.* 63, 238–245. doi: 10.1139/cjm-2016-0449

Lozupone, C., Lladser, M. E., Knights, D., Stombaugh, J., and Knight, R. (2011). UniFrac: an effective distance metric for microbial community comparison. *ISME J.* 5, 169–172. doi: 10.1038/ismej.2010.133

Marasco, R., Mosqueira, M. J., Fusi, M., Ramond, J. B., Merlino, G., Booth, J. M., et al. (2016). Salicornia strobilacea (synonym of *Halocnemum strobilaceum*) grown under different tidal regimes selects rhizosphere bacteria capable of promoting plant growth. *Front. Microbiol.* 7:1286. doi: 10.3389/fmicb.2016.01286

Mukhtar, S., Mehnaz, S., Mirza, M. S., and Malik, K. A. (2019). Isolation and characterization of halophilic bacteria from the rhizosphere of halophytes and non-rhizospheric soil samples. *Braz. J. Microbiol.* 50, 85–97.

Mukhtar, S., Mirza, B. S., Mehnaz, S., Mirza, M. S., Mclean, J., and Malik, K. A. (2018). Impact of soil salinity on the structure and composition of rhizosphere microbiome. *World J. Microbiol. Biotech.* 34:136.

Mukhtar, S., Mirza, M. S., Awan, H. A., Maqbool, A., Mehnaz, S., and Malik, K. A. (2016). Microbial diversity and metagenomic analysis of the rhizosphere of Para Grass (*Urochloa mutica*) growing under saline conditions. *Pak. J. Bot.* 48, 779–791.

Mukhtar, S., Ishaq, A., Hassan, S., Mehnaz, S., Mirza, M. S., and Malik, K. A. (2017a). Comparison of microbial communities associated with halophyte (*Salsola stocksii*) and non-halophyte (*Triticum aestivum*) using culture-independent approaches. *Pol. J. Microbiol.* 66, 375–386.

Mukhtar, S., Shahid, I., Mehnaz, S., and Malik, K. A. (2017b). Assessment of two carrier materials for phosphate solubilizing biofertilizers and their effect on growth of wheat (*Triticum aestivum*). *Microbiol. Res.* 205, 107–117. doi: 10.1016/j.micres.2017.08.011

Mukhtar, S., Zareen, M., Khaliq, Z., Mehnaz, S., and Malik, K. A. (2020). Phylogenetic analysis of halophyte-associated rhizobacteria and effect of halotolerant and halophilic phosphate-solubilizing biofertilizers on maize growth under salinity stress conditions. *J. Appl. Microbiol.* 128, 556–573. doi: 10.1111/jam.14497

Olsen, S. R., Cole, C. V., Watanabe, F. S., and Dean, L. A. (1954). *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*. Washington D.C: USDA Circular, Printing Office,

Oren, A. (2013). Life at high salt concentrations, intracellular KCl concentrations, and acidic proteomes. *Front. Microbiol.* 4:315. doi: 10.3389/fmicb.2013.00315

Oren, A. (2015). Halophilic microbial communities and their environments. *Curr. Opin. Microbiol.* 33, 119–124. doi: 10.1016/j.copbio.2015.02.005

Pan, Y., Cassman, N., Hollander, M., Mendes, L. W., Korevaar, H., et al. (2014). Impact of long-term N, P, K, and NPK fertilization on the composition and potential functions of the bacterial community in grassland soil. *FEMS Microbiol. Ecol.* 90, 195–205. doi: 10.1111/1574-6941.12384

Ramadoss, D., Lakkineni, V. K., Bose, P., Ali, S., and Annapurna, K. (2013). Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. *Springerplus.* 2:6. doi: 10.1186/2193-1801-2-6

Rengasamy, R. (2006). World salinization with emphasis on Australia. *J. Exp. Bot.* 57, 1017–1023. doi: 10.1093/jxb/erj108

Santhanam, R., Oh, Y., Kumar, R., Weinhold, A., Luu, V. T., Groten, K., et al. (2017). Specificity of root microbiomes in native-grown *Nicotiana attenuata* and plant responses to UVB increase Deinococcus colonization. *Mol. Ecol.* 26, 2543–2562. doi: 10.1111/mec.14049

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W., et al. (2011). Metagenomic biomarker discovery and explanation. *Gen. Biol.* 12, R60.

Tian, X. Y., and Zhang, C. S. (2017). Illumina-based analysis of endophytic and rhizosphere bacterial diversity of the coastal halophyte Messerschmidia sibirica. *Front. Microbiol.* 8:2288. doi: 10.3389/fmicb.2017.02288

Tian, Y. Q., and Gao, L. H. (2014). Bacterial diversity in the rhizosphere of cucumbers grown in soils covering a wide range of cucumber cropping histories and environmental conditions. *Microbial. Ecol.* 68, 794–806. doi: 10.1007/s00248-014-0461-y

Vogel, H. (1978). *Vogel's Textbook of Quantitative Inorganic Analysis*, 4th Edn. London: Longman.

Walkley, A., and Black, I. A. (1934). An examination of degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37, 29–37. doi: 10.1097/00010694-193401000-00003

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73, 5261–5267. doi: 10.1128/aem.00062-07

Yan, N., Marschner, P., Cao, W., Zuo, C., and Qin, W. (2015). Influence of salinity and water content on soil microorganisms. *ISWCR* 3:316323.

Yuan, Z., Druzhinina, I. S., Labbé, J., Redman, R., Qin, Y., Rodriguez, R., et al. (2016). Specialized microbiome of a halophyte and its role in helping non-host plants to withstand salinity. *Sci. Rep.* 6:32467.

Zenga, Y., Fengb, F., Medováa, H., Deana, J., and Koblížeka, M. (2014). Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum Gemmatimonadetes. *PNAS.* 111, 7795–7800. doi: 10.1073/pnas.1400295111

Zhang, H., Wang, Z., Zhang, Y., Ding, M., and Li, L. (2015). Identification of traffic-related metals and the effects of different environments on their enrichment in roadside soils along the Qinghai-Tibet highway. *Sci. Total Environ.* 522, 160–172. doi: 10.1016/j.scitotenv.2015.03.054

Zhao, S., Zhou, N., Zhao, Z. Y., Zhang, K., Wu, G. H., and Tian, C. Y. (2016). Isolation of endophytic plant growth-promoting bacteria associated with the halophyte Salicornia europaea and evaluation of their promoting activity under salt stress. *Curr. Microbiol.* 73, 574–581. doi: 10.1007/s00284-016-1096-7

Zhou, N., Zhao, S., and Tian, C. Y. (2017). Effect of halotolerant rhizobacteria isolated from halophytes on the growth of sugar beet (*Beta vulgaris* L.) under salt stress. *FEMS Microbiol. Lett.* 364:fnx091.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 20 April 2021
doi: 10.3389/fmicb.2021.648455

Resistance, Resilience, and Recovery of Dryland Soil Bacterial Communities Across Multiple Disturbances

Blaire Steven[1], Michala L. Phillips[2], Jayne Belnap[2], La Verne Gallegos-Graves[3], Cheryl R. Kuske[3] and Sasha C. Reed[2]*

[1] Department of Environmental Sciences, Connecticut Agricultural Experiment Station, New Haven, CT, United States, [2] United States Geological Survey, Southwest Biological Science Center, Moab, UT, United States, [3] Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM, United States

Dryland ecosystems are sensitive to perturbations and generally slow to recover post disturbance. The microorganisms residing in dryland soils are especially important as they contribute to soil structure and nutrient cycling. Disturbance can have particularly strong effects on dryland soil structure and function, yet the natural resistance and recovery of the microbial components of dryland soils has not been well documented. In this study, the recovery of surface soil bacterial communities from multiple physical and environmental disturbances is assessed. Samples were collected from three field sites in the vicinity of Moab, UT, United States, 6 to 7 years after physical and climate disturbance manipulations had been terminated, allowing for the assessment of community recovery. Additionally, samples were collected in a transect that included three habitat patches: the canopy zone soils under the dominant shrubs, the interspace soils that are colonized by biological soil crusts, and edge soils at the plot borders. Field site and habitat patch were significant factors structuring the bacterial communities, illustrating that sites and habitats harbored unique soil microbiomes. Across the different sites and disturbance treatments, there was evidence of significant bacterial community recovery, as bacterial biomass and diversity were not significantly different than control plots. There was, however, a small number of 16S rRNA gene amplicon sequence variants that distinguished particular treatments, suggesting that legacy effects of the disturbances still remained. Taken together, these data suggest that dryland bacterial communities may possess a previously unappreciated potential to recover within years of the original disturbance.

Keywords: dryland, biological soil crust, resilience, recovery, bacteria, arid soils

OPEN ACCESS

Edited by:
Mathew A. Leibold, University of Florida, United States

Reviewed by:
Roey Angel, Centre for Biology, Academy of Sciences of the Czech Republic (ASCR), Czechia
Claudia Coleine, University of Tuscia, Italy

****Correspondence:***
Blaire Steven
blaire.steven@ct.gov

Specialty section:
This article was submitted to Terrestrial Microbiology, a section of the journal Frontiers in Microbiology

Received: *31 December 2020*
Accepted: *18 March 2021*
Published: *20 April 2021*

Citation:
Steven B, Phillips ML, Belnap J, Gallegos-Graves LV, Kuske CR and Reed SC (2021) Resistance, Resilience, and Recovery of Dryland Soil Bacterial Communities Across Multiple Disturbances. Front. Microbiol. 12:648455. doi: 10.3389/fmicb.2021.648455

INTRODUCTION

Soil microorganisms are central to many biogeochemical cycles, including those for carbon, nitrogen, and phosphorus. Given the significance of these populations and the processes they help control, there is a rich literature describing how microbial communities respond to various disturbances (Allison and Martiny, 2008; Shade et al., 2012; Evans and Wallenstein, 2014).

The capacity of a community to recover from a disturbance is referred to as the community's resilience (Baho et al., 2012). Thus, a community's resilience defines the severity of population shifts in response to a disturbance and the time scales at which recovery can be expected to occur. This, in turn, informs the scale and resources that should be dedicated to management or intervention efforts to restore degraded ecosystems (van de Leemput et al., 2018). Information on the resilience of soil microbial communities is severely lacking (García-García et al., 2019; Rath et al., 2019; Uritskiy et al., 2019). A recent meta-analysis of microbial disturbance and recovery found that only a fraction of studies undertake assessing the recovery of microbial communities as an objective, and only a small number of those studies measure or detect a return to pre-disturbance community composition and function. Thus, the authors posit that this either reflects that microbial communities have not been sampled with enough replication or duration to assess recovery, or that microbial populations in fact have a low resilience to disturbance (Shade et al., 2012). Consequently, there is considerable uncertainty regarding the resilience of microbial communities to the multitude of disturbances they face in a changing environment and under altered land use.

Drylands account for approximately 40% of the terrestrial surface, and are expected to expand under a regime of climate warming (Cherlet et al., 2018). Arid ecosystems are among the most fragile areas on the planet, and thought to be exquisitely sensitive to perturbations due to their low and limited resource availability (Archer and Predick, 2008; Poulter et al., 2014). This resource scarcity leads to generally slow recovery rates of arid ecosystems post disturbance. For example, estimates for plant recovery in arid ecosystems ranges from 60 to >100 years depending on factors such as elevation, temperature, and rainfall (Estruch et al., 2018; Monroe et al., 2020). Similar recovery times have been proposed for surface soil autotroph communities (i.e., biological soil crusts) with full recovery proposed to occur in a decadal or even century timeframes (Weber et al., 2016; Williams et al., 2018). However, these studies have often focused on late successional dryland soil communities such as mosses and lichens with relatively little information on the pioneering soil bacterial communities.

In 2015, we reported the response of dryland soil bacteria to multiple climate change and physical disturbance manipulations (Steven et al., 2015). These consisted of chronic physical trampling, a 4°C above ambient warming, altered precipitation consisting of increased frequency of small precipitation events, and the warming and precipitation manipulation in combination. The disturbances generally resulted in significant declines in bacterial biomass and shifts in the relative abundance of hundreds of bacterial taxa (Steven et al., 2015). In several cases the disturbances resulted in a total collapse of the surface biological soil crust communities, which are composed of cyanobacteria, algae, lichens, mosses and their associated heterotrophic fungi, bacteria, and archaea (Housman et al., 2007; Steven et al., 2012a, 2014; Steven, 2017). Yet, we found that each disturbance resulted in a unique microbial community composition, which was not apparent at the visual-scale (Ferrenberg et al., 2015). Given the severity and differing responses of the communities to the perturbations, we undertook a study to characterize the recovery of the bacterial populations following the termination of these disturbances. Chronic trampling and the precipitation pulse disturbances were terminated 6–7 years prior to sampling, while the warming treatment was maintained. In this manner, we could assess if increased temperatures would influence the rate or success of recovery of the soil bacterial communities. We further investigated three habitat patches: (1) root zone soils under the canopy of the local shrubs, (2) interspace soils colonized by biological soil crusts, and (3) the edge soils at the border of the plot. With this approach, we could test if recovery was happening equally across the plot or if particular environmental niches fostered a faster recovery.

MATERIALS AND METHODS

Sample Sites and Field Manipulations

Three long-term *in situ* experiments located in otherwise undisturbed natural dryland landscapes were used for this study. The effects of chronic physical disturbance by annual foot trampling were tested at two sites, one located in Arches National Park (termed Arches here; 38.72662 N, 109.5434 W, elevation 1,490 m). At Arches, soil depth ranged from 30 to 100 cm and was of the Mido-Sazi complex, gray soil, loamy sand. The dominant shrub was *Coleogyne ramosissima* (blackbrush).

The second trampling site was located in the Island in the Sky district of Canyonlands National Park (termed ISKY here; 38.45756 N, 109.5411 W, elevation 1,800 m). The ISKY soil profile was 10–25 cm in depth and consisted of Rizno dry rock outcrop complex, light blond soil, loamy sand. The dominant shrub was also *C. ramosissima* (blackbrush). Both trampled sites harbored biological soil crusts that differed in development and composition of lichens and cyanobacteria, where cyanobacteria were more abundant and developed at the ISKY site.

The third site, Castle Valley, UT, United States (termed CV here; 38.6748 N, 109.4163 W, elevation 1,310 m), was about 10 km from the Arches and ISKY sites. Soil depth ranged from 17 to 122 cm and was a Lithic Torriorthent, a sandy loam. The dominant shrub was *Atriplex confertifolia*. Here, the effects of year-round warming and altered frequency of summer precipitation were tested in a long-term field experiment. Increased warming with infrared (IR) lamps was delivered to five replicate plots, resulting in an approximately 4°C soil warming to a depth of 5 cm. Altered precipitation was delivered as 1.2-mm water pulses in an average of 35 events during the summer months, resulting in an approximate 4-fold increase in the number of small precipitation events. Separate plots received year-round warming and altered precipitation treatments in combination. Control plots consisted of untreated plots with a non-energized IR lamp housing. Plots were 2 m × 5 m in size with five replicates per treatment per site in a randomly assigned full factorial design.

Across the three field sites, annual precipitation from 2005 to 2018 ranged from 137 to 375 mm with an average of 246 mm. The manipulations employed in these studies were within the range of climate or land use changes already occurring in these regions or

predicted to occur within the next few decades (Wang J. et al., 2007). Samples were collected from Arches and from ISKY on October 1st, 2018. Both sites received a cumulative 16 years of annual spring trampling treatments. At the time of sampling the treatments had been ended for 7 years. At the CV site, samples were collected on October 2nd after 6 years of treatment. All treatments, excluding warming, were terminated in 2012.

Soil Collection for Microbial Community Analysis

Soil samples consisted of the upper 0–2 cm of soil collected with a sterile spatula. Soil samples were immediately placed on dry-ice and were kept frozen during transport to the Los Alamos National Laboratory. The samples were stored in a −80°C freezer until sample processing. The soil samples were collected in a roughly north to south transect from each plot encompassing the three habitat patches: the edge of the plot, canopy zone soils, and interspace soils, with two samples per habitat (**Figure 1**). Four replicate plots were sampled per treatment resulting in 8 replicate soil samples per habitat zone per treatment.

