RESEARCH ARTICLE

Biological invasions alter environmental microbiomes: A meta-analysis

Antonino Malacrinò[ID]¹*, Victoria A. Sadowski¹, Tvisha K. Martin², Nathalia Cavichiolli de Oliveira³, Ian J. Brackett¹, James D. Feller¹, Kristian J. Harris¹, Orlando Combita Heredia[ID]¹, Rosa Vescio¹,⁴, Alison E. Bennett¹

1 Department of Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus, OH, United States of America, 2 College of Food, Agricultural and Environmental Sciences, The Ohio State University, Columbus, OH, United States of America, 3 Department of Entomology, University of Sao Paulo, Piracicaba (SP), Brazil, 4 Dipartimento AGRARIA, Università degli Studi Mediterranea, Reggio Calabria, Italy

* malacrino.1@osu.edu, antonino.malacrino@gmail.com

Abstract

Biological invasions impact both agricultural and natural systems. The damage can be quantified in terms of both economic loss and reduction of biodiversity. Although the literature is quite rich about the impact of invasive species on plant and animal communities, their impact on environmental microbiomes is underexplored. Here, we re-analyze publicly available data using a common framework to create a global synthesis of the effects of biological invasions on environmental microbial communities. Our findings suggest that non-native species are responsible for the loss of microbial diversity and shifts in the structure of microbial populations. Therefore, the impact of biological invasions on native ecosystems might be more pervasive than previously thought, influencing both macro- and micro-biomes. We also identified gaps in the literature which encourage research on a wider variety of environments and invaders, and the influence of invaders across seasons and geographical ranges.

Introduction

Biological invasions have severe impacts on biodiversity, community composition and ecosystem functions [1–5]. Invasive plants can alter many important ecosystem functions including the nitrogen cycle [6], carbon cycle, and decomposition. For example, invasion by the plant Amur Honeysuckle altered the decomposition rate in the invaded environment likely through changes in litter quality [7]. Exotic snails have been found to alter carbon and nitrogen fluxes in freshwater systems through their consumption/excretion activity [8]. These functions are provided by environmental microbiomes. Yet, despite the implications for ecosystem functioning, we are still learning the consequences of biological invasions on environmental microbiomes.

Previous studies have shown biological invasions can impact the diversity and taxonomical structure of environmental microbiomes. For example, we often see a shift in soil microbiota following invasion by non-native plant species [9–19]. Removal of feral pigs increased the

diversity of soil bacterial communities and shifted their structure [20], and invasive crustaceans [21], mussels [22] and jellyfish [23] produce changes in the structure of water microbiomes. However, shifts in environmental microbiome as consequence of biological invasions do not always occur. For example, invasion by the plants *Robinia pseudoacacia* [24], *Eucalyptus* sp. [25], and *Vincetoxicum rossicum* [26] did not alter the structure of soil microbial communities. Also, some microcosms exposed to the simultaneous invasion of multiple plant species [27–29] did not alter soil microbiomes. Similarly, soil microbiome structure in microcosms did not change with the addition of the invasive earthworm *Aporrectodea trapezoides* [30]. Several of these studies used techniques (e.g. DGGE, PLFA, t-RFLP) that limit fine scale investigations of biological invasions on environmental microbiome diversity and taxonomical composition. Among the studies using high-throughput amplicon-sequencing techniques, most did not find changes in microbiome diversity [13, 15, 18, 19, 21, 25, 27, 28, 30], few reported a decrease of microbial diversity in response to invasion [16, 20, 29], and fewer still reported an increase [11, 14]. Thus, there is little consensus on the effects of biological invasions on the diversity and taxonomical structure of the environmental microbiomes, both tied to the stability and function of microbial communities [31, 32].

Our ability to draw broad conclusions from published studies is limited, because individual studies have occurred within a limited geographical range or with a limited group of species. Meta-analyses of published biological means have long enabled more robust conclusions than individual studies [33–36]. However, the meta-analytic approach has less frequently been applied to amplicon-sequencing data that represent environmental microbiome community composition. The majority of meta-analytic metabarcoding studies have occurred in the medical sciences [37–45]. This approach can be successfully used to address ecological questions. For example, meta-analytic metabarcoding studies have found common patterns in the structure of indoor microbiomes [46] and freshwater eukaryotes [47]. Shade et al. [48] also used a meta-analysis of metabarcoding datasets from different environments highlighting a time-dependent structure of microbiomes. A meta-analytic approach has also been used to test the effects of stressors (e.g. water availability, temperature, heavy metals) on environmental microbiomes [49]. Thus meta-analyses on microbiome data have a striking potential to address global-scale questions, generate new hypotheses and model common patterns [50], because they provide across study comparisons [39, 51, 52].

Here, we aim to test whether the effect of biological invasion on environmental microbiomes can be generalized or is idiosyncratic. To do so, we collected publicly available data and re-analyzed this data under a common framework. We tested the effect of invasive species on the diversity and structure of environmental microbiomes, with the hypothesis that the presence of invasive species will decrease microbial diversity and alter the composition of the environmental microbiome. We then investigated whether certain taxonomical groups are more responsive to biological invasions.