DNA Extraction and Quantitative PCR

Total DNA was extracted with the Qiagen DNeasy PowerSoil HTP 96 Kit following the manufacturer's protocols. Prior to amplification, DNA samples were normalized to a concentration of 1 ng μl^{-1} as measured by a Qubit Fluorometer. Reaction volumes of 24 µl were prepared as follows: 12.5 µl iQ Syber Green Super Mix, 0.25 µl BSA, 0.7 µl forward primer (338-Eub 5′CTC CTA CGG GAG GCA GCA CT 3′), 0.7 µl reverse primer (513-Eub 5′ATT ACC GCG GCT GCT GG 3′), 9.85 µl H_2O. Thermal cycling consisted of initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s; 55°C for 30 s (data collection step); 72°C for 30 s. A melting curve was performed consisting of 80 cycles at 55°C for 10 s. A six-point standard curve was prepared using a 10X dilution series of *Microcoleus vaginatus* DNA.

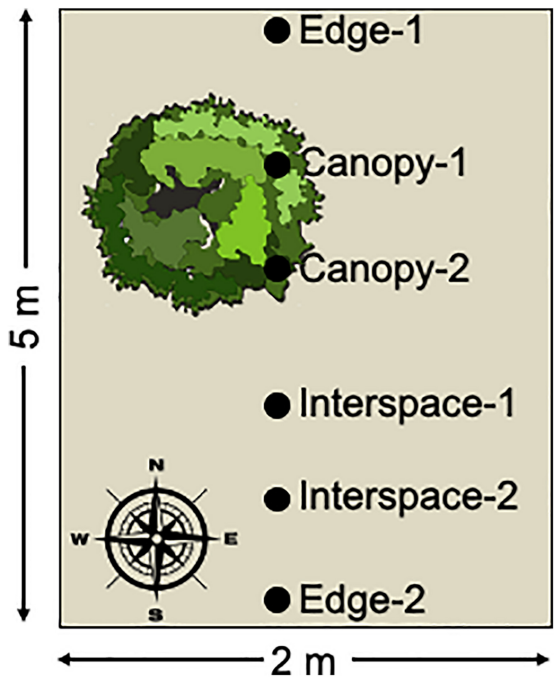


FIGURE 1 | Schematic diagram of sample collection. Surface soil samples were collected along a roughly north to south transect from each sampled plot. The transect encompassed three habitat patches: canopy zone soils in the dripline of the dominant shrubs, interspace soils in the unvegetated patches of the plot, and edge soils at the plot boundaries. Two replicate samples were collected for each habitat patch and four plots were sampled per treatment, for a total of 8 biological replicates per site, treatments, and habitat patch, resulting in a total of 192 soil samples collected.

16S rRNA Gene Amplification and Sequencing

DNA extractions were processed at the University of Connecticut's Microbial Analysis, Resources, and Services facility for 16S rRNA gene amplifications and sequencing. Bacterial 16S rRNA genes were amplified using 30 ng of extracted DNA from each of the 192 soil samples. The V4 region was amplified using primers 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) with Illumina adapters and dual indices. Samples were amplified in triplicate using GoTaq (Promega) with the addition of 10 µg BSA (New England BioLabs), and the three independent PCR reactions were pooled prior to sequencing. The PCR reaction was incubated at 95°C for 3.5 min, then 30 cycles of 30 s at 95.0°C, 30 s at 50.0°C and 90 s at 72.0°C, followed by final extension as 72.0°C for 10 min. PCR products were pooled for quantification and visualization using the QIAxcel DNA Fast Analysis (Qiagen). PCR products were normalized based on the concentration of DNA then pooled using the QIAgility liquid handling robot. The pooled PCR products were cleaned using the Mag-Bind RxnPure Plus (Omega Bio-tek) according to the manufacturer's protocol. The cleaned pool was sequenced on the MiSeq platform using v2 2 × 250 chemistry (Illumina, Inc).

Bioinformatic and Statistical Analyses

Sequencing reads were assembled into contigs and quality screened by using mothur v1.39.5 (Schloss et al., 2009). Sequences that were at least 253 bp in length, contained no ambiguous bases, and no homopolymers of more than 8 bp were used in the analysis. Chimeric sequences were identified by using the VSEARCH (v2.15.2) as implemented in mothur (Rognes et al., 2016), and all potentially chimeric sequences were removed. To maintain a similar sampling effort between samples, datasets with less than 1,000 sequences per sample were also removed. After removal a total of 172 samples remained from the original 192 samples (**Figure 1**). Thus, the majority of samples contained 8 biological replicates across site, treatment and habitat patch, with the smallest number of replicates being 6. The resulting list of unique sequences was considered the set of amplicon sequence variants (ASVs). Taxonomic classification of sequences was performed with the Ribosomal Database Project classifier against the SILVA v132 reference alignment in mothur (Wang Q. et al., 2007; Quast et al., 2012).

Sequence data were imported into the phylsoseq package in R (v. 1.34.0) for exploratory and statistical analyses (McMurdie and Holmes, 2013). For calculation of Shannon's diversity, data were randomly subsetted to the size of the smallest dataset (1,417 sequences). The subsetted data were used to calculate Bray-Curtis similarities for sample distances. Statistically significant differences in clustering were determined by permutational multivariate analysis of variance (PERMANOVA) calculated with the adonis function in the VEGAN R software package (Dixon and Palmer, 2003, v. 2.5–7).

Differentially Abundant Amplicon Sequence Variants (DA ASVs) were determined using unnormalized ASV counts with the ALDEx2 (v. 1.18.0) software package (Fernandes et al., 2013), after ASVs consisting of five or fewer sequences were removed. Briefly, a Dirichlet-multinomial model to infer abundance from counts was employed. For three-way comparisons among the field sites significant differences were identified with the Kruskal-Wallace and generalized linear model tests with raw *P*-values corrected with Benjamini-Hochberg correction. Significant ASVs had corrected *P*-values < 0.05 for both tests. For two-way comparisons among treatments the results of the Wilcoxon Rank Sum were used to identify DA ASVs. *P*-values were corrected with the Benjamini-Hochberg correction for multiple testing. Reported DA ASVs had a corrected *P*-value < 0.05. Statistically significant differences in phylum level taxonomic bins were identified using the STAMP software package (v.2.1.3) using an ANOVA test followed by a Games-Howell *post hoc* test. *P*-values were corrected for multiple testing employing Storey's FDR method (Parks et al., 2014).

RESULTS

Bacterial Community Composition, Diversity, and Biomass

Canonical analysis of principal coordinates (CAP) analysis was employed to investigate the relationship between sequence datasets (**Figure 2A**). The largest factor explaining the variation between datasets was field site, with the samples from the three sites clustering independently (Adonis R^2 0.067, $P < 0.001$). The different treatments also clustered independently (Adonis R^2 0.071, $P < 0.001$). For example, the Arches and ISKY trampled datasets clustered distinctly, indicating that the field site differences were still apparent even in the disturbed soils (**Figure 2A**). Similar dynamics are observed for the CV field site, with clear differences between the warming (W), precipitation (P), and warming and precipitation (W+P) datasets (**Figure 2**).

To test if there were differences in bacterial diversity among the sites Shannon's diversity index was calculated (**Figure 2B**). There was a significant reduction in diversity between the control and trampled datasets at the Arches field site ($P < 0.001$). Similarly, there was a reduction in diversity at the ISKY site with trampling, although the difference from the ISKY controls was not significant. Across the CV manipulations there was a general trend in diversity increasing with the manipulations, although again the differences were not significant. Taken together, these observations suggest that bacterial diversity was generally similar between the control and disturbed soils, with the exception of the trampling manipulation, particularly at the Arches site. Finally, we employed quantitative PCR (qPCR) as a measure of bacterial biomass in the soil samples (**Figure 2C**). Across the experiment there were no significant differences in 16S rRNA gene copy numbers between field sites or manipulations, indicating that bacterial biomass was similar across filed site and manipulations.

Biogeography of Soil Communities Among Field Sites

Given that field site was found to be a large factor in structuring the bacterial communities (**Figure 2A**), differences in bacterial populations among field sites were investigated. The control sequence datasets were bioinformatically separated and compared by non-metric multidimensional scaling (NMDS; **Figure 3A**). Clustering of the datasets was readily apparent and significant ($P < 0.001$), indicating that the natural state of the soil microbiome differs among the field sites.

To characterize the populations that account for field site differences the sequences were classified to the phylum level (**Figure 3B**). The relative abundance of Cyanobacteria was higher at ISKY than at Arches or CV ($P < 0.001$ and $P < 0.01$, respectively). This translated into significantly lower relative abundances of the Actinobacteria at ISKY in comparison to the other field sites.

Finally, the data were interrogated for differentially abundant (DA) 16S rRNA gene ASVs. A total of 28 ASVs were identified as being significantly different in relative abundance among the sites (**Figure 3C**). The DA ASVs belonged to four bacterial phyla the Acidobacteria, Actinobacteria, Proteobacteria, and Cyanobacteria. Patterns in the identified ASVs matched observations for the phylum level bins. For instance, all of the ASVs related to the Cyanobacteria were enriched at ISKY, which also showed the highest proportions of cyanobacteria-related sequences (**Figure 3B**). Several of the DA ASVs were classified to similar taxonomic ranks. Of the 9 DA ESVs classified to the Actinobacteria, six were identified as belonging to the family *Geodermatophilaceae*. Similarly, among the eight DA ASVs related to the Proteobacteria, three belonged to the same genus, *Microvirga* (**Supplementary Table 1**). Thus, there was a conserved taxonomic signal among those ASVs that differed in relative abundance among field sites.

Differentially Abundant Amplicon Sequence Variants in Response to Disturbance

To assess the recovery of the soil bacterial communities from the different manipulations, DA ASVs between the control and treatment datasets were determined. The trampling treatments demonstrated a small number of DA ASVs with 6 and 11 in the Arches and ISKY sites, respectively (**Figure 4**). At both sites the majority of the ASVs were enriched in the control plots. In fact only a single ASV, classified to the cyanobacterial genus *Tychonema*, was found to be more abundant in the trampled plots at the Arches site.

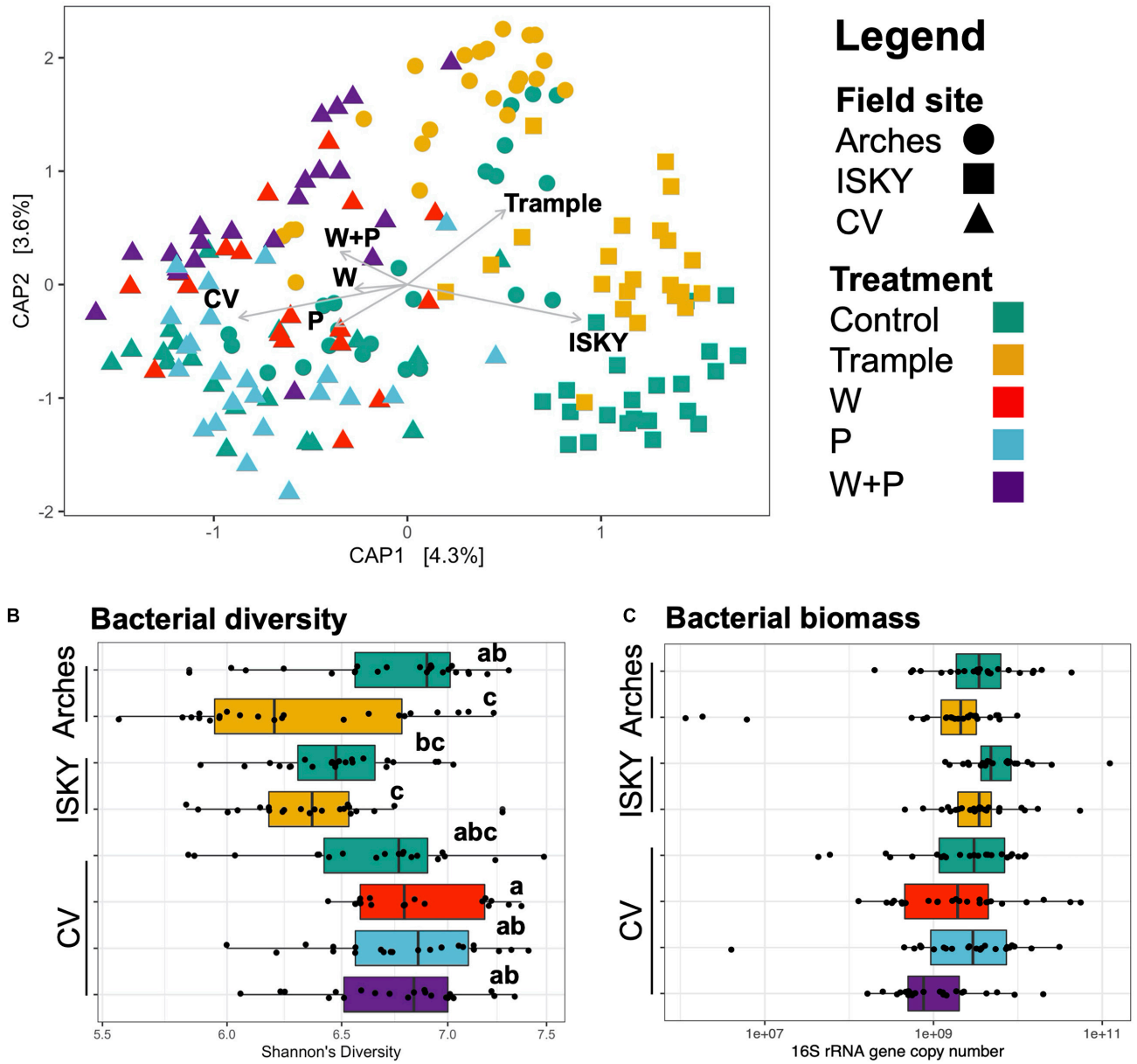


FIGURE 2 | Bacterial composition, diversity, and biomass across the datasets. **(A)** Canonical analysis of principal coordinates ordination of sequence datasets. Axes labels denote the percent variation explained by principal components. Arrows indicate the direction and magnitude of the variance explained by field site and manipulation treatments. **(B)** Bacterial diversity. Diversity was assessed by the non-parametric Shannon's diversity index. Boxplots show the mean (solid bar) and interquartile range of the values. Each individual sample is represented by the black points. Samples labeled with different letters were significantly different by ANOVA testing of means followed by a *post hoc* Tukey-Kramer test. **(C)** Bacterial abundance as determined by qPCR of bacterial 16S rRNA genes. Boxplots show the mean (solid bar) and interquartile range of the values. Each individual sample is represented by the black points. No significant differences between datasets were identified.

Interestingly four DA ASVs were identified in both the Arches and ISKY field sites (**Supplementary Table 2**). These ASVs were identified to the genera *Solirubrobacter* (phylum Actinobacteria), *Geodermatophilus* (phylum Actinobacteria), *Rubellimicrobium* (phylum Proteobacteria), and *Mastigocladopsis* (phylum Cyanobacteria). Thus, there appears to be common core of bacteria that show a similar response to trampling between the two field sites. A full list of DA ASVs is shown in **Supplementary Table 2**.

At the CV field climate change manipulation experiment, there were no DA ASVs associated with the warming (W) or altered precipitation (P) manipulations, suggesting that the soil bacterial communities have largely recovered or were resilient to the disturbances (**Figure 4**). In the combined treatment

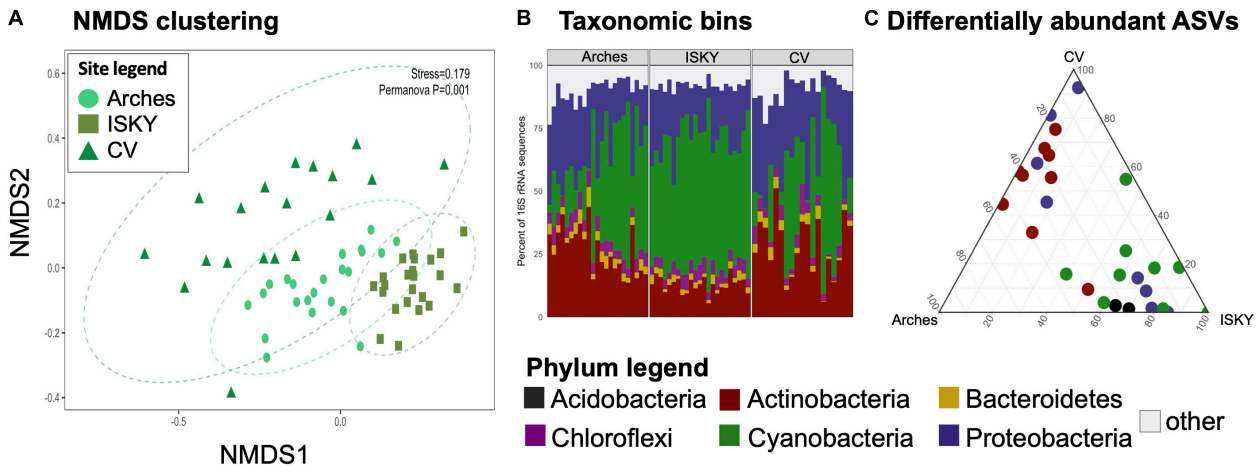


FIGURE 3 | Field site biogeography. For each dataset the control samples were compared to investigate if the normal state of the soil microbiome differed among sites. **(A)** NMDS clustering. The stress value of the ordination is indicated. Ellipses indicate the 95% confidence interval of the group centroids. PERMANOVA statistical testing identified field site as a significant factor in discriminating the bacterial communities ($P = 0.001$). **(B)** Phylum level taxonomic bins in the datasets. Stacked bar graphs representing the relative abundance of the six most predominant bacterial phyla in the datasets. Each phylum accounted for at least 1% of the recovered sequences across the individual samples. The remaining taxa were assigned to the category "other." Four phyla were identified as significantly different in relative abundance between sites, Actinobacteria, Bacteroidetes, Cyanobacteria, and Proteobacteria. **(C)** Ternary diagram of differentially abundant ASVs. Each point represents an ASV identified as significantly different in relative abundance among the sites. Points are colored by the Phylum to which they belong. A full list of the differentially abundant ASVs are listed in **Supplementary Table 1**.

(W + P) a small number of ASVs were depleted relative to the control plots (**Figure 4**), suggesting there were still legacy effects of the W + P treatment 6 years after the altered precipitation ceased. Of note is that two of the DA ASVs were also common to the trampling manipulations. These ASVs were classified to the genus *Rubellimicrobium* (phylum Proteobacteria) and family *Micromonosporaceae* (phylum Actinobacteria), further supporting that particular bacterial taxa are consistently identified as associated with legacy effects of disturbance.

Influence of Habitat Patches on Community Composition

Finally, we assessed three different habitat patches -canopy zone soils, interspace soils, and soils from the edge of the plot – in an attempt to test if habitat niches affect community composition or recovery. CAP clustering was employed to investigate community similarity amongst the habitat patches under the different treatments. Across the datasets the control canopy zone soils clustered independently from interspace and edge soils ($P \leq 0.01$; **Supplementary Table 3**), indicating significant differences in community composition between the environments. There was a more pronounced overlap between the interspace and edge samples (**Figure 5** and **Supplementary Table 3**). This is somewhat expected as the edge samples were interspace soils that happened to occur at the plot borders, thus it is not surprising that they represent a similar environment. Some site specific patterns were apparent. For example, in the warming plots the edge samples clustered distinctly and significantly from the other soil patches (**Figure 5**, $P < 0.05$; **Supplementary Table 3**). This may represent an experimental artifact, as the IR lamp is over the center of the plot, thus warming may have attenuated at the plot edges resulting in an unequal disturbance across the plot. In this regard, the plot interspaces potentially receive more IR warming manifesting as a larger community shift. Yet, in general, the different habitat patches shifted in the same direction and with relatively similar magnitude, suggesting that the different types of disturbance affected the soil communities to a similar degree. Thus, these data indicate that while there are differences in soil microbial populations between the habitat patches, these populations show a similar sensitivity, response, and recovery from the various manipulations performed in this study. These data also suggest that recovery was similar across the niches in the plot. For instance, the edges did not show a higher recovery than the interspaces, suggesting that recovery did not occur through migration of healthy populations from adjacent healthy soils.

DISCUSSION

Biogeographic Patterns in Soil Communities

To properly assess the resilience and recovery of microbial communities, the natural state of the community first needed to be defined. This included the biogeographic factors that can influence community composition. Previous work has shown that soil edaphic factors, climatic legacies, and various landscape features all act to influence the structure of dryland soil microbial populations (Briggs and Morgan, 2008; Steven et al., 2013; Gombeer et al., 2015; Eldridge and Delgado-Baquerizo, 2019). In line with these observations, we show the bacterial populations

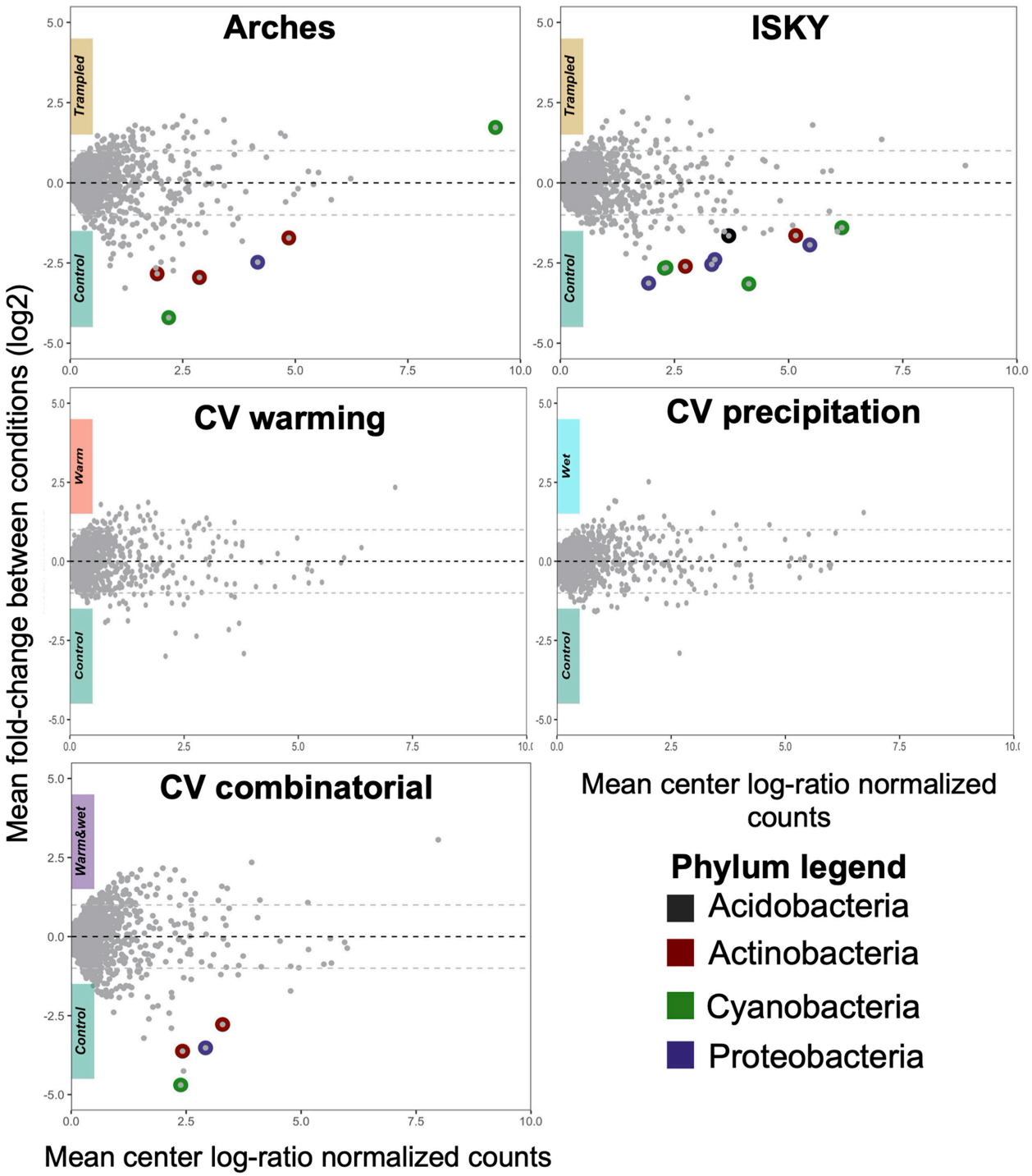


FIGURE 4 | Differentially abundant ASVs due to treatment. Each panel represents a Bland-Altman plot for the abundance and fold change of each ASV in the dataset. The *x*-axis indicates the center log-ratio normalized counts of each ASV in the data, while the *y*-axis shows the fold-change in relative abundance due to the treatments. ASVs that showed a statistically significant difference in relative abundance are highlighted by colors representing the Phylum to which they belong. A full list of the differentially abundant ASVs is listed in **Supplementary Table 2**.

were largely differentiated among field sites in the absence of disturbance (**Figures 2A**, **3**). One of the ways in which the community differences were manifest was an increase in spatial heterogeneity at particular sites. For instance, the inter-sample distances between the CV replicate datasets was larger than for ISKY, with Arches falling between the two (**Figure 3A**). This is

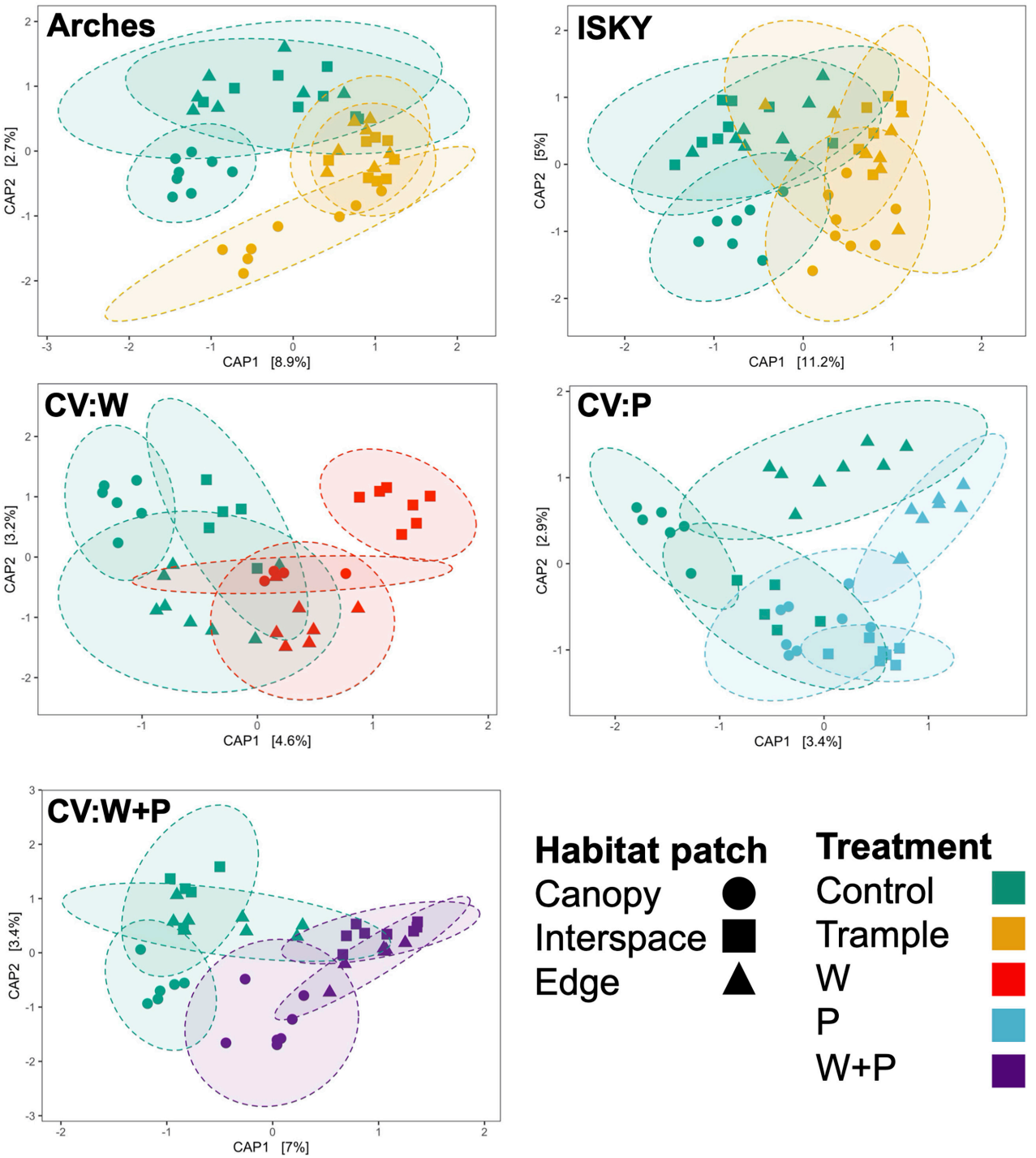


FIGURE 5 | Influence of habitat patch on community composition. Canonical analysis of principal coordinates ordination of sequence datasets for each treatment. Axes labels denote the percent variation explained by principal components. Ellipses indicate the 95% confidence interval of the group centroids. A table of PERMANOVA statistical results of between group comparisons is presented in **Supplementary Table 3**.

also apparent for the taxonomic bins identified in the datasets. The proportion of Cyanobacteria-related sequences varied widely between CV samples, while remaining more stable at the ISKY sites (**Figure 3B**). These observations support and build substantially on previous reports describing the biogeographic patterns that shape dryland surface soil bacterial communities (Steven et al., 2013; Wang et al., 2017; Ji et al., 2020). These data will improve our understanding of how factors such as

climate and soil type impact dryland soil bacterial community composition and will inform forecasts of how these microbial populations may respond to the variety of disturbances they face in a changing future.

A second biogeographic pattern related to the different habitat patches was also apparent. In general, communities from the canopy zone and interspace soils clustered distinctly from one another, indicating differing composition (**Figure 5** and **Supplementary Table 3**). This matches previous observations supporting that habitat patches in arid landscapes harbor differing resources and microbial populations (Schade and Hobbie, 2005; Koyama et al., 2018). Specifically, shrubs in arid ecosystems foster a collection of resources through intercepting wind carried dust, depositing carbon and nutrients to soil through litter, concentrating precipitation, and providing shade which reduces evaporation and protects from ultraviolet radiation (Coppinger et al., 1991; Martinez-Meza and Whitford, 1996; Dean et al., 1999). This generally results in an increase in microbial activity in the canopy zone soils (Conant et al., 1998; Su et al., 2004). As such, canopy zones have been referred to as "islands of fertility" (Schlesinger et al., 1990). Yet, although the composition of soil communities differed, canopy zone and interspace soils have been found to harbor an overlapping set of bacteria, suggesting that different habitat patches largely consist of a common core of species that differ in their relative abundance rather than presence or absence (Steven et al., 2012a, 2014). A similar pattern is noted here. For instance, several ASVs were identified that differentiated the field sites (**Figure 3**). Yet, these ASVs differed in relative abundance not presence or absence. Thus, these data suggest that a population of bacterial species is relatively ubiquitous across the landscape, but these populations shift in abundance in relation to biographic factors related to soil edaphic properties, local climates, and/or small-scale environmental niches. These are all important factors when considering the resilience of microbial communities as the different starting points for each community will likely influence how these communities respond to and recover from various disturbances.