Methods

Data collection

We searched for metabarcoding studies that evaluated the effect of biological invasions on environmental microbiomes, and compared invaded and non-invaded habitats. Our literature search for this study was conducted using Web of Science Core Collection (accessed on March 6th, 2020) using the keywords "Invasive speci*" and "microbio*" published between 2010–2020, and found 1,471 studies. Two additional studies were added by searching the same keywords on Google Scholar (S1 Fig). Records were manually filtered based on the study design appropriate for our research question. This step yielded 22 studies, and we further filtered

these studies based on data availability in public repositories. When data was not available, we attempted to contact the corresponding author. Finally we selected only studies that used the 16S rRNA marker gene, primer pair 515F/806R [53] or 341F/785R [54], and Illumina MiSeq sequencing platform. After discarding studies that failed quality checks (see below), we were able to include a total of five studies (Table 1), summing up to a total of 356 samples. The study by Gibbons et al. [28] tested the impact of five invasive plant species (*Agropyron cristatum*, *Bromus tectorum*, *Sisymbrium altissimum*, *Erodium cicutarium* and *Poa bulbosa*) on soil microbiome using microcosms, comparing monocultures of each one of them towards a mixture of eight native plant species. A similar question was tested in Rodrigues et al. [19] in field condition. They identified three locations invaded by three different exotic plant species (*Microstegium vimineum*, *Rhamnus davurica* and *Ailanthus altissima*) and, within each location, they sampled soil form an invaded area and a non-invaded area for comparisons. Similarly, Collins et al. [11] compared the soil microbial community of field sites invaded by *Artemisia rothrockii* to non-invaded sites. The study by Wehr et al. [20] focused on the effects of feral pig (*Sus scrofa*) invasion on soil microbiome comparing invaded areas to those where pigs were removed over a ~25 year chronosequence. Finally, the only study performed in an aquatic environment [22] compared water samples collected in lake areas invaded by the exotic mussel *Dreissena bugensis* to non-invaded sites. Three studies focused on invasive plants, and the remaining studies focused on a mammal and a mussel (Table 1).

We took the following steps to alleviate some of the potential sources of bias due to studies performed in different labs, using different protocols and sequenced on different instruments. First, all studies included were performed using the Illumina MiSeq platform, in order to reduce the potential bias that might be generated by directly comparing data obtained from different platforms. Second, all studies targeted the same region of the 16S rRNA, as several primer pairs targeting different regions are currently published and widely used. Three out of five papers we considered in our analysis used the 515F/806R primer pair [53], while two used the 341F/785R [54]. Because these primer pairs overlap in the V4 region of 16S rRNA we feel confident that the chance of including spurious OTUs in our analysis is quite negligible. To account for study-specific variances due to small differences in sampling procedures and lab protocols, we also included the study itself, the environment where the study was performed (i.e., soil or water) and the identity of the invasive species as stratification variables in the PERMANOVA and as random factors in our linear model. This allowed us to ensure that our results are not biased by study-specific features.

Once the papers were selected, we assigned each a "Study ID" and collected meta-data from each sample in each study (invasive species, type of organism, invaded environment). We then downloaded data from repositories using SRA Toolkit 2.10.4 for data on the SRA databases, or by manually downloading files from the MG-RAST database.

Table 1. Summary of studies included in the meta-analysis.

Study ID	Invasive organism	Species	Invaded environment	Reference
MPG13011	Plant	*Agropyron cristatum*, *Bromus tectorum*, *Sisymbrium altissimum*, *Erodium cicutarium* and *Poa bulbosa*	Soil	Gibbons et al. [28]
MPG87547	Mammal	*Sus scrofa*	Soil	Wehr et al. [20]
PRJNA296487	Plant	*Microstegium vimineum*, *Rhamnus davurica* and *Ailanthus altissima*	Soil	Rodrigues et al. [19]
PRJNA320310	Plant	*Artemisia rothrockii*	Soil	Collins et al. [11]
PRJNA385848	Mussel	*Dreissena bugensis*	Water	Denef et al. [22]

https://doi.org/10.1371/journal.pone.0240996.t001

Data processing and analysis

Paired-end reads were merged using FLASH 1.2.11 [55] and data were processed using QIIME 1.9.1 [56]. Quality-filtering of reads was performed using default parameters, binning OTUs and discarding chimeric sequences identified with VSEARCH 2.14.2 [57]. Taxonomy for representative sequences was determined by querying against the SILVA database v132 [58] using the BLAST method. A phylogeny was obtained by aligning representative sequences using MAFFT v7.464 [59] and reconstructing a phylogenetic tree using FastTree [60].

Data analysis was performed using R statistical software 3.5 [61] with the packages phyloseq [62] and vegan [63]. Read counts were normalized using DESeq2 v1.22.2 [64] prior to data analysis. Singletons and sequences classified as chloroplast were excluded, as well as samples which had less than 5000 sequence counts. Shannon diversity was fit to a linear mixed-effects model specifying *sample type* (invaded or control), *organism* (plant, mammal, mussel), and their interactions as fixed factors. We included *studyID* or both *studyID* and *environment* (soil or water) as random factors, and both models reported similar results (S2 Table). We focused on the one with only *studyID* as random effect due to the lower AIC value. Models were fit using the *lmer*() function under the *lme4* package [65] and the package *emmeans* was used to infer pairwise contrasts (corrected using False Discovery Rate, FDR). Furthermore, we explored the effects of *sample type* and *organism* on the structure of the microbial communities using a multivariate approach. Distances between pairs of samples, in terms of community composition, were calculated using a Unifrac matrix, and then visualized using an RDA procedure. Differences between sample groups were inferred through PERMANOVA multivariate analysis (999 permutations). We ran two different PERMANOVA models: in one we stratified permutations at level of *studyID* and *identity of invasive species*, and in the other we stratified permutations at level of *studyID*, *environment* and *identity of invasive species*, obtaining similar results (S3 Table). Pairwise contrasts from PERMANOVA were subjected to FDR correction. Finally, the relative abundance of each bacterial family was fit using the *lmer*() function to test the effects of *sample type* (invaded or control) on individual taxa. We ran two different linear mixed-effects models, one including *studyID*, *organism* (plant, mammal, mussel) and *environment* as random factors, and another with *studyID* and *organism* as random factors, obtaining similar results (S4 Table).

Results

Our search yielded 5 studies with an appropriate experimental design and available data, for a total of 356 samples. A few samples failed quality checks and we further considered 335 samples for downstream analyses. Sequences clustered into 22831 OTUs, after quality checks, removal of singletons and "chloroplast" reads, with an average of 61776.92 reads per sample. This high OTU count is likely a result of increased richness from analyzing samples across multiple environments (soil and water) and from different geographical regions.

Biological invasions led to a reduction in Shannon diversity (Control = 6.92±0.06, Invaded = 6.73±0.07, χ^2 = 3.85, df = 1, P = 0.04). We also found biological invasions altered microbiome community composition in the invaded environment compared to the control (Table 2 and Fig 1). The type of invasive organism (plant, mammal, or mussel) produced a different community structure (pairwise $P<0.01$, FDR corrected). A deeper analysis of bacterial families (S5 Table) revealed that some taxonomic groups are significantly more abundant in invaded environments (Blastocatellaceae, Chitinophagaceae, Nitrosomonadaceae, Pirellulaceae, Sphingomonadaceae), while others are more abundant in non-invaded samples (Acetobacteraceae, Beijerinckiaceae, Gemmataceae, Micromonosporaceae, Pedosphaeraceae, Solibacteraceae, Solirubrobacteraceae).

Table 2. Results from PERMANOVA analysis testing the effects of *sample type* (invaded/control), *organism group* (plant, mammal, mussel) and their interaction on microbial community composition. The factors *studyID* (unique for each study) was used as strata to constrain permutations.

Factor	df	R^2	F	P
Sample type (Invaded/Control)	1	0.011	6.68	<0.001
Organism group (plant, mammal, mussel)	3	0.411	118.86	<0.001
Sample type × Organism group	3	0.007	2.1	0.01

https://doi.org/10.1371/journal.pone.0240996.t002

Discussion

Here we show biological invasions decrease the diversity of environmental microbiomes. While several studies have investigated the effects of species invasions on environmental microbiomes, we still lack a generalized consensus across different environmental microbiomes and systems. Previous studies have found that invasive species increased environmental microbial diversity [11, 14], while others reported a decrease [16, 20, 29]. However, the majority of studies did not analyze the microbial diversity, as they used techniques that did not allow for such analysis, or reported no changes [9, 10, 12, 13, 15, 17–19, 21–28, 30]. Within the studies included in our analysis, invasion by feral pigs decreased soil microbial diversity, while invasion by *Artemisia rothrockii* increased soil microbial diversity. The remaining three studies in our analysis reported no effects of biological invasions on environmental microbiome diversity. Our analysis was constrained in terms of sampled environment (soil) and invasive organism (plants), and an expanded dataset would be beneficial to generalize our results. Microbial diversity is tied to the function of microbiomes, and changes in diversity can reflect changes in function [66–68]. Changes in microbial diversity and function do not always have the same direction [69], which might explain the discrepancy between our results and other studies.
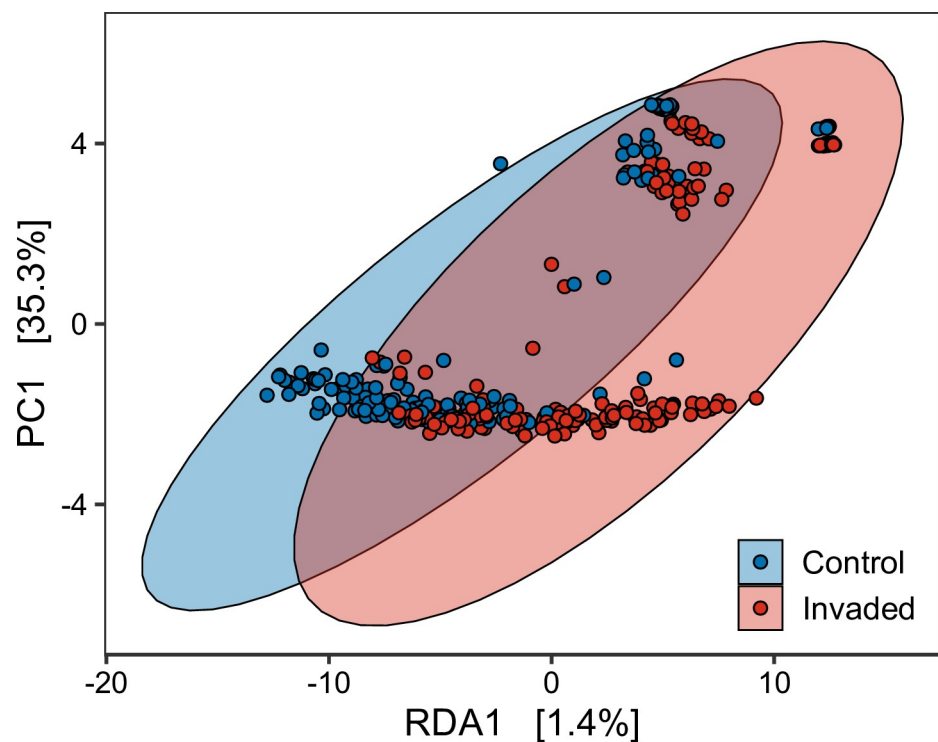

Fig 1. RDA ordination using a Bray-Curtis distance matrix of samples.

https://doi.org/10.1371/journal.pone.0240996.g001

While we observed a relatively small reduction of the Shannon diversity index in invaded environments compared to non-invaded environment, this matches with the changes we observed in terms of community composition.