Recovery From Chronic Trampling

Chronic physical trampling was carried out at two field sites for 15 years. Samples collected during this disturbance showed a collapse of the soil microbial community, with reduced bacterial biomass and shifts in the abundance of dominant bacterial populations (Kuske et al., 2012; Ferrenberg et al., 2015; Steven et al., 2015, 2018). In 2015, we reported a significant decrease (approximately 2 fold lower) in 16S rRNA gene copy number in response to trampling before recovery was initiated (Steven et al., 2015). In this study, 7 years after the trampling ceased, this biomass difference was no longer apparent (**Figure 2C**). Yet, there were still significant differences in bacterial diversity (**Figure 2B**), clustering of sequence datasets (**Figure 2A**), and a number of DA ASVs that distinguished the control and trampled datasets (**Figure 4**). Therefore, while bacterial biomass may be showing signs of recovery, community composition still reflects legacy effects of the trampling disturbance. An interesting observation from the community differences due trampling was that despite biogeographic differences between the Arches and ISKY field sites (**Figure 2**), an overlapping set of four ASVs, all enriched in the control sites, were identified as DA at both sites (**Supplementary Table 2**), indicating a congruent response to chronic physical disturbance. These observations suggest that certain taxa are slower to recover and may be indicative of legacy effects of a severe disturbance.

Response to Chronic Warming

The 4°C warming was the only manipulation that was still in operation at the time of sampling. Yet, bacterial 16S rRNA gene copies (**Figure 2C**), bacterial diversity (**Figure 2B**), and ASV relative abundance (**Figure 4**) were all similar between control and warmed plots. In 2015, we reported a limited influence of warming on the soil bacterial community. There was no significant difference in bacterial biomass between warmed and control plots, and only a small number of taxa (5 genera) showed a significant difference in relative abundance (Steven et al., 2015). This points to these soil bacterial populations being largely resistant to warming above ambient temperatures. It is important to note that the visible (non-microscopic) biological soil crust community of mosses and lichens did respond to this warming treatment (Ferrenberg et al., 2015), suggesting that various members of dryland soil communities may display differing capacities to resist disturbance.

Larger magnitude warming regimes, in the scale of 13°C, have been shown to induce a shift in the dominant bacterial populations in these systems, namely an ecological replacement of the dominant cyanobacterial population (Garcia-Pichel et al., 2013). However, these temperature shifts are significantly higher than those used in our studies, or those likely to occur with near-term climate change. This suggests that these dryland soil bacterial populations are largely resilient to the temperature increases expected to occur with near future climate warming. While changes in fungi and archaea could follow distinct patterns, these results add to a growing list of disturbances for which bacterial communities show resistance, including elevated atmospheric CO_2 and nitrogen deposition (Steven et al., 2012b; Mueller et al., 2015). Taken together, these observations indicate that despite the well-documented sensitivity of arid ecosystems to disturbance, there are particular perturbations to which the surface soil microbial communities are resistant.

Recovery From Altered Precipitation and Altered Precipitation and Warming in Combination

The precipitation treatment resulted in an increase in the overall amount of applied water but delivered in numerous small pulses. After 2 years of this treatment there was a significant decline in biological soil crust cover and bacterial biomass, indicating a detrimental effect on the soil populations (Ferrenberg et al., 2015; Steven et al., 2018). It was hypothesized that the declines in biomass and shifts in community composition were driven by carbon starvation induced by an increase in the

number of precipitation events that do not meet a threshold to support viable photosynthesis (Coe et al., 2012). In this model, a precipitation event is associated with a rapid burst of respiration, followed by photosynthesis and carbon fixation. However, the community needs to stay hydrated long enough for photosynthesis to recuperate carbon loss induced during respiration. If the precipitation events are particularly small or of short duration a negative carbon balance can occur, resulting in carbon starvation and cell death (Reed et al., 2012). The additive effects of these small precipitation pulses delivered over multiple years leads to shifts in the composition and biomass of the soil community (Ferrenberg et al., 2015; Steven et al., 2015). Here we show that 6 years after the precipitation treatments have ceased, bacterial biomass (**Figure 2C**), bacterial diversity (**Figure 2B**), and microbial community composition (**Figure 4**), have largely recuperated. This suggests that the biological soil crusts exposed to an altered precipitation regime are on a trajectory to full recovery.

When the warming and precipitation treatments were combined the collapse of the soil communities was catastrophic. After 2 years of the combinatorial treatment, healthy late successional biological soil crusts were eliminated in the interspace soils. This was verified through significant reductions in cyanobacterial biomass, and a dysbiosis in the soil microbial community with hundreds of taxa displaying significant shifts in relative abundance in comparison to control plots (Steven et al., 2015). A 1° temperature warming can increase soil evaporation by 3–5% (Le Houérou, 1996). By combining a 4°C warming with frequent small precipitation events, the number of deleterious precipitation events may be greatly increased, producing detrimental results for the soil communities. When the precipitation treatment was removed (leaving the warming in place), the soil communities showed signs of recovering. Bacterial biomass (**Figure 2C**) and composition (**Figure 3**) were similar to control plots, although some legacy effects are apparent. Four ASVs were still significantly different between the W + P and control plots (**Figure 4** and **Supplementary Table 2**). Collectively, these data show that the interactions among changing temperature and precipitation regimes that may occur in this region have the potential to influence the soil microbial communities, and further demonstrate how environmental disturbances can act in concert to drive microbial communities to states that would not be predicted from investigating individual disturbances in isolation. Finally, we also show that when one of the disturbances is removed the community retains the capacity for recovery.

CONCLUSION

The observations of this study support the idea that these dryland soil bacterial communities show a significant resistance and resilience to a number of disturbances, in that they either were recalcitrant to change in the face of the disturbance (i.e., warming) or have begun to show a process of recovery when the disturbances end. Interestingly, this was true for two very different forms of disturbance: physical trampling and altered precipitation. Thus, the natural recovery of dryland soil bacterial communities may be occurring at previously unrecognized scales. Instead of decades to centuries we are witnessing significant recovery in just 6–7 years. However, these results need to be treated with caution. The data presented here suggest that the taxonomic composition of the communities is recovering but does not address their functional attributes. Recently it was shown that disturbed arid soil communities differed significantly in their transcriptional response to a wetting pulse in comparison to undisturbed control soils, despite harboring a similar community composition (Steven et al., 2018). For instance, in control surface soils thousands of transcripts, related to processes such as photosynthesis and the Calvin-Benson Cycle were differentially expressed due to a short 1-h wetting pulse. In contrast, trampled soils showed relatively few differentially expressed genes, suggesting a muted response to wetting (Steven et al., 2018). Thus, the metabolic capacity of disturbed soil communities may differ even when the community composition is similar.

These arid soil communities provide a wealth of ecosystem services to drylands, including soil stabilization, nitrogen fixation, and moisture retention among others (Neilson et al., 2017; Bowker et al., 2018; Feng et al., 2020). Measuring the true recovery of these communities will require addressing if they are functionally, as well as taxonomically, equivalent to undisturbed populations. Finally, this study only addresses the bacterial component of the community. Other members such as fungi, lichens, and mosses also play a significant role in dryland ecology (Xiao and Veste, 2017; Green et al., 2018), but were not addressed in this study. Taken together, these data all point to significant knowledge gaps in the natural recovery rate and resilience of dryland soil populations. In particular, the data presented here suggest the sensitivity and recovery of these communities has been largely underestimated, and more data are needed to determine the consistency and drivers of recovery patterns across drylands. Through a long-term program tracking of the recovery of these systems to a multitude of disturbances we can better inform management and restoration efforts in order to protect these important ecosystems.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, PRJNA682175.

AUTHOR CONTRIBUTIONS

BS, JB, CK, and SR contributed to study design, data analysis, and manuscript writing. MP contributed to experimental methods, data interpretation, and manuscript editing. LG-G contributed to experimental methods and data analysis. All authors contributed to the article and approved the submitted version.

FUNDING

Participation of CK, LG-G, and BS was supported in part by a Department of Energy Science Focus Area grant to CK (#F260LANL2015). This research was supported by the Office of Science (Office of Biological and Environmental Research; DE-SC-0008168) United States Department of Energy Terrestrial Ecosystem Science program and the United States Geological Survey. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the United States Government. This manuscript is Los Alamos National Laboratory Unclassified Report # 20-30348.

ACKNOWLEDGMENTS

We are indebted to the many field technicians who helped with the project and E. Grote for plot instrumentation and quality control.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.648455/full#supplementary-material

REFERENCES

Allison, S. D., and Martiny, J. B. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc. Nat. Acad. Sci.U.S.A.* 105, 11512–11519.

Archer, S. R., and Predick, K. I. (2008). Climate change and ecosystems of the Southwestern United States. *Rala* 30, 23–28.

Baho, D. L., Peter, H., and Tranvik, L. J. (2012). Resistance and resilience of microbial communities–temporal and spatial insurance against perturbations. *Environ. Microbiol.* 14, 2283–2292. doi: 10.1111/j.1462-2920.2012.02754.x

Bowker, M. A., Reed, S. C., Maestre, F. T., and Eldridge, D. J. (2018). Biocrusts: the living skin of the earth. *Plant Soil* 429, 1–7. doi: 10.1007/s11104-018-3735-1

Briggs, A., and Morgan, J. W. (2008). Morphological diversity and abundance of biological soil crusts differ in relation to landscape setting and vegetation type. *Aust. J. Bot.* 56, 246–253. doi: 10.1071/BT07194

Cherlet, M., Hutchinson, C., Reynolds, J., Hill, J., Sommer, S., and von Maltitz, G. (2018). *World Atlas of Desertification*. Luxembourg: Publication Office of the European Union.

Coe, K. K., Belnap, J., and Sparks, J. P. (2012). Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. *Ecology* 93, 1626–1636. doi: 10.1890/11-2247.1

Conant, R. T., Klopatek, J. M., Malin, R. C., and Klopatek, C. C. (1998). Carbon pools and fluxes along an environmental gradient in northern Arizona. *Biogeochemistry* 43, 43–61. doi: 10.1023/A:1006004110637

Coppinger, K. D., Reiners, W. A., Burke, I. C., and Olson, R. K. (1991). Net erosion on a sagebrush steppe landscape as determined by Cesium-137 distribution. *Soil Sci. Soc. Am. J.* 55:254. doi: 10.2136/sssaj1991.03615995005500010043x

Dean, W. R. J., Milton, S. J., and Jeltsch, F. (1999). Large trees, fertile islands, and birds in arid savanna. *J. Arid Environ.* 41, 61–78.

Dixon, P., and Palmer, M. W. (2003). VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* 14, 927–930.

Eldridge, D. J., and Delgado-Baquerizo, M. (2019). The influence of climatic legacies on the distribution of dryland biocrust communities. *Glob. Chang. Biol.* 25, 327–336. doi: 10.1111/gcb.14506

Estruch, C., Lozano, Y. M., Armas, C., and Pugnaire, F. I. (2018). Plant community changes after land abandonment control CO2 balance in a dry environment. *Plant Soil* 425, 253–264. doi: 10.1007/s11104-018-3581-1

Evans, S. E., and Wallenstein, M. D. (2014). Climate change alters ecological strategies of soil bacteria. *Ecol. Lett.* 17, 155–164. doi: 10.1111/ele.12206

Feng, W., Zhang, Y., Yan, R., Lai, Z., Qin, S., Sun, Y., et al. (2020). Dominant soil bacteria and their ecological attributes across the deserts in northern China. *Eur. J. Soil Sci.* 71, 524–535. doi: 10.1111/ejss.12866

Fernandes, A. D., Macklaim, J. M., Linn, T. G., Reid, G., and Gloor, G. B. (2013). ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. *PLoS One* 8:e67019. doi: 10.1371/journal.pone.0067019

Ferrenberg, S., Reed, S. C., and Belnap, J. (2015). Climate change and physical disturbance cause similar community shifts in biological soil crusts. *Proc. Nat. Acad. Sci.U.S.A.* 112, 12116–12121. doi: 10.1073/pnas.1509150112

García-García, N., Tamames, J., Linz, A. M., Pedrós-Alió, C., and Puente-Sánchez, F. (2019). Microdiversity ensures the maintenance of functional microbial communities under changing environmental conditions. *ISME J.* 13, 2969–2983. doi: 10.1038/s41396-019-0487-8

Garcia-Pichel, F., Loza, V., Marusenko, Y., Mateo, P., and Potrafka, R. M. (2013). Temperature drives the continental-scale distribution of key microbes in topsoil communities. *Science* 340, 1574–1577. doi: 10.1126/science.1236404

Gombeer, S., Ramond, J.-B., Eckardt, F. D., Seely, M., and Cowan, D. A. (2015). The influence of surface soil physicochemistry on the edaphic bacterial communities in contrasting terrain types of the Central Namib Desert. *Geobiology* 13, 494–505. doi: 10.1111/gbi.12144

Green, T. G. A., Pintado, A., Raggio, J., and Sancho, L. G. (2018). The lifestyle of lichens in soil crusts. *Lichenologist Camb.* 50, 397–410. doi: 10.1017/S0024282918000130

Housman, D. C., Yeager, C. M., Darby, B. J., Sanford, R. L., Kuske, C. R., Neher, D. A., et al. (2007). Heterogeneity of soil nutrients and subsurface biota in a dryland ecosystem. *Soil Biol. Biochem.* 39, 2138–2149. doi: 10.1016/j.soilbio.2007.03.015

Ji, M., Kong, W., Stegen, J., Yue, L., Wang, F., Dong, X., et al. (2020). Distinct assembly mechanisms underlie similar biogeographical patterns of rare and abundant bacteria in Tibetan Plateau grassland soils. *Environ. Microbiol.* 22, 2261–2272. doi: 10.1111/1462-2920.14993

Koyama, A., Harlow, B., Kuske, C. R., Belnap, J., and Evans, R. D. (2018). Plant and microbial biomarkers suggest mechanisms of soil organic carbon accumulation in a Mojave Desert ecosystem under elevated CO2. *Soil Biol. Biochem.* 120, 48–57. doi: 10.1016/j.soilbio.2018.01.033

Kuske, C. R., Yeager, C. M., Johnson, S., Ticknor, L. O., and Belnap, J. (2012). Response and resilience of soil biocrust bacterial communities to chronic physical disturbance in arid shrublands. *ISME J.* 6, 886–897. doi: 10.1038/ismej.2011.153

Le Houérou, H. N. (1996). Climate change, drought and desertification. *J. Arid Environ.* 34, 133–185.

Martinez-Meza, E., and Whitford, W. G. (1996). Stemflow, throughfall and channelization of stemflow by roots in three Chihuahuan desert shrubs. *J. Arid Environ.* 32, 271–287.