Indeed, our report of changes in community composition was relatively consistent with the published literature and the individual results of the studies we analyzed. Most studies of the influence of biological invasions on environmental microbiomes found that biological invasions alter environmental microbial community composition. However, some previous reports did not report changes [24–30], including the study by Gibbons et al. [28] considered in our analysis. This variation may be due to individual effects of organisms on the environment. For example, invasive plants may alter soil microbiome composition through root exudates [5], and invasive mussels may alter water microbiome composition via bacterial removal through their feeding activity [22]. Thus, reported influences on community composition are more consistent. Alternatively, changes in community composition might be due to the response of some bacterial groups to environmental disturbance. The bacterial families that we found to be differentially abundant between the invaded and control environments have diverse ecological functions ranging from nitrogen fixation and carbohydrate metabolism to antimicrobial properties. Although the differences in relative abundance we found might be relatively small, they can have an important impact on the functions of the environmental microbiome [70]. Many of the families that showed a significant difference in abundance between invaded and control environments include taxa that play important roles at various points during nitrogen and carbon cycling (i.e. Nitrosomonadaceae, Acetobacteraceae, Chitinophagaceae, Micromonosporaceae, Gemmataceae, Beijerinckiaceae, Pirellulaceae) [71–81]. However, nitrogen-fixing and carbohydrate-degrading bacteria did not have a unified response to invaded environments as some increased and others decreased in abundance in invaded environments. Many nitrogen-fixing bacteria have been shown to respond to environmental disturbance, such as Acidobacteria abundances during forest to pasture conversions or Pirellulaceae's response to the presence of microplastics [82, 83]. Thus, changes in environmental microbiome community composition appear to be linked to changes in ecosystem functions, although this pattern is not yet predictable across functions and taxa.

Few previous studies on biological invasions have reported details on the differential abundance of taxa, and among these we found limited general consensus. For example, some studies report a decrease in abundance of bacteria associated with nitrogen cycling (e.g. Nitrosphaeria, Nitrospira, Nitrosomonadales) [13, 14, 19], while others report an increase of Nitrosomonadaceae following invasion [30]. In our study some groups associated with the nitrogen cycle were positively associated with biological invasions (i.e. Nitrosomonadaceae, Pirellulaceae, Chitinophagaceae) while others were negatively associated (Beijerinckiaceae, Micromonosporaceae). Unfortunately, amplicon-based sequencing has a limited power to infer changes in the functions of microbiomes. Future metagenomic and metatranscriptomic studies are needed to investigate whether biological invasions alter gene content or gene expression of environmental microbiomes, and whether this reflects changes in biogeochemical cycling.

Meta-analyses are also useful to highlight gaps in the literature, and here we highlight some aspects that warrant further investigation. First, we identified a large gap in the availability of sequencing data from multiple types of environments and types of invasive species. For our analysis almost all available data came from two environments: four sets of data came from soil and one came from freshwater. Greater effort is needed for sample collection from invasions in both freshwater and marine environments. Without sufficient diversity of sample environments, it is impossible to tell whether microbial shifts following an invasion are unique to an invaded environment. Second, in our analysis the majority of data came from one type of invasive species: plants. Noticeably absent from our dataset were invasions by insects, fish, and

amphibians. Sequencing data is needed from a larger number of invasive species to allow us to broadly assess shifts in microbial community structure. A third gap we identified was the lack of spatial and temporal resolution. Almost all of the initially identified 22 studies we assessed were also restricted to one season of sampling and were conducted in the Northern Hemisphere. Thus, it is infeasible with existing datasets to validate the influence of latitude or explore how seasonality and biological invasions interact to modulate microbial communities. Thus, there are a number of opportunities for future research on how biological invasions alter environmental microbial communities.

Here we analyzed 16S amplicon sequencing data from five studies and show that biological invasions influence both the diversity and the structure of environmental microbiomes. Understanding the impact of biological invasions on environmental microbiomes is of high priority to preserve ecosystem functions [84]. We identified a number of gaps in our knowledge, including the need to assess a wider range of environments, invasive species, temporal variation, and latitudinal variation. We also demonstrate the power of re-analysis of publicly available datasets using a common pipeline which benefited from open-data initiatives.

Supporting information

S1 Fig. PRISMA workflow. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097.
(PDF)

S1 Table. PRIMA checklist. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097.
(PDF)

S2 Table. Comparison of two different linear mixed-effects models testing the effect of biological *sample type* (invaded or control), *organism* (plant, mammal, mussel), and their interactions, on Shannon diversity index of the environmental microbiome. In Model 1 we included *studyID* as random factor, while in Model 2 we included both *studyID* and *environment* (soil or water) as random factors.
(PDF)

S3 Table. Comparison of two different PERMANOVA models testing the effect of biological *sample type* (invaded or control), *organism* (plant, mammal, mussel), and their interactions, on the structure of the environmental microbiome. In Model 1 we included *studyID* and *speciesID* as stratification factor, while in Model 2 we included *studyID*, *speciesID* and *environment* (soil or water) as stratification factors.
(PDF)

S4 Table. Results from two different the linear mixed-effects model testing the abundance of each bacterial family against *sample type* (invaded or control), *organism* (plant, mammal, mussel), and their interactions. In Model 1 we included *studyID* as random factor, while in Model 2 we included both *studyID* and *environment* (soil or water) as random factors.
(PDF)

S5 Table. Comparison of the relative proportion of each bacterial family between control and invaded environments. Differences are assessed using a linear mixed-effects model testing the normalized proportions of each bacterial family against *sample type* (Model 1 in S4 Table).
(PDF)

Acknowledgments

We would like to thank Kali Mattingly (Ohio State University, USA) and Davide Rassati (Università degli Studi di Padova, Italy) for their helpful comments on the manuscript.

Author Contributions

Conceptualization: Antonino Malacrinò, Victoria A. Sadowski, Tvisha K. Martin, Nathalia Cavichiolli de Oliveira, Ian J. Brackett, James D. Feller, Kristian J. Harris, Orlando Combita Heredia, Rosa Vescio, Alison E. Bennett.

Data curation: Antonino Malacrinò, Victoria A. Sadowski, Tvisha K. Martin, Nathalia Cavichiolli de Oliveira, Ian J. Brackett, James D. Feller, Kristian J. Harris, Orlando Combita Heredia, Rosa Vescio.

Formal analysis: Antonino Malacrinò, Victoria A. Sadowski, Tvisha K. Martin, Nathalia Cavichiolli de Oliveira, Ian J. Brackett, James D. Feller, Kristian J. Harris, Orlando Combita Heredia, Rosa Vescio.

Methodology: Antonino Malacrinò.

Supervision: Antonino Malacrinò, Alison E. Bennett.

Writing – original draft: Antonino Malacrinò.

Writing – review & editing: Antonino Malacrinò, Victoria A. Sadowski, Tvisha K. Martin, Nathalia Cavichiolli de Oliveira, Ian J. Brackett, James D. Feller, Kristian J. Harris, Orlando Combita Heredia, Rosa Vescio, Alison E. Bennett.

References

1. Chapin III FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, et al. Consequences of changing biodiversity. Nature. 2000; 405: 234–242. https://doi.org/10.1038/35012241 PMID: 10821284

2. Pejchar L, Mooney HA. Invasive species, ecosystem services and human well-being. Trends Ecol Evol. 2009; 24: 497–504. https://doi.org/10.1016/j.tree.2009.03.016 PMID: 19577817

3. Shackleton RT, Shackleton CM, Kull CA. The role of invasive alien species in shaping local livelihoods and human well-being: A review. J Environ Manage. 2019; 229: 145–157. https://doi.org/10.1016/j.jenvman.2018.05.007 PMID: 30049620

4. Charles H, Dukes JS. Impacts of Invasive Species on Ecosystem Services. Biological Invasions. Berlin, Heidelberg: Springer Berlin Heidelberg; 2008. pp. 217–237. https://doi.org/10.1007/978-3-540-36920-2_13

5. Weidenhamer JD, Callaway RM. Direct and Indirect Effects of Invasive Plants on Soil Chemistry and Ecosystem Function. J Chem Ecol. 2010; 36: 59–69. https://doi.org/10.1007/s10886-009-9735-0 PMID: 20077127

6. Lee MR, Bernhardt ES, van Bodegom PM, Cornelissen JHC, Kattge J, Laughlin DC, et al. Invasive species' leaf traits and dissimilarity from natives shape their impact on nitrogen cycling: a meta-analysis. New Phytol. 2017; 213: 128–139. https://doi.org/10.1111/nph.14115 PMID: 27501517

7. Arthur MA, Bray SR, Kuchle CR, McEwan RW. The influence of the invasive shrub, Lonicera maackii, on leaf decomposition and microbial community dynamics. Plant Ecol. 2012; 213: 1571–1582. https://doi.org/10.1007/s11258-012-0112-7

8. Hall RO Jr, Tank JL, Dybdahl MF. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. Front Ecol Environ. 2003; 1: 407–411. https://doi.org/10.1890/1540-9295(2003)001[0407:ESDNAC]2.0.CO;2

9. Lazzaro L, Giuliani C, Fabiani A, Agnelli AE, Pastorelli R, Lagomarsino A, et al. Soil and plant changing after invasion: The case of Acacia dealbata in a Mediterranean ecosystem. Sci Total Environ. 2014;497–498: 491–498. https://doi.org/10.1016/j.scitotenv.2014.08.014 PMID: 25151267

10. Malinich E, Lynn-Bell N, Kourtev PS. The effect of the invasive Elaeagnus umbellata on soil microbial communities depends on proximity of soils to plants. Ecosphere. 2017; 8: e01827. https://doi.org/10.1002/ecs2.1827

11. Collins CG, Carey CJ, Aronson EL, Kopp CW, Diez JM. Direct and indirect effects of native range expansion on soil microbial community structure and function. Austin A, editor. J Ecol. 2016; 104: 1271–1283. https://doi.org/10.1111/1365-2745.12616

12. Lorenzo P, Pereira CS, Rodríguez-Echeverría S. Differential impact on soil microbes of allelopathic compounds released by the invasive Acacia dealbata Link. Soil Biol Biochem. 2013; 57: 156–163. https://doi.org/10.1016/j.soilbio.2012.08.018

13. Parsons LS, Sayre J, Ender C, Rodrigues JLM, Barberán A. Soil microbial communities in restored and unrestored coastal dune ecosystems in California. Restor Ecol. 2020; rec.13101. https://doi.org/10.1111/rec.13101

14. Piper CL, Siciliano SD, Winsley T, Lamb EG. Smooth brome invasion increases rare soil bacterial species prevalence, bacterial species richness and evenness. De Deyn G, editor. J Ecol. 2015; 103: 386–396. https://doi.org/10.1111/1365-2745.12356

15. Zhang H-Y, Goncalves P, Copeland E, Qi S-S, Dai Z-C, Li G-L, et al. Invasion by the weed Conyza canadensis alters soil nutrient supply and shifts microbiota structure. Soil Biol Biochem. 2020; 143: 107739. https://doi.org/10.1016/j.soilbio.2020.107739

16. Le Roux JJ, Ellis AG, van Zyl L-M, Hosking ND, Keet J-H, Yannelli FA. Importance of soil legacy effects and successful mutualistic interactions during Australian acacia invasions in nutrient-poor environments. Aerts R, editor. J Ecol. 2018; 106: 2071–2081. https://doi.org/10.1111/1365-2745.12965