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. doi: 10.1371/journal.pone.0061217

Monroe, A. P., Aldridge, C. L., O'Donnell, M. S., Manier, D. J., Homer, C. G., and Anderson, P. J. (2020). Using remote sensing products to predict recovery of vegetation across space and time following energy development. *Ecol. Indic.* 110:105872. doi: 10.1016/j.ecolind.2019.105872

Mueller, R. C., Belnap, J., and Kuske, C. R. (2015). Soil bacterial and fungal community responses to nitrogen addition across soil depth and microhabitat in an arid shrubland. *Front. Microbiol.* 6:891. doi: 10.3389/fmicb.2015.00891

Neilson, J. W., Califf, K., Cardona, C., Copeland, A., Treuren, W., van Josephson, K. L., et al. (2017). Significant impacts of increasing aridity on the arid soil microbiome. *mSystems* 2:e00195. doi: 10.1128/mSystems.00195-16

Parks, D. H., Tyson, G. W., Hugenholtz, P., and Beiko, R. G. (2014). STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* 30, 3123–3124. doi: 10.1093/bioinformatics/btu494

Poulter, B., Frank, D., Ciais, P., Myneni, R. B., Andela, N., Bi, J., et al. (2014). Contribution of semi-arid ecosystems to interannual variability of the global carbon cycle. *Nature* 509, 600–603. doi: 10.1038/nature13376

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucl. Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219

Rath, K. M., Fierer, N., Murphy, D. V., and Rousk, J. (2019). Linking bacterial community composition to soil salinity along environmental gradients. *ISME J.* 13, 836–846. doi: 10.1038/s41396-018-0313-8

Reed, S. C., Coe, K. K., Sparks, J. P., Housman, D. C., Zelikova, T. J., and Belnap, J. (2012). Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. *Nat. Clim. Chang.* 2, 752–755. doi: 10.1038/nclimate1596

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ.* 4:e2584. doi: 10.7717/peerj.2584

Schade, J. D., and Hobbie, S. E. (2005). Spatial and temporal variation in islands of fertility in the Sonoran Desert. *Biogeochemistry* 73, 541–553. doi: 10.1007/s10533-004-1718-1

Schlesinger, W. H., Reynolds, J. F., Cunningham, G. L., Huenneke, L. F., Jarrell, W. M., Virginia, R. A., et al. (1990). Biological feedbacks in global desertification. *Science* 247, 1043–1048. doi: 10.1126/science.247.4946.1043

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537–7541. doi: 10.1128/AEM.01541-09

Shade, A., Peter, H., Allison, S. D., Baho, D., Berga, M., Buergmann, H., et al. (2012). Fundamentals of microbial community resistance and resilience. *Front. Microbiol.* 3:417. doi: 10.3389/fmicb.2012.00417

Steven, B. (2017). "1. An introduction to arid soils and their biology," in *The Biology of Arid Soils*, ed. B. Steven (Boston: De Gruyter), 1–14. doi: 10.1515/9783110419047-001

Steven, B., Belnap, J., and Kuske, C. R. (2018). Chronic physical disturbance substantially alters the response of biological soil crusts to a wetting pulse, as characterized by metatranscriptomic sequencing. *Front. Microbiol.* 9:2382. doi: 10.3389/fmicb.2018.02382

Steven, B., Gallegos-Graves, L. V., Belnap, J., and Kuske, C. R. (2013). Dryland soil microbial communities display spatial biogeographic patterns associated with soil depth and soil parent material. *FEMS Microbiol. Ecol.* 86, 101–113. doi: 10.1111/1574-6941.12143

Steven, B., Gallegos-Graves, L. V., Starkenburg, S. R., Chain, P. S., and Kuske, C. R. (2012a). Targeted and shotgun metagenomic approaches provide different descriptions of dryland soil microbial communities in a manipulated field study. *Environ. Microbiol. Rep.* 4, 248–256. doi: 10.1111/j.1758-2229.2012.00328.x

Steven, B., Gallegos-Graves, L. V., Yeager, C. M., Belnap, J., Evans, R. D., and Kuske, C. R. (2012b). Dryland biological soil crust cyanobacteria show unexpected decreases in abundance under long-term elevated CO_2. *Environ. Microbiol.* 14, 3247–3258. doi: 10.1111/1462-2920.12011

Steven, B., Gallegos-Graves, L. V., Yeager, C. M., Belnap, J., and Kuske, C. R. (2014). Common and distinguishing features of the bacterial and fungal communities in biological soil crusts and shrub root zone soils. *Soil Biol. Biochem.* 69, 302–312.

Steven, B., Kuske, C. R., Reed, S. C., and Belnap, J. (2015). Climate change and physical disturbance manipulations result in distinct biological soil crust communities. *Appl. Environ. Microbiol.* 81, 7448–7459.

Su, Y., Zhao, H., Li, Y., and Cui, J. (2004). Carbon mineralization potential in soils of different habitats in the semiarid horqin sandy land: a laboratory experiment. *Arid Land Res. Manag.* 18, 39–50. doi: 10.1080/15324980490244997

Uritskiy, G., Getsin, S., Munn, A., Gomez-Silva, B., Davila, A., Glass, B., et al. (2019). Halophilic microbial community compositional shift after a rare rainfall in the Atacama Desert. *ISME J.* 13, 2737–2749. doi: 10.1038/s41396-019-0468-y

van de Leemput, I. A., Dakos, V., Scheffer, M., and van Nes, E. H. (2018). Slow recovery from local disturbances as an indicator for loss of ecosystem resilience. *Ecosystems* 21, 141–152. doi: 10.1007/s10021-017-0154-8

Wang, J., Anderson, B. T., and Salvucci, G. D. (2007). Stochastic modeling of daily summertime rainfall over the Southwestern United States. Part II: intraseasonal variability. *J. Hydrometeorol.* 8, 938–951. doi: 10.1175/JHM592.1

Wang, Q., Garrity, G. M., Tiedje, J. M., and Cole, J. R. (2007). Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73, 5261–5267. doi: 10.1128/AEM.00062-07

Wang, X.-B., Lü, X.-T., Yao, J., Wang, Z.-W., Deng, Y., Cheng, W.-X., et al. (2017). Habitat-specific patterns and drivers of bacterial β-diversity in China's drylands. *ISME J.* 11, 1345–1358. doi: 10.1038/ismej.2017.11

Weber, B., Bowker, M., Zhang, Y., and Belnap, J. (2016). "Natural recovery of biological soil crusts after disturbance," in *Biological Soil Crusts: An Organizing Principle in Drylands* Ecological Studies, eds B. Weber, B. Büdel, and J. Belnap (Cham: Springer International Publishing), 479–498. doi: 10.1007/978-3-319-30214-0_23

Williams, L., Jung, P., Zheng, L.-J., Maier, S., Peer, T., Grube, M., et al. (2018). Assessing recovery of biological soil crusts across a latitudinal gradient in Western Europe. *Restor. Ecol.* 26, 543–554. doi: 10.1111/rec.12579

Xiao, B., and Veste, M. (2017). Moss-dominated biocrusts increase soil microbial abundance and community diversity and improve soil fertility in semi-arid climates on the Loess Plateau of China. *Appl. Soil Ecol.* 117–118, 165–177. doi: 10.1016/j.apsoil.2017.05.005

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 09 June 2021
doi: 10.3389/fmicb.2021.661410

Short-Term Effect in Soil Microbial Community of Two Strategies of Recovering Degraded Area in Brazilian Savanna: A Pilot Case Study

Priscila Jane Romano Gonçalves Selari[1], Luiz Ricardo Olchanheski[2], Almir José Ferreira[3], Tiago do Prado Paim[4], Guido Calgaro Junior[4], Flavio Lopes Claudio[4], Estenio Moreira Alves[4], Darliane de Castro Santos[5], Welington Luiz Araújo[3] and Fabiano Guimarães Silva[6]*

[1] Laboratory of Microbiology, Department of Biology, Instituto Federal de Educação, Ciência e Tecnologia Goiano (Federal Institute of Education, Science and Technology Goiano), Ceres, Brazil, [2] Laboratory of Microbiology, Department of Structural and Molecular Biology and Genetics, State University of Ponta Grossa (UEPG), Ponta Grossa, Brazil, [3] Laboratory of Molecular Biology and Microbial Ecology, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo (USP), São Paulo, Brazil, [4] Laboratory of Education in Agriculture Production, Instituto Federal de Educação, Ciência e Tecnologia Goiano (Federal Institute of Education, Science and Technology Goiano), Iporá, Brazil, [5] Laboratory of Agricultural Chemistry, Instituto Federal de Educação, Ciência e Tecnologia Goiano (Federal Institute of Education, Science and Technology Goiano), Rio Verde, Brazil, [6] Laboratory of Plant Tissue and Culture, Instituto Federal de Educação, Ciência e Tecnologia Goiano (Federal Institute of Education, Science and Technology Goiano), Rio Verde, Brazil

OPEN ACCESS

Edited by:
Brendan J. M. Bohannan, University of Oregon, United States

Reviewed by:
Damien Robert Finn, Arizona State University, United States
Gwen-Aelle Grelet, Manaaki Whenua – Landcare Research, New Zealand

***Correspondence:**
Priscila Jane Romano Gonçalves Selari
priscila.goncalves@ifgoiano.edu.br

Specialty section:
This article was submitted to Terrestrial Microbiology, a section of the journal Frontiers in Microbiology

Received: *30 January 2021*
Accepted: *14 May 2021*
Published: *09 June 2021*

Citation:
Selari PJRG, Olchanheski LR, Ferreira AJ, Paim TdP, Calgaro Junior G, Claudio FL, Alves EM, Santos DdC, Araújo WL and Silva FG (2021) Short-Term Effect in Soil Microbial Community of Two Strategies of Recovering Degraded Area in Brazilian Savanna: A Pilot Case Study. Front. Microbiol. 12:661410. doi: 10.3389/fmicb.2021.661410

The Brazilian Cerrado is a highland tropical savanna considered a biodiversity hotspot with many endemic species of plants and animals. Over the years, most of the native areas of this biome became arable areas, and with inadequate management, some are nowadays at varying levels of degradation stage. Crop-livestock integrated systems (CLIS) are one option for the recovery of areas in degradation, improving the physicochemical and biological characteristics of the soil while increasing income and mitigating risks due to product diversification. Little is known about the effect of CLIS on the soil microbial community. Therefore, we perform this pilot case study to support further research on recovering degraded areas. The bacterial and fungal soil communities in the area with CLIS were compared to an area under moderate recovery (low-input recovering - LI) and native savanna (NS) area. Bacterial and fungal communities were investigated by 16S and ITS rRNA gene sequencing (deep rRNA sequencing). Ktedonobacteraceae and AD3 families were found predominantly in LI, confirming the relationship of the members of the Chloroflexi phylum in challenging environmental conditions, which can be evidenced in LI. The CLIS soil presented 63 exclusive bacterial families that were not found in LI or NS and presented a higher bacterial richness, which can be related to good land management. The NS area shared 21 and 6 families with CLIS and LI, respectively, suggesting that the intervention method used in the analyzed period brings microbial diversity closer to the conditions of the native area, demonstrating a trend of approximation between NS and CLIS even in the short term. The most abundant fungal phylum in NS treatment was Basidiomycota and Mucoromycota, whereas Ascomycota predominated in CLIS and LI. The fungal community needs more time to recover and to approximate from the native area than the bacterial community. However, according to the analysis of bacteria, the CLIS

area behaved differently from the LI area, showing that this treatment induces a faster response to the increase in species richness, tending to more accelerated recovery. Results obtained herein encourage CLIS as a sustainable alternative for recovery and production in degraded areas.

Keywords: crop-livestock integrated system, land use, metagenomics, soil microbiome, sustainability

INTRODUCTION

The Cerrado (Brazilian savanna) is the second largest biome in Brazil, covering approximately 24% of the country's territory. The climate of the region is marked by high temperatures and long periods of drought. The soils are naturally acidic, poor in nutrients, and rich in aluminum, with tropical savanna vegetation and distinct physiognomies ranging from pastures to forests (Souza et al., 2016). The most abundant type of vegetation is the Cerrado *stricto sensu* which is characterized by a forest savanna with low and twisted trees, shrubs, and large grasslands (Araujo et al., 2012). The "Cerradão" is a forest floristically similar to the Cerrado *stricto sensu*, with trees ranging from 8 to 15 m tall and from 50 to 90% cover (Dall'Agnol et al., 2016).

Despite being considered a global hotspot of biodiversity, approximately 50% of the Cerrado native areas have been transformed into mechanized agriculture, forestry, and intensive livestock areas over the years (López-Poma et al., 2020). The inadequate management of production systems can lead to the degradation of these areas and reduce their productivity. There are several strategies for recovering these areas under degradation, and the choice between these strategies relies on the stage of degradation, financial resources available, and other soil and topographic limitations for mechanization.

The crop-livestock integrated system (CLIS) involves intercropping, crop rotation, and succession with livestock grazing in the same area. This system aims to improve agricultural productivity and recover degraded land, due to improvements in soil physical, chemical, and biological characteristics (Lisboa et al., 2014; Sarto et al., 2020). CLIS enables a higher yield and more stable income due to the diversity of products generated (mainly, cereals, legumes and animal products, meat, and milk), which can reduce the pressure for deforestation in other areas (Damian et al., 2020). However, the first year of CLIS implantation involves a series of soil disturbances like limestone and gypsum application and plowing with different methodologies. This management is required to prepare the soil physically and chemically to crop seeding. On the other hand, these soil managements can negatively impact soil biological conditions (Sándor et al., 2020).

Other recovering strategies propose minor soil interventions with lower inputs (fertilizer, machinery, and money), taking more time to reach the full recovery of the area. These strategies could preserve soil biological content, which would help in the recovering process.

Microorganisms are essential for soil productivity and fertility because they perform several important functions, such as decomposition of organic matter, nutrient solubilization, and cycling, and synthesis of phytohormones and antimicrobial compounds, thus promoting plant growth and protection (Nannipieri et al., 2017). Different land uses and management systems can modify the structure and functioning of the soil microbial community (Hermans et al., 2020), thus affecting plant productivity.

There are several studies reporting changes in the microbial diversity in Brazilian Cerrado soil due to land use transformations (Castro et al., 2016; Souza et al., 2016; Araujo et al., 2017; Castañeda and Barbosa, 2017; Vieira et al., 2018; Silva et al., 2019). Studies of the soil of the Brazilian Cerrado under CLIS using crop-dependent methods revealed a reduction in the microbial diversity and enzymatic activity in the forest component of the CLIS compared to the crop rows. In addition, the microbial biomass and abundance of bacteria and fungi were higher in the native Cerrado than in the CLIS and conventional monoculture system (Sarto et al., 2020). In contrast, an CLIS in temperate agroforestry systems increased the abundance of various bacteria and fungi in the soil compared to the conventional monoculture system, increasing soil fertility (Beule et al., 2020). The soil microbiome in Cerrado has been studied under different points of view, including the impact of agriculture on taxonomic and functional microbial diversity (Souza et al., 2016), mining areas under revegetation (Vieira et al., 2018), high yield and degraded pasture areas (Silva et al., 2019), different vegetation physiognomies (Castro et al., 2016; Araujo et al., 2017), and even in the search for new lipolytic enzymes with biotechnological potential (Istvan et al., 2018). However, little is known about the short-term effect of CLIS implementation on the soil microbial community.

Thus, the objective of this pilot case study was to evaluate the short-term effects of recovering degraded areas in the composition and structure of soil bacterial and fungal communities. The present study evaluated this soil biological condition in fields under recovery through CLIS and under a moderate intervention recovering comparing to native vegetation in the Brazilian savanna (Cerrado).

MATERIALS AND METHODS

Characterization of the Study Area

The study was conducted at the "Boa Esperança" Teaching, Research, and Extension Unit (UEPE Boa Esperança for its acronym in Portuguese; 16°26′34.951″N 51°1′2.539″E) of the Institutional Project for Integrated Agricultural Production Systems of the Goiano Federal Institute (IF GOIANO) located in Iporá city, state of Goiás, Brazil (**Figure 1**). The soil was classified as typical dystrophic Dark Red Latosol, moderate A horizon,

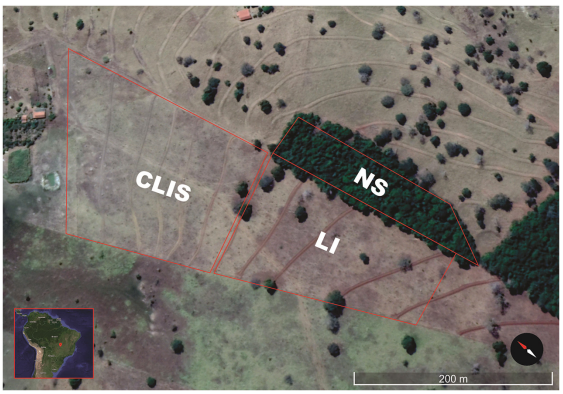


FIGURE 1 | Image of UEPE Boa Esperança, Iporá, Goiás, Brazil, in June of 2018. The demarcated fields represent the treatments: NS - native savanna, LI - low-input recovering, and CLIS - crop-livestock integrated system.

mixed texture (IUSS Working Group WRB, 2015), and flat relief (<6% slope), at 495 m of altitude.

The climate of the region is classified as tropical savanna (AW, Köppen), being 24.7°C the annual average temperature and 1,369 mm the rainfall average. The dry season is from May to September (winter) and the rainfall average is 62 mm. The rainy season is from October to April (summer) and the rainfall average is 1,307 mm[1].