17. Brunel C, Beifen Y, Pouteau R, Li J, van Kleunen M. Responses of Rhizospheric Microbial Communities of Native and Alien Plant Species to Cuscuta Parasitism. Microb Ecol. 2020; 79: 617–630. https://doi.org/10.1007/s00248-019-01438-z PMID: 31598761

18. Pickett B, Irvine IC, Bullock E, Arogyaswamy K, Aronson E. Legacy effects of invasive grass impact soil microbes and native shrub growth. Invasive Plant Sci Manag. 2019; 12: 22–35. https://doi.org/10.1017/inp.2018.32

19. Rodrigues RR, Pineda RP, Barney JN, Nilsen ET, Barrett JE, Williams MA. Plant Invasions Associated with Change in Root-Zone Microbial Community Structure and Diversity. Liu J, editor. PLoS One. 2015; 10: e0141424. https://doi.org/10.1371/journal.pone.0141424 PMID: 26505627

20. Wehr NH, Kinney KM, Nguyen NH, Giardina CP, Litton CM. Changes in soil bacterial community diversity following the removal of invasive feral pigs from a Hawaiian tropical montane wet forest. Sci Rep. 2019; 9: 14681. https://doi.org/10.1038/s41598-019-48922-7 PMID: 31604976

21. Boeker C, Geist J. Effects of invasive and indigenous amphipods on physico-chemical and microbial properties in freshwater substrates. Aquat Ecol. 2015; 49: 467–480. https://doi.org/10.1007/s10452-015-9539-y

22. Denef VJ, Carrick HJ, Cavaletto J, Chiang E, Johengen TH, Vanderploeg HA. Lake Bacterial Assemblage Composition Is Sensitive to Biological Disturbance Caused by an Invasive Filter Feeder. Kent AD, editor. mSphere. 2017;2. https://doi.org/10.1128/mSphere.00189-17 PMID: 28593195

23. Manzari C, Fosso B, Marzano M, Annese A, Caprioli R, D'Erchia AM, et al. The influence of invasive jellyfish blooms on the aquatic microbiome in a coastal lagoon (Varano, SE Italy) detected by an Illumina-based deep sequencing strategy. Biol Invasions. 2015; 17: 923–940. https://doi.org/10.1007/s10530-014-0810-2

24. Lazzaro L, Mazza G, D'Errico G, Fabiani A, Giuliani C, Inghilesi AF, et al. How ecosystems change following invasion by Robinia pseudoacacia: Insights from soil chemical properties and soil microbial, nematode, microarthropod and plant communities. Sci Total Environ. 2018;622–623: 1509–1518. https://doi.org/10.1016/j.scitotenv.2017.10.017 PMID: 29054645

25. Hernández-Gómez O, Byrne AQ, Gunderson AR, Jenkinson TS, Noss CF, Rothstein AP, et al. Invasive vegetation affects amphibian skin microbiota and body condition. PeerJ. 2020; 8: e8549. https://doi.org/10.7717/peerj.8549 PMID: 32117625

26. Thompson GL, Bell TH, Kao-Kniffin J. Rethinking Invasion Impacts across Multiple Field Sites Using European Swallowwort (Vincetoxicum rossicum) as a Model Invader. Invasive Plant Sci Manag. 2018; 11: 109–116. https://doi.org/10.1017/inp.2018.22

27. Carey CJ, Beman JM, Eviner VT, Malmstrom CM, Hart SC. Soil microbial community structure is unaltered by plant invasion, vegetation clipping, and nitrogen fertilization in experimental semi-arid grasslands. Front Microbiol. 2015;6. https://doi.org/10.3389/fmicb.2015.00006 PMID: 25713560

28. Gibbons SM, Lekberg Y, Mummey DL, Sangwan N, Ramsey PW, Gilbert JA. Invasive Plants Rapidly Reshape Soil Properties in a Grassland Ecosystem. Shade A, editor. mSystems. 2017;2. https://doi.org/10.1128/mSystems.00178-16 PMID: 28289729

29. Gomes SIF, Merckx VSFT, Hynson NA. Biological invasions increase the richness of arbuscular mycorrhizal fungi from a Hawaiian subtropical ecosystem. Biol Invasions. 2018; 20: 2421–2437. https://doi.org/10.1007/s10530-018-1710-7 PMID: 30956539

30. de Menezes AB, Prendergast-Miller MT, Macdonald LM, Toscas P, Baker G, Farrell M, et al. Earthworm-induced shifts in microbial diversity in soils with rare versus established invasive earthworm populations. FEMS Microbiol Ecol. 2018;94. https://doi.org/10.1093/femsec/fiy051 PMID: 29579181

31. Bier RL, Bernhardt ES, Boot CM, Graham EB, Hall EK, Lennon JT, et al. Linking microbial community structure and microbial processes: an empirical and conceptual overview. Muyzer G, editor. FEMS Microbiol Ecol. 2015; 91: fiv113. https://doi.org/10.1093/femsec/fiv113 PMID: 26371074

32. Gibbons SM, Gilbert JA. Microbial diversity—exploration of natural ecosystems and microbiomes. Curr Opin Genet Dev. 2015; 35: 66–72. https://doi.org/10.1016/j.gde.2015.10.003 PMID: 26598941

33. Gurevitch J, Koricheva J, Nakagawa S, Stewart G. Meta-analysis and the science of research synthesis. Nature. 2018; 555: 175–182. https://doi.org/10.1038/nature25753 PMID: 29517004