Design and Characterization of Treatments

Three areas with different land use strategies were evaluated: (a) recovery of an area in degradation via CLIS, (b) recovery of an area in degradation using a moderate intervention strategy (low-input recovering, LI), and (c) native Cerrado control area (native savanna, NS).

The CLIS field received 1.8 Mg.ha^{-1} of dolomitic limestone (85% of Relative Power of Total Neutralization), and 4.00 Mg.ha^{-1} of poultry litter applied on the surface. After, chisel plow at 0.25 m depth and 0.6 m spacing was applied. The remaining plants in the area (predominantly *Urochloa brizantha* and *Urochloa humidicola*) were desiccated with 1,440 g of glyphosate.ha^{-1}. Seven days after desiccation, a triple intercropping with corn (*Zea mays* cv. "AG1051"), pigeon pea (*Cajanus cajan* cv. "Super N"), and Tamani grass (*Megathyrsus maximus* cv. "BRS Tamani") were simultaneously sowed. The sowing was made with a 5-row mechanical seed drill using three maize rows intercalated with two pigeon pea rows. An additional fine seeds distribution system in the same machine sowed Tamani grass (5 kg of pure viable seeds.ha^{-1}). The pigeon pea had a final population of 28,250 plants.ha^{-1} and maize had 67,000 plants.ha^{-1}. The fertilization used was 200 kg.ha^{-1} of 8-41-5 (nitrogen-phosphorus-potassium). Topdressing fertilizations with N were performed in the corn vegetative stages of two (V2) and four (V4) fully expanded true leaves using 46 kg.ha^{-1} of N (100 kg.ha^{-1} of urea) in each operation.

The intercropped forage was harvested for silage production in February 2018 (90 days after sowing). The forage collected was composed of all biomass available (corn, pigeon pea, and Tamani grass) in 2 m^2. The material was ground and homogenized, then ensiled in micro-silos (polyvinyl chloride material, 10 cm × 30 cm) for anaerobic fermentation. After 60 days, the micro-silos were opened, and the material was dried at 65°C by 72 h. The dry matter (DM) content was determined, and the DM yield per hectare was calculated. The dry material was grounded in a 1-mm Wiley mill to determine the levels of calcium (Ca), phosphorus (P), crude protein (CP), crude fiber (CF), ether extract (EE), mineral matter (MM), total digestible nutrients (TDN), acid detergent fiber (ADF), and neutral detergent fiber (NDF) (AOAC, 1995).

Thirty days after harvesting, the area was divided into 12 paddocks (3,125 m^2). A total of 15 animals (Girolando dairy cows) were used in a rotational grazing scheme according to target sward height management, 35 cm pre-grazing, and 25 cm post-grazing (Tesk et al., 2020).

The LI field also received 1.8 Mg.ha^{-1} of dolomitic limestone superficially spread followed by chisel plow at 0.25 m depth and 0.6 m spacing. The forage species (*Urochloa brizantha*) present in the field was maintained and used for livestock grazing during the following year. The area was divided into eight paddocks (4,687 m^2 each) and grazed by 15 animals (Girolando dairy cows) in a rotational grazing scheme following the same management (Tesk et al., 2020).

The Native savanna area sampled is located at the side of the two recovering fields. The collection points were 100 to 160 m away, which ensure the same soil source and type at all three sites evaluated here.

Soil Sampling and Analyses

The soil samples for chemical analyses were composed of five subsamples collected in each area (NS, CLIS, and LI) at 0–20 cm depth that were homogenized prior to measurement. These samples were used for the characterization of the soil before the beginning of the recovering process (collected in June 2017) and after the first year of recovery management (July 2018). The average soil textures for clay, silt, and sand were 24, 12, and 64% (0–20 cm), respectively. The average chemical composition was 4.6 pH ($CaCl_2$), 0.75 Ca (cmolc dm^{-3}), 0.3 Mg (cmolc dm^{-3}), 1.05 Ca + Mg (cmolc dm^{-3}), 0.1 Al (cmolc dm^{-3}), 2.55 H + Al (cmolc dm^{-3}), 3.8 CEC (cmolc dm^{-3}), 1 P Melich (mg dm^{-3}), 70 K (mg dm^{-3}), 14 Organic Matter (g.kg^{-1}), 11.5 Aluminium saturation (%), and 31% of Base saturation (V%).

A new soil collection was performed in July 2018 for DNA extraction of the microbial community, collecting four samples in each site (NS, CLIS, and LI). Each sample consisted of three subsamples obtained within a radius of 3 m, which were duly homogenized. The collection points of the samples between treatments were less than 160 m

[1]https://pt.climate-data.org/america-do-sul/brasil/goias/ipora-43430/

apart. The samples for microbial diversity analyses were at 5–10 cm depth.

Soil DNA Extraction and Next-Generation Sequencing

The sampled soil was sieved (2-mm-mesh sieve) and placed in 50-mL tubes, which were properly sealed, identified, and immediately placed in liquid nitrogen. In the laboratory, the samples were stored in a freezer at −80°C until processing.

Total DNA was extracted from each of the 12 soil samples using the DNeasy PowerSoil Kit (Qiagen, San Francisco, CA, United States) following the manufacturer's instructions. For bacteria, the V3-V4 regions of the bacterial 16S rRNA gene were amplified using the universal primers 515F (Parada et al., 2016) and 806R (Apprill et al., 2015) with an Illumina adaptor. For fungi, the primers ITS1F and ITS2 (Hoggard et al., 2018) with an Illumina adaptor were used to amplify the Internal Transcribed Sequence 1 (ITS-1) of the ribosomal gene cluster. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, San Francisco, CA, United States) before performing agarose gel electrophoresis. The concentrations of the purified amplicon products were determined by NanoDrop™ 1000 spectrophotometry (Thermo Fisher Scientific, Waltham, MA, United States) and sequenced on an Illumina MiSeq PE3000 platform (Illumina, San Diego, CA, United States) and CD Genomics (Shirley, New York, NY, United States).

Soil Microbial Community Analyses

The sequence data demultiplexed were imported into the Quantitative Insights into Microbial Ecology 2 (QIIME2, version 2021.2.0; Caporaso et al., 2010). The Divisive Amplicon Denoising Algorithm 2 (DADA2; Callahan et al., 2016) was used to quality filter, trim, denoise, merge the pairs of reads, and remove chimeric sequences (15 nucleotides were removed from the forward and reverse reads, according to visual inspection of the quality at each nucleotide). The taxonomic assignment was carried out using the feature-classifier plugin against SILVA SSU 138 non-redundant database for bacterial taxonomy (Quast et al., 2013; Bokulich et al., 2018), and UNITE 8.2 (Abarenkov et al., 2020) for fungi taxonomy.

Species richness, diversity (Shannon and Simpson indices), and Pielou evenness indices were estimated for each of the samples from the ASV table using the software R v.4.0.2 (R Core Development Team, 2019) through the "vegan" package (Oksanen et al., 2013). MANOVA was conducted to explore the differences between land management. Means were compared with the "ScottKnott" package (Jelihovschi et al., 2014). Normality and homogeneity of the residual distribution were inspected. Non-metric multidimensional scaling (NMDS) of the weighted Unifrac distance matrix was used to visualize the bacterial community structure. The NMDS analyses were performed using the packages "ggord" (Beck, 2016), "vegan" and "factoextra" (Kassambara and Mundt, 2017) and the graphical representation used the "ggplot2" package (Wickham, 2009). The Permutational Multivariate Analysis of Variance (PERMANOVA) was performed using the package "vegan." Venn diagrams were made using the R VennDiagram package to analyze each taxonomic level shared among the NS, CLIS, and LI areas (Chen and Boutros, 2011).

The crude reads were deposited at the National Center for Biotechnology Information (NCBI) with BioProject accession number PRJNA669331.

RESULTS

The results of the soil chemical analysis in the first year of recovering process are shown in **Table 1**. These results represent a characterization of the chemical composition of the soil in each area and are shown to demonstrate the contrasting soil conditions in each land management system.

Crop-livestock integrated system represented the crop-livestock integrated system using a triple intercropping of corn, pigeon pea, and grass. The forage yield was 7.2 $Mg.ha^{-1}$ of corn, 1.7 $Mg.ha^{-1}$ of pigeon pea, and 4.9 $Mg.ha^{-1}$ grass, totaling 13.8 $Mg.ha^{-1}$ of total forage, on a dry matter basis. Therefore, the silage dry matter was composed of 52.2% of corn, 12.3% of pigeon pea, and 35.5% of Tamani grass. The chemical composition of the silage was 0.3, 0.1, 7.8, 28.2, 3.5, 5.8, 64.1, 42.6, and 59.9% for Ca, P, CP, CF, EE, MM, TDN, ADF, and NDF, respectively. These results demonstrate the capacity of CLIS to produce high-quality forage in a great amount.

Bacterial Soil Community

About 2.3 million reads were obtained from the sequencing of the 16S rRNA. After applying quality filters, merging reads, and chimera removal, the 873,043 bacterial sequences were grouped into 12,299 ASVs distributed in 944 taxonomic groups at level 7 (species). From species-level classification, 733 species

TABLE 1 | Analysis of soil chemical attributes in July 2018 (one year after recovering process started) at a depth of 0–20 cm in the different treatments evaluated (NS - native savanna, LI - low-input recovering, CLIS - crop-livestock integrated system).

Attributes	NS	CLIS	LI
pH ($CaCl_2$) (un)	4.4	5.0	5.4
Ca (cmolc dm^{-3})	0.4	1.5	1.4
Mg (cmolc dm^{-3})	0.2	0.4	0.4
Ca + Mg (cmolc dm^{-3})	0.6	1.9	1.8
Al (cmolc dm^{-3})	0.3	0.0	0.0
H + Al (cmolc dm^{-3})	2.8	2.1	1.5
CEC (cmolc dm^{-3})	3.6	4.2	3.4
P (Melich I) (mg dm^{-3})	3.0	4.0	2.0
K (cmolc dm^{-3})	0.20	0.22	0.11
K (mg dm^{-3})	80.0	88.0	44.0
Organic Matter (g kg^{-1})	23.0	12.0	12.0
Sat. Al (M%)	27.0	0.0	0.0
Base Saturation (V%)	23.0	51.0	56.0
Ca/Mg	2.0	3.8	3.5
Ca/CEC	11.1	35.7	41.2
Mg/CEC	5.6	9.5	11.8
K/CEC	5.7	5.4	3.3

were found in the CLIS sample, representing the most diverse sample, followed by the LI sample (563 species), and NS (494 species). Among the species observed, 33.5% (316) corresponded to the bacterial core present in all sampled areas, regardless of the treatment. In another hand, 229, 82, and 103 OTUs were exclusive for CLIS, LI, and NS areas, respectively. Unclassified sequences, which corresponded to 0.012% of the total, were excluded from the analyses.

In order to verify the difference in the abundance of taxonomic groups among the different land managements, a non-metric multidimensional scaling analysis (NMDS) was applied. Phylum, Class, Order, and Family were able to group the samples according to land management, verified by PERMANOVA ($p = 0.001$).

The sequencing of the 16S rRNA allowed the identification of 35 phyla (**Supplementary Figure 1**). The first component of NMDS analyses separated NS from the other two. The second component demonstrated some variability inside CLIS and LI. The NS area shared four phyla exclusively with the CLIS and no phylum was shared between NS and LI and between LI and CLIS, demonstrating a closer relation of CLIS with NS. Among the phyla, two were represented by the Archaea domain, with a predominance of Proteobacteria (22.8%), Actinobacteria (22.7%), Chloroflexi (14.5%), and Acidobacteriota (13.5%). Native savanna areas were associated with higher Acidobacteriota (17.2%), Verrucomicrobiota (13.7%), and Planctomycetota (8.5%), compared to the other cultivated areas. Higher Actinobacteriota were found in CLIS and LI. The highest Chloroflexi abundance was observed in LI and the lowest in NS, while CLIS had an intermediate position. Chloroflexi was the only phyla that differentiate between the three land management systems (**Supplementary Figure 1**).

Analyzing class-level taxonomy, there were found 76 classes, the most abundant being Alphaproteobacteria (19.3%), Actinobacteria (10.6%), Acidobacteriaceae (8.7%), Verrucomicrobiae (8.1%), Thermoleophila (7.9%), and Ktedonobacteria (6.6%). The classes Verrucomicrobiae (13.4%), Acidobacteria (12.5%), Planctomycetes (8.1%), and Gammaproteobacteria (5%), were significantly more abundant in the NS area than in the CLIS (5.8, 7.1, 4.8, and 2.7%, respectively) and LI treatments (5.1, 6.5, 5.4, and 2.7%, respectively) ($p = 0.05$). These classes were also positively correlated with the first component in NMDS. Thermoleophilia, Actinobacteria, and Bacilli were more predominant in CLIS and LI and negatively correlated with the first component in NMDS. Ktedonobacteria were found significantly more abundant in LI ($p = 0.05$) and positively correlated with the second dimension in NMDS (**Supplementary Figure 2**).

In NS, the orders Chthoniobacterales (12.2%) and Acidobacteriales (6%) were significantly more abundant than in the CLIS (5.1 and 3.9%, respectively) and LI (4.6 and 3.4%, respectively) treatments. Solirubrobacterales were less abundant in the NS area. NMDS based on bacterial orders showed a higher separation of CLIS and LI in the second dimension, with only one observation of LI at the positive side. Ktedonobacterales abundance was higher in LI samples and negatively correlated with NMDS2, explaining the separation of LI to CLIS (**Supplementary Figure 3**). Rhizobiales had the highest positive correlation with NNMDS2, which can indicate higher abundance in CLIS than LI. CLIS had the highest number of exclusive orders and shared 16 and 15 orders exclusively with NS and LI, respectively, demonstrating the higher diversity in the soil of this land management system.

Non-metric multidimensional scaling analysis of soil bacterial community showed that dissimilarities at the family level were driven by sampling area (**Figure 2**), very similar to the results based on the order. Ktedonobacteraceae and AD3 had higher abundance in LI compared to NS and CLIS, being negatively correlated with NMDS2 (**Figures 2A,F**).

The NS treatment shared 21 families with CLIS and six families with LI. The CLIS and LI areas shared 32 families. Twenty families were exclusive to the NS, 18 families were exclusive to LI, and 63 families were exclusive to the CLIS (**Figure 2B**). Xanthobacteraceae was the most abundant family (**Figure 2E**) found in all the studied areas (NS = 11%; CLIS = 13%, and LI = 10.1%) and contributes positively with the second dimension in NMDS, representing the separation between CLIS and LI (**Figures 2A,C**). Relative abundances of Chthoniobacteraceae and Acidothermaceae were higher in NS (7 and 3.2%, respectively) than CLIS (2.2 and 1.5%) and LI (1.8 and 1.8%). On the other hand, TK10 and Bacillaceae were less abundant in NS than CLIS and LI areas.

Fungal Soil Community

About 2.6 million reads were obtained from the sequencing of the ITS region. After applying quality filters, merging reads, and chimera removal, the 1,716,917 fungal sequences were grouped into 12,610 ASVs distributed in 1094 taxonomic groups at level 7 (species). From species-level classification, 671 species were found in the NS, representing the most diverse sample, followed by the LI sample (505 species), and CLIS (499 species). Among the species observed, 15.3% (167) corresponded to the bacterial core present in all sampled areas, regardless of the treatment. In another hand, 149, 151, and 380 species were exclusive for CLIS, LI, and NS areas, respectively. Of the reads approved in the quality index, 26.4% were not classifiable from the database used and were not included in other analyses.

Abundances of Basidiomycota and Mucoromycota were higher, while Ascomycota was less abundant in NS compared to CLIS and LI areas ($p = 0.05$), both phyla were positively correlated with the first dimension, related to separation between NS and the others (**Supplementary Figure 4**). Ascomycota and Glomeromycota were negatively correlated with the second dimension of NMDS (**Supplementary Figure 4**), representing the separation between CLIS and LI.