34. Nakagawa S, Poulin R. Meta-analytic insights into evolutionary ecology: an introduction and synthesis. Evol Ecol. 2012; 26: 1085–1099. https://doi.org/10.1007/s10682-012-9593-z

35. Nakagawa S, Santos ESA. Methodological issues and advances in biological meta-analysis. Evol Ecol. 2012; 26: 1253–1274. https://doi.org/10.1007/s10682-012-9555-5

36. Senior AM, Grueber CE, Kamiya T, Lagisz M, O'Dwyer K, Santos ESA, et al. Heterogeneity in ecological and evolutionary meta-analyses: its magnitude and implications. Ecology. 2016; 97: 3293–3299. https://doi.org/10.1002/ecy.1591 PMID: 27912008

37. Lozupone CA, Stombaugh J, Gonzalez A, Ackermann G, Wendel D, Vazquez-Baeza Y, et al. Meta-analyses of studies of the human microbiota. Genome Res. 2013; 23: 1704–1714. https://doi.org/10.1101/gr.151803.112 PMID: 23861384

38. Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with obesity and IBD. FEBS Lett. 2014; 588: 4223–4233. https://doi.org/10.1016/j.febslet.2014.09.039 PMID: 25307765

39. Duvallet C, Gibbons SM, Gurry T, Irizarry RA, Alm EJ. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. Nat Commun. 2017; 8: 1784. https://doi.org/10.1038/s41467-017-01973-8 PMID: 29209090

40. Koren O, Knights D, Gonzalez A, Waldron L, Segata N, Knight R, et al. A Guide to Enterotypes across the Human Body: Meta-Analysis of Microbial Community Structures in Human Microbiome Datasets. Eisen JA, editor. PLoS Comput Biol. 2013; 9: e1002863. https://doi.org/10.1371/journal.pcbi.1002863 PMID: 23326225

41. Krych L, Hansen CHF, Hansen AK, van den Berg FWJ, Nielsen DS. Quantitatively Different, yet Qualitatively Alike: A Meta-Analysis of the Mouse Core Gut Microbiome with a View towards the Human Gut Microbiome. Bereswill S, editor. PLoS One. 2013; 8: e62578. https://doi.org/10.1371/journal.pone.0062578 PMID: 23658749

42. Wang J, Kurilshikov A, Radjabzadeh D, Turpin W, Croitoru K, Bonder MJ, et al. Meta-analysis of human genome-microbiome association studies: the MiBioGen consortium initiative. Microbiome. 2018; 6: 101. https://doi.org/10.1186/s40168-018-0479-3 PMID: 29880062

43. Jiao N, Baker SS, Nugent CA, Tsompana M, Cai L, Wang Y, et al. Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. Physiol Genomics. 2018; 50: 244–254. https://doi.org/10.1152/physiolgenomics.00114.2017 PMID: 29373083

44. Wirbel J, Pyl PT, Kartal E, Zych K, Kashani A, Milanese A, et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. Nat Med. 2019; 25: 679–689. https://doi.org/10.1038/s41591-019-0406-6 PMID: 30936547

45. Bisanz JE, Upadhyay V, Turnbaugh JA, Ly K, Turnbaugh PJ. Meta-Analysis Reveals Reproducible Gut Microbiome Alterations in Response to a High-Fat Diet. Cell Host Microbe. 2019; 26: 265-272.e4. https://doi.org/10.1016/j.chom.2019.06.013 PMID: 31324413

46. Adams RI, Bateman AC, Bik HM, Meadow JF. Microbiota of the indoor environment: a meta-analysis. Microbiome. 2015; 3: 49. https://doi.org/10.1186/s40168-015-0108-3 PMID: 26459172

47. Debroas D, Domaizon I, Humbert J-F, Jardillier L, Lepère C, Oudart A, et al. Overview of freshwater microbial eukaryotes diversity: a first analysis of publicly available metabarcoding data. FEMS Microbiol Ecol. 2017;93. https://doi.org/10.1093/femsec/fix023 PMID: 28334157

48. Shade A, Gregory Caporaso J, Handelsman J, Knight R, Fierer N. A meta-analysis of changes in bacterial and archaeal communities with time. ISME J. 2013; 7: 1493–1506. https://doi.org/10.1038/ismej.2013.54 PMID: 23575374

49. Rocca JD, Simonin M, Blaszczak JR, Ernakovich JG, Gibbons SM, Midani FS, et al. The Microbiome Stress Project: Toward a Global Meta-Analysis of Environmental Stressors and Their Effects on Microbial Communities. Front Microbiol. 2019;9. https://doi.org/10.3389/fmicb.2019.00009 PMID: 30713525

50. Duvallet C. Meta-analysis generates and prioritizes hypotheses for translational microbiome research. Microb Biotechnol. 2018; 11: 273–276. https://doi.org/10.1111/1751-7915.13047 PMID: 29349912

51. Drewes JL, White JR, Dejea CM, Fathi P, Iyadorai T, Vadivelu J, et al. High-resolution bacterial 16S rRNA gene profile meta-analysis and biofilm status reveal common colorectal cancer consortia. npj Biofilms Microbiomes. 2017; 3: 34. https://doi.org/10.1038/s41522-017-0040-3 PMID: 29214046

52. Ramirez KS, Knight CG, de Hollander M, Brearley FQ, Constantinides B, Cotton A, et al. Detecting macroecological patterns in bacterial communities across independent studies of global soils. Nat Microbiol. 2018; 3: 189–196. https://doi.org/10.1038/s41564-017-0062-x PMID: 29158606

53. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci. 2011; 108: 4516–4522. https://doi.org/10.1073/pnas.1000080107 PMID: 20534432

54. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013; 41: e1–e1. https://doi.org/10.1093/nar/gks808 PMID: 22933715

55. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011; 27: 2957–2963. https://doi.org/10.1093/bioinformatics/btr507 PMID: 21903629

56. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012; 6: 1621–1624. https://doi.org/10.1038/ismej.2012.8 PMID: 22402401

57. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016; 4: e2584. https://doi.org/10.7717/peerj.2584 PMID: 27781170

58. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2012; 41: D590–D596. https://doi.org/10.1093/nar/gks1219 PMID: 23193283

59. Katoh K, Standley DM. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol Biol Evol. 2013; 30: 772–780. https://doi.org/10.1093/molbev/mst010 PMID: 23329690

60. Price MN, Dehal PS, Arkin AP. FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. Mol Biol Evol. 2009; 26: 1641–1650. https://doi.org/10.1093/molbev/msp077 PMID: 19377059

61. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2016. 2017.

62. McMurdie PJ, Holmes S. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. Watson M, editor. PLoS One. 2013; 8: e61217. https://doi.org/10.1371/journal.pone.0061217 PMID: 23630581

63. Dixon P. VEGAN, a package of R functions for community ecology. J Veg Sci. 2003; 14: 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

64. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014; 15: 550. https://doi.org/10.1186/s13059-014-0550-8 PMID: 25516281

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

66. Delgado-Baquerizo M, Maestre FT, Reich PB, Jeffries TC, Gaitan JJ, Encinar D, et al. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat Commun. 2016; 7: 10541. https://doi.org/10.1038/ncomms10541 PMID: 26817514

67. Miki T, Yokokawa T, Matsui K. Biodiversity and multifunctionality in a microbial community: a novel theoretical approach to quantify functional redundancy. Proc R Soc B Biol Sci. 2014; 281: 20132498. https://doi.org/10.1098/rspb.2013.2498 PMID: 24352945

68. Philippot L, Spor A, Hénault C, Bru D, Bizouard F, Jones CM, et al. Loss in microbial diversity affects nitrogen cycling in soil. ISME J. 2013; 7: 1609–1619. https://doi.org/10.1038/ismej.2013.34 PMID: 23466702

69. Mendes LW, Tsai SM, Navarrete AA, de Hollander M, van Veen JA, Kuramae EE. Soil-Borne Microbiome: Linking Diversity to Function. Microb Ecol. 2015; 70: 255–265. https://doi.org/10.1007/s00248-014-0559-2 PMID: 25586384

70. Shi Y, Delgado-Baquerizo M, Li Y, Yang Y, Zhu Y-G, Peñuelas J, et al. Abundance of kinless hubs within soil microbial networks are associated with high functional potential in agricultural ecosystems. Environ Int. 2020; 142: 105869. https://doi.org/10.1016/j.envint.2020.105869 PMID: 32593837

71. Chen J, Mo L, Zhang Z, Nan J, Xu D, Chao L, et al. Evaluation of the ecological restoration of a coal mine dump by exploring the characteristics of microbial communities. Appl Soil Ecol. 2020; 147: 103430. https://doi.org/10.1016/j.apsoil.2019.103430

72. Cheng J, Chen Y, He T, Liao R, Liu R, Yi M, et al. Soil nitrogen leaching decreases as biogas slurry DOC/N ratio increases. Appl Soil Ecol. 2017; 111: 105–113. https://doi.org/10.1016/j.apsoil.2016.12.001

73. Warneke C, Karl T, Judmaier H, Hansel A, Jordan A, Lindinger W, et al. Acetone, methanol, and other partially oxidized volatile organic emissions from dead plant matter by abiological processes: Significance for atmospheric HO x chemistry. Global Biogeochem Cycles. 1999; 13: 9–17. https://doi.org/10.1029/98GB02428

74. Kämpfer P, Lodders N, Falsen E. Hydrotalea flava gen. nov., sp. nov., a new member of the phylum Bacteroidetes and allocation of the genera Chitinophaga, Sediminibacterium, Lacibacter, Flavihumibacter, Flavisolibacter, Niabella, Niastella, Segetibacter, Parasegetibacter, Terrimonas, Fer. Int J Syst Evol Microbiol. 2011; 61: 518–523. https://doi.org/10.1099/ijs.0.023002-0 PMID: 20382796

75. Madhaiyan M, Poonguzhali S, Senthilkumar M, Pragatheswari D, Lee J-S, Lee K-C. Arachidicoccus rhizosphaerae gen. nov., sp. nov., a plant-growth-promoting bacterium in the family Chitinophagaceae isolated from rhizosphere soil. Int J Syst Evol Microbiol. 2015; 65: 578–586. https://doi.org/10.1099/ijs.0.069377-0 PMID: 25404481

76. Reis VM, Teixeira KR dos S. Nitrogen fixing bacteria in the family Acetobacteraceae and their role in agriculture. J Basic Microbiol. 2015; 55: 931–949. https://doi.org/10.1002/jobm.201400898 PMID: 25736602

77. Trujillo ME, Bacigalupe R, Pujic P, Igarashi Y, Benito P, Riesco R, et al. Genome Features of the Endophytic Actinobacterium Micromonospora lupini Strain Lupac 08: On the Process of Adaptation to an Endophytic Life Style? Brüggemann H, editor. PLoS One. 2014; 9: e108522. https://doi.org/10.1371/journal.pone.0108522 PMID: 25268993

78. Carro L, Nouioui I, Sangal V, Meier-Kolthoff JP, Trujillo ME, Montero-Calasanz M del C, et al. Genome-based classification of micromonosporae with a focus on their biotechnological and ecological potential. Sci Rep. 2018; 8: 525. https://doi.org/10.1038/s41598-017-17392-0 PMID