According to the taxonomic analysis of classes, Agaricomycetes was the most abundant class in NS (64%). CLIS and LI showed less than 20% of the abundance of the Agaricomycetes. Eurotiomycetes, Sordariomycetes, and Dothideomycetes were less abundant in NS (**Supplementary Figure 5**).

Non-metric multidimensional scaling analysis for fungal orders showed a clear separation among areas (**Figures 3A,C,D**).

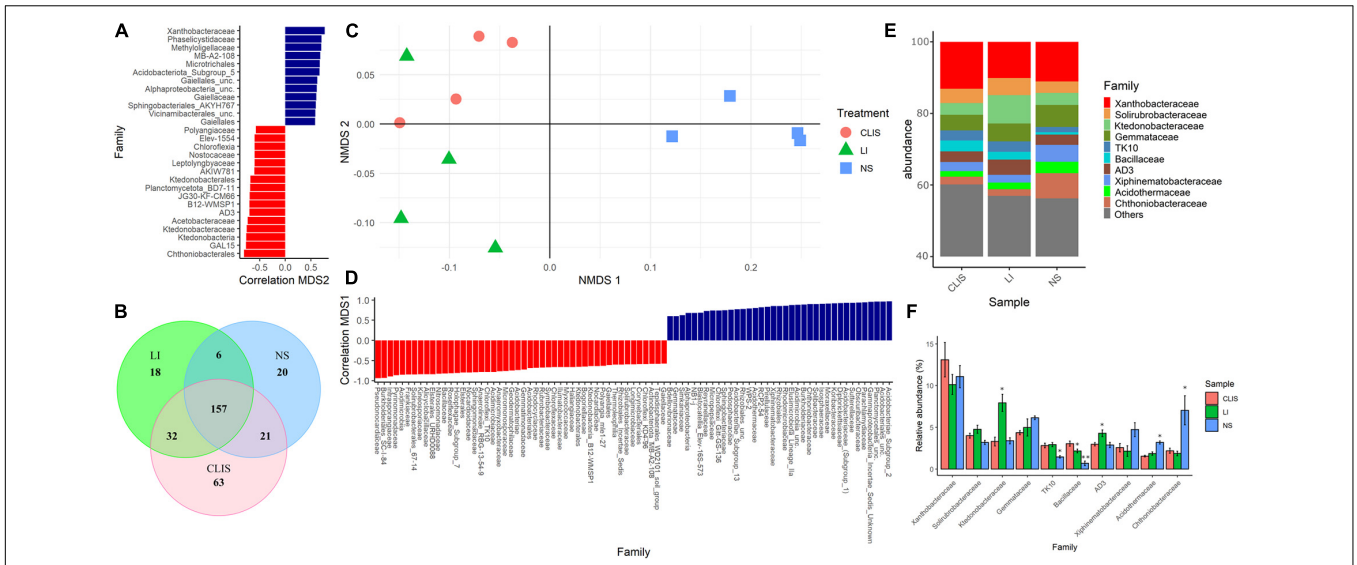


FIGURE 2 | Evaluation of the bacterial community (Family) in the native savanna (NS), low-input recovering (LI), and crop-livestock integrated system (CLIS) areas obtained by sequencing of 16S rRNA genes. **(A)** Family with significant correlation with the second component of NMDS. **(B)** Venn diagram obtained from Family data. **(C)** Individual coordinates from NMDS results. **(D)** Family with significant correlation with the first component of NMDS. **(E)** Relative abundance of bacterial families in each area. **(F)** Percentage of relative abundance of bacterial families in each area. Asterisks represent significance according to the Scott-Knott method $p < 0.05$.

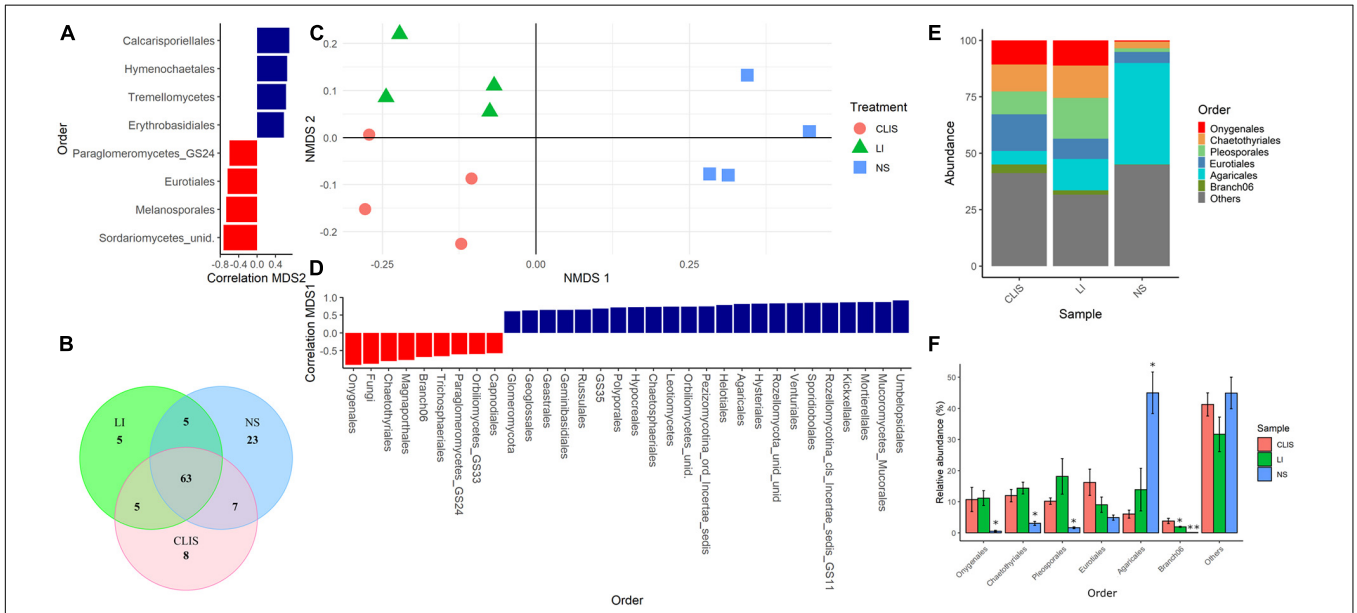


FIGURE 3 | Evaluation of the fungal community (Order) in the native savanna (NS), low-input recovering (LI), and crop-livestock integrated system (CLIS) areas obtained by sequencing of ITS rRNA genes. **(A)** Order with significant correlation with the second component of NMDS. **(B)** Venn diagram obtained from Order data. **(C)** Individual coordinates from NMDS results. **(D)** Order with significant correlation with the first component of NMDS. **(E)** Relative abundance of fungal orders in each area. **(F)** Percentage of relative abundance of fungal families in each area. Asterisks represent significance according to the Scott-Knott method $p < 0.05$.

The NS area shared seven orders with CLIS and five orders with LI. The CLIS and LI areas shared five orders among themselves. Twenty-three orders were exclusive to the NS, five to LI, and eight to CLIS (**Figure 3B**). Agaricales was predominant in NS (45%). Onygenales, Chaetothyriales, Pleosporales, and Branch 06 were less abundant in NS (0.5, 3, 1.6, and 0.1%, respectively) when compared to CLIS (10.7, 12, 18, and 3.8%, respectively) and LI (11.1, 14.3, 18.1, and 1.9%, respectively) (**Figures 3E,F**). Eight fungal orders were significantly correlated with the second dimension, which divided the CLIS and LI. Sordariomycetes, Melanosporales, Eurotiales, and glomeromycetes were higher in CLIS while

Calcarisporiellales, Hymenochaetales, Tremellomycetes, and Erythrobasidiales were higher in LI (**Figure 3A**).

The NMDS with fungal family data separated the three areas, the first dimension separated NS from others and the second dimension separated LI from CLIS (**Supplementary Figure 6**). In NS, sequences of unidentified families were more abundant (30.7%), followed by Hygrophoraceae (31.7%), which represented only 0.9% in CLIS and 7.75% in LI. Herpotrichiellaceae was less abundant in NS (1.78%) compared to the other areas (CLIS = 7.8%; LI = 8.6%). Those families were important to the separation between CLIS and NS in the second component of NMDS (**Supplementary Figure 6**).

The abundances of the microbial communities obtained in each sample are shown in **Supplementary Figures 7–10**. In general, the abundances were consistent across repetitions.

Diversity Index of Soil Microbial Community

The bacterial diversity (Shannon-Wiener and Simpson indices) and Pielou evenness indices showed no differences among the sampled areas. However, the bacterial richness was higher in the CLIS than in the LI and NS. In the NS area, the fungal diversity and Pielou evenness indices were smaller than in CLIS and LI, while the fungal richness was higher in native savanna in comparison to CLIS and LI treatments (**Table 2**).

DISCUSSION

Since the experiment was conducted on a commercial farm, there were limitations to develop an experimental design with traditional repetitions. Therefore, we provided here a pilot case study to explore the potential impact of CLIS management on soil biodiversity and microbial structure, and the results obtained here were representative for the studied place, which is inserted in the Cerrado biome.

The productivity achieved in three months, which was verified through phytotechnology and bromatological analyses of the forage, was included in this work to prove that the forage produced at CLIS is of higher quality compared to traditional production systems. Thus, it reinforces our hypothesis that CLIS promotes soil regeneration, besides being able to represent economic gains to the producer (Salton et al., 2014). The results obtained were similar to those observed by Ligoski et al. (2020) that studied the same intercropping system. These authors showed that this intercropped silage can be a good ruminant feedstuff and decrease animal methane emissions due to ruminal fermentation.

The culture-independent analysis revealed that the predominant phyla of bacteria were Proteobacteria, Actinobacteria, and Acidobacteria, which varied in abundance between the sampled areas. The differences in relative abundances suggest the influence of the different conditions of each area, such as soil pH, nutrients, and vegetation. Moisture, temperature, soil composition, and pH shape the bacterial community in the soil, and pH is one of the main influencers of bacterial diversity and richness (Alsharif et al., 2020). Zhalnina et al. (2014) also state that pH directs the structure of the soil microbial community because it affects the availability of nutrients for microorganisms.

Proteobacteria, Actinobacteria, and Acidobacteria are often abundant phyla in Cerrado soil (Silva et al., 2019). Among the Proteobacteria classes, there is a prevalence of the class Alphaproteobacteria in the Cerrado (Araujo et al., 2017). Actinobacteria are often found in arid and semiarid environments. The members of this phylum are versatile and can grow under extreme conditions of salinity, temperature, radiation, pH, and low water availability (Alsharif et al., 2020). The species of the phylum Actinobacteria are often related to hostile environmental conditions and weathering (O'Brien et al., 2019), which are conditions that can be found in CLIS and LI and explain the high incidence of this phylum in those areas.

The phylum Acidobacteria was found in greater abundance in the NS, which can be explained by the more acidic pH found in this area since the representatives of this group have tolerance to lower pH (Araujo et al., 2017), which had a pH 4.4 (**Table 1**). Verrucomicrobia was another phylum with a higher incidence in the native area, which includes oligotrophic bacteria found in soil regions that are poor in nutrients, conditions found in native soils of Cerrado (Silva et al., 2019). Firmicutes had a higher incidence in CLIS and LI than NS (**Supplementary Figure 1**). This phylum contains representatives resistant to unfavorable conditions (Araujo et al., 2017). In addition, the class Bacilli, which has important species involved in the promotion of plant growth through different mechanisms (Chaudhry et al., 2017), was more abundant in CLIS and LI, demonstrating ecological changes and possible improvements in soil functions.

TABLE 2 | Analysis of richness (Chao1, ACE), diversity (Shannon and Simpson), and evenness (Pielou) in the 16S rRNA genes of bacteria and the ITS region of fungi, as well as the number of OTUs, observed in each treatment (NS, native savanna, LI, low-input recovering, and CLIS, crop-livestock integrated system).

Sequence	Treatment	Shannon	Simpson	Pielou	Richness
16S	NS	4.87 ± 0.26^{a}	0.98 ± 0.005^{a}	0.85 ± 0.01^{a}	305.50 ± 71.73^{b}
	CLIS	5.17 ± 0.15^{a}	0.98 ± 0.003^{a}	0.85 ± 0.02^{a}	449.00 ± 20.61^{a}
	LI	5.09 ± 0.14^{a}	0.98 ± 0.002^{a}	0.87 ± 0.01^{a}	345.00 ± 54.67^{b}
ITS	NS	3.15 ± 0.45^{b}	0.86 ± 0.07^{b}	0.54 ± 0.08^{b}	346.25 ± 52.08^{a}
	CLIS	3.76 ± 0.21^{a}	0.94 ± 0.01^{a}	0.68 ± 0.02^{a}	256.50 ± 38.51^{b}
	LI	3.81 ± 0.33^{a}	0.94 ± 0.02^{a}	0.69 ± 0.04^{a}	253.00 ± 42.60^{b}

Means followed by the standard deviation with the same letter are not significantly different according to the Scott-Knott method $p < 0.05$.

The phylum Chloroflexi was less abundant in NS and CLIS than LI. Representants of this phylum are markedly found in anaerobic habitats where play a role in fermentation and degradation of organic compounds to support their growth and that of other bacterial populations (Speirs et al., 2019). It was expected to find more anaerobic conditions in the LI area since is not yet fully recovered and presents greater degradation than the other areas. In terms of the abundance of this phylum, in particular, it seems that CLIS with the intervention is getting closer to the NS area. This idea was reinforced by the Venn diagram, which shows four phylum sharing between CLIS and NS.

The family Chthoniobacteraceae (phylum Verrucomicrobia) was most abundant in NS, while CLIS and LI showed abundances three times lower of this family. Granada et al. (2019) related the high incidence of members of the family Chthoniobacteraceae in areas with higher levels of organic matter, as seems to be the case when comparing NS with CLIS and LI. A similar pattern was observed for the family Acidothermaceae, which was most frequent in NS than CLIS and LI. In turn, the abundances of Ktedonobacteraceae and AD3 were greater in LI. Ktedonobacteraceae belongs to the phylum Chloroflexi and are often found in a higher abundance in extreme environments (Yabe et al., 2017). AD3 is related to sandy soils, highly weathered soils, and soils poor in organic C (Brewer et al., 2019). The members of this family are commonly adapted to conditions of nutritional limitation and present characteristics such as spore formation, synthesis and storage of carbohydrates, and use of carbon monoxide as an energy source (Brewer et al., 2019). Considering that the LI area is not yet fully recovered and presents greater degradation than the other areas, this would explain the higher incidence of AD3. Associated with this we can highlight that NS shared a larger number of families with CLIS than with LI area. And thus, although the CLIS treatment did not present the same parameters as the native area, there is evidence that the intervention method used in the analyzed period brings microbial diversity closer to the conditions of the native area.

The variations in the abundances of the families found in the studied areas reinforce the idea of a seed microbial bank, which refers to the ability of an organism to decrease its metabolic activity in the face of adverse conditions while still living in that environment and later being reactivated under favorable conditions (Lennon and Jones, 2011). This would explain the higher incidence of certain families in the areas, since they showed differences in the management, in addition to explaining the persistence of rare groups. Our results provide evidence that the different taxa respond differently to the types of management, which affects the functioning and restoration of the ecosystem.

The CLIS had the highest bacterial richness among the studied areas. This result can be explained by the fact that the area contains cultivated plants in addition to animals. Different plants and root exudates attract different groups of microorganisms to the rhizosphere (Alsharif et al., 2020; Pascale et al., 2020).

According to the NMDS analyses, the three areas analyzed were grouped in different clusters. There was a more pronounced separation of the NS from the two recovered areas (CLIS and LI) when analyzing bacterial data. The fungal data showed a more pronounced separation of LI from the other two.

The difference between the CLIS and LI areas shows that the compositions of the fungal and bacterial communities differed. Regarding the analysis of fungal sequences, the NS showed a dominance of the phylum Basidiomycota. The phylum Ascomycota predominated in CLIS and LI, which suggests that degradation in the areas could lead to the loss of basidiomycetes, and this niche is occupied by ascomycete fungi. The prevalence of ascomycetes has been correlated with human activity and the high tolerance to environmental stresses by representatives of the phylum (Souza et al., 2016). The change in agricultural management of Cerrado soil caused changes in the structure of the fungal community of the soil, mainly due to changes in soil properties, such as nutritional levels, CEC, and base saturation (Valadares-Pereira et al., 2017).

Studies on microbial diversity in Cerrado *stricto sensu* areas have shown that the predominant fungal phyla in dry seasons are Ascomycota and Basidiomycota (Castro et al., 2016), which is in agreement with the results obtained in this study. However, the relative abundance of basidiomycetes in the rainy season increases, while the relative abundance of ascomycetes decreases, compared to that in the dry season; this demonstrates the strong effect of soil water content on the fungal community structure (Castro et al., 2016). The conditions in the NS might have contributed to its higher moisture levels than those in the LI and CLIS treatments, which contributed, together with other factors, to a greater development of basidiomycetes in NS.

The dominant family in NS was Hygrophoraceae, whose representatives are primarily found in undisturbed grassland habitats and are much rarer or absent in grasslands subject to agricultural intensification, which can characterize them as ecological indicators (Halbwachs et al., 2018). Thus, it is evident that the fungal community needs more time to recover and to approximate from the native area than the bacterial community, mainly because those are more sensitive to environmental changes (Genevieve et al., 2019). This explains also the higher fungal richness and the smaller fungal diversity in the NS area compared to CLIS and LI. Although there is a greater richness in NS, the evenness is lower (confirmed by the diversity and Pielou indexes). That is, in a native environment, certain species are prevalent, which is not being observed in the regenerating areas. It is likely due to the kinetics of nutrients and altered environmental conditions for the recovery of the areas, which makes the "disturbance" in the areas (or lack of environmental stability) prevent the development of certain species at the expense of others.

CONCLUSION

This study evaluated the taxonomic composition of the bacterial and fungal communities of the soil in CLIS compared to LI and NS areas. The land-use type affected the taxonomic diversity of the soil microbiota. The analyses of the microbial community showed that after one year of intervention in the degraded area, the CLIS area already showed a more accelerated recovery than

the LI area, mainly marked by the bacterial communities. The results presented here contribute to a better understanding of the factors that help to model the microbial communities in strategic systems used for the recovery of degraded areas and encourage the use of CLIS for those areas.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in NCBI under BioProject accession number PRJNA669331.

AUTHOR CONTRIBUTIONS

PS, TP, GJ, FC, EA, and DS contributed to the conception and design of the study. GJ, FC, and EA conducted the field work and the soil sampling. PS performed the DNA extraction and soil and DNA analysis. LO and AF conducted the bioinformatics. LO ran the statistical analysis. PS, LO, and EA wrote the manuscript. TP, EA, DS, WA, and FS contributed to the manuscript revision. FS contributed to the funding acquisition and supervision. All authors read and approved the submitted manuscript.

FUNDING

National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq) (grant number 400901/2019-6) and Instituto Federal de Educação, Ciência e Tecnologia Goiano (Federal Institute of Education, Science and Technology Goiano) (contract number no 24/2018).

ACKNOWLEDGMENTS

We would like to thank IF Goiano for the support and the family that owns the Boa Esperança Farm for supporting and providing the area for the research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.661410/full#supplementary-material

REFERENCES

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R. H., et al. (2020). *UNITE QIIME release for Fungi*. London: UNITE Community.

Alsharif, W., Saad, M. M., and Hirt, H. (2020). Desert Microbes for Boosting Sustainable Agriculture in Extreme Environments. *Front. Microbiol.* 11:1666. doi: 10.3389/fmicb.2020.01666

AOAC, (1995). *Official methods of analysis of the association of analytical chemists*. Washington, DC: Association of Official Analytical Chemists.

Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137. doi: 10.3354/ame01753

Araujo, A. S. F., Bezerra, W. M., dos Santos, V. M., Rocha, S. M. B., Carvalho, N. et al. (2017). Distinct bacterial communities across a gradient of vegetation from a preserved Brazilian Cerrado. *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* 110, 457–469. doi: 10.1007/s10482-016-0815-1

Araujo, J. F., de Castro, A. P., Costa, M. M. C., Togawa, R. C., Júnior, G. J. P., Quirino, B. F., et al. (2012). Characterization of Soil Bacterial Assemblies in Brazilian Savanna-Like Vegetation Reveals Acidobacteria Dominance. *Microb. Ecol.* 64, 760–770. doi: 10.1007/s00248-012-0057-3

Beck, M. W. (2016). *ggord: Ordination Plots with ggplot2. R Packag. version 0.11*. Vienna: R Core Team.

Beule, L., Lehtsaar, E., Corre, M. D., Schmidt, M., Veldkamp, E., and Karlovsky, P. (2020). Poplar Rows in Temperate Agroforestry Croplands Promote Bacteria, Fungi, and Denitrification Genes in Soils. *Front. Microbiol.* 10:3108. doi: 10.3389/fmicb.2019.03108

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., et al. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2′s q2-feature-classifier plugin. *Microbiome* 6:90. doi: 10.1186/s40168-018-0470-z

Brewer, T. E., Aronson, E. L., Arogyaswamy, K., Billings, S. A., Botthoff, J. K., Campbell, A. N., et al. (2019). Ecological and Genomic Attributes of Novel Bacterial Taxa That Thrive in Subsurface Soil Horizons. *MBio* 10, e1318–e1319. doi: 10.1128/mBio.01318-19

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi: 10.1038/nmeth.3869

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335–336. doi: 10.1038/nmeth.f.303

Castañeda, L. E., and Barbosa, O. (2017). Metagenomic analysis exploring taxonomic and functional diversity of soil microbial communities in Chilean vineyards and surrounding native forests. *PeerJ* 30:e3098. doi: 10.7717/peerj.3098

Castro, A. P., Da Silva, M. R. S., Quirino, B. F., Da Cunha, Bustamante, M. M., and Krüger, R. H. (2016). Microbial diversity in Cerrado biome (neotropical savanna) soils. *PLoS One* 11:1–16. doi: 10.1371/journal.pone.0148785

Chaudhry, V., Sharma, S., Bansal, K., and Patil, P. B. (2017). Glimpse into the Genomes of Rice Endophytic Bacteria: Diversity and Distribution of Firmicutes. *Front. Microbiol.* 7:2115. doi: 10.3389/fmicb.2016.02115

Chen, H., and Boutros, P. C. (2011). VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinformatics* 12:35. doi: 10.1186/1471-2105-12-35

Dall'Agnol, R. F., Plotegher, F., Souza, R. C., Mendes, I. C., dos Reis Junior, F. B., et al. (2016). *Paraburkholderia nodosa* is the main N2 -fixing species trapped by promiscuous common bean (*Phaseolus vulgaris* L.) in the Brazilian 'Cerradão.'. *FEMS Microbiol. Ecol.* 92:fiw108. doi: 10.1093/femsec/fiw108

Damian, J. M., Firmano, R. F., Cherubin, M. R., Pavinato, P. S., de Marchi Soares, T., Paustian, K., et al. (2020). Changes in soil phosphorus pool induced by pastureland intensification and diversification in Brazil. *Sci. Total Environ.* 703:135463. doi: 10.1016/j.scitotenv.2019.135463

Genevieve, L., Pierre-Luc, C., Roxanne, G.-T., Amélie, M., Danny, B., Vincent, M., et al. (2019). Estimation of Fungal Diversity and Identification of Major Abiotic Drivers Influencing Fungal Richness and Communities in Northern Temperate and Boreal Quebec Forests. *Forests* 10:1096. doi: 10.3390/f10121096

Granada, C. E., Vargas, L. K., Lisboa, B. B., Giongo, A., Martinho, C. T., Pereira, L. D. M., et al. (2019). Bacterial and Archaeal Communities Change With Intensity of Vegetation Coverage in Arenized Soils From the Pampa Biome. *Front. Microbiol.* 10:497. doi: 10.3389/fmicb.2019.00497

Halbwachs, H., Easton, G. L., Bol, R., Hobbie, E. A., Garnett, M. H., Peršoh, D., et al. (2018). Isotopic evidence of biotrophy and unusual nitrogen nutrition in soil-dwelling Hygrophoraceae. *Environ. Microbiol.* 20, 3573–3588. doi: 10.1111/1462-2920.14327

Hermans, S. M., Taylor, M., Grelet, G., Curran-Cournane, F., Buckley, H. L., Handley, K. M., et al. (2020). From pine to pasture: land use history has long-term impacts on soil bacterial community composition and functional potential. *FEMS Microbiol. Ecol.* 96:fiaa041. doi: 10.1093/femsec/fiaa041

Hoggard, M., Vesty, A., Wong, G., Montgomery, J. M., Fourie, C., Douglas, R. G., et al. (2018). Characterizing the human mycobiota: A comparison of small subunit rRNA, ITS1, ITS2, and large subunit rRNA genomic targets. *Front. Microbiol.* 9:2208. doi: 10.3389/fmicb.2018.02208

Istvan, P., Souza, A. A., Garay, A. V., dos Santos, D. F. K., de Oliveira, G. M., Santana, R. H., et al. (2018). Structural and functional characterization of a novel lipolytic enzyme from a Brazilian Cerrado soil metagenomic library. *Biotechnol. Lett.* 40, 1395–1406. doi: 10.1007/s10529-018-2598-0

IUSS Working Group WRB, (2015). *International soil classification system for naming soils and creating legends for soil maps*. Available online at: https://www.iuss.org/int-year-of-soils-2015/ (accessed November 18, 2020)

Jelihovschi, E., Faria, J. C., and Allaman, I. B. (2014). ScottKnott: A Package for Performing the Scott-Knott Clustering Algorithm in R. *TEMA* 15:003. doi: 10.5540/tema.2014.015.01.0003

Kassambara, A., and Mundt, F. (2017). *Package "factoextra" for R: Extract and Visualize the Results of Multivariate Data Analyses. R Packag. version*. Vienna: R Core Team.

Lennon, J. T., and Jones, S. E. (2011). Microbial seed banks: The ecological and evolutionary implications of dormancy. *Nat. Rev. Microbiol.* 9, 119–130. doi: 10.1038/nrmicro2504

Ligoski, B., Gonçalves, L. F., Claudio, F. L., Alves, E. M., Krüger, A. M., Bizzuti, B. E., et al. (2020). Silage of Intercropping Corn, Palisade Grass, and Pigeon Pea Increases Protein Content and Reduces *In Vitro* Methane Production. *Agronomy* 10:1784. doi: 10.3390/agronomy10111784

Lisboa, F. J. G., Chaer, G. M., Fernandes, M. F., Berbara, R. L. L., and Madari, B. E. (2014). The match between microbial community structure and soil properties is modulated by land use types and sample origin within an integrated agroecosystem. *Soil Biol. Biochem.* 78, 97–108. doi: 10.1016/j.soilbio.2014.07.017

López-Poma, R., Pivello, V. R., de Brito, G. S., and Bautista, S. (2020). Impact of the conversion of Brazilian woodland savanna (cerradão) to pasture and *Eucalyptus* plantations on soil nitrogen mineralization. *Sci. Total Environ.* 704:135397. doi: 10.1016/j.scitotenv.2019.135397

Nannipieri, P., Ascher, J., Ceccherini, M. T., Landi, L., Pietramellara, G., and Renella, G. (2017). Microbial diversity and soil functions. *Eur. J. Soil Sci.* 68, 12–26. doi: 10.1111/ejss.4_12398

O'Brien, F. J. M., Almaraz, M., Foster, M. A., Hill, A. F., Huber, D. P., King, E. K., et al. (2019). Soil Salinity and pH Drive Soil Bacterial Community Composition and Diversity Along a Lateritic Slope in the Avon River Critical Zone Observatory, Western Australia. *Front. Microbiol.* 10:1486. doi: 10.3389/fmicb.2019.01486

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., and O'Hara, R. B. (2013). *vegan: Community Ecology Package*. Vienna: R Cor e Team.

Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* 18, 1403–1414. doi: 10.1111/1462-2920.13023

Pascale, A., Proietti, S., Pantelides, I. S., and Stringlis, I. A. (2020). Modulation of the Root Microbiome by Plant Molecules: The Basis for Targeted Disease Suppression and Plant Growth Promotion. *Front. Plant Sci.* 10:1741. doi: 10.3389/fpls.2019.01741

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucl. Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219

R Core Development Team, (2019). *R: A language and environment for statistical computing*. Vienna: R Core Development Team.

Salton, J. C., Mercante, F. M., Tomazi, M., Zanatta, J. A., Concenço, G., Silva, W. M., et al. (2014). Integrated crop–livestock system in tropical Brazil: toward a sustainable production system. *Agric. Ecosyst. Environ.* 190, 70–79. doi: 10.1016/j.agee.2013.09.023

Sándor, Z., Tállai, M., Kincses, I., et al. (2020). Effect of various soil cultivation methods on some microbial soil properties. *DRC Sustainab. Fut.* 1, 14–20. doi: 10.37281/DRCSF/1.1.3

Sarto, M. V. M., Borges, W. L. B., Sarto, J. R. W., Pires, C. A. B., Rice, C. W., and Rosolem, C. A. (2020). Soil microbial community and activity in a tropical integrated crop-livestock system. *Appl. Soil Ecol.* 145:103350. doi: 10.1016/j.apsoil.2019.08.012

Silva, M. R. S. S., Pereira, de Castro, A., Krüger, R. H., and Bustamante, M. (2019). Soil bacterial communities in the Brazilian Cerrado: Response to vegetation type and management. *Acta Oecol.* 100:103463. doi: 10.1016/j.actao.2019.103463

Souza, R. C., Mendes, I. C., Reis-Junior, F. B., Carvalho, F. M., Nogueira, M. A., Vasconcelos, A. T. R., et al. (2016). Shifts in taxonomic and functional microbial diversity with agriculture: How fragile is the Brazilian Cerrado? *BMC Microbiol.* 16:42. doi: 10.1186/s12866-016-0657-z

Speirs, L. B. M., Rice, D. T. F., Petrovski, S., and Seviour, R. J. (2019). The Phylogeny, Biodiversity, and Ecology of the Chloroflexi in Activated Sludge. *Front. Microbiol.* 10:2015. doi: 10.3389/fmicb.2019.02015

Tesk, C. R. M., Cavalli, J., Pina, D. S., et al. (2020). Herbage responses of Tamani and Quênia guineagrasses to grazing intensity. *Agronomy* 112, 2081–2091. doi: 10.1002/agj2.20189

Valadares-Pereira, A. A., Oliveira, E. C., Navarrete, A. A., Oliveira Junior, W. P. et al. (2017). Fungal community structure as an indicator of soil agricultural management effects in the cerrado. *Rev. Bras. Cienc. Solo* 41, 1–12. doi: 10.1590/18069657rbcs20170489

Vieira, C. K., Borges, L. G., Marconatto, L., Giongo, A., and Stürmer, S. L. (2018). Microbiome of a revegetated iron-mining site and pristine ecosystems from the Brazilian Cerrado. *Appl. Soil Ecol.* 131, 55–65. doi: 10.1016/j.apsoil.2018.07.011

Wickham, H. (2009). *ggplot2*. New York, NY: Springer, doi: 10.1007/978-0-387-98141-3

Yabe, S., Sakai, Y., Abe, K., and Yokota, A. (2017). Diversity of Ktedonobacteria with Actinomycetes-Like Morphology in Terrestrial Environments. *Microbes Environ.* 32, 61–70. doi: 10.1264/jsme2.ME16144

Zhalnina, K., Dias, R., de Quadros, P. D., Davis-Richardson, A., Camargo, A. O., Clark, I. M., et al. (2014). Soil pH determines microbial diversity and composition in the park grass experiment. *Microb. Ecol.* 69, 395–406. doi: 10.1007/s00248-014-0530-2

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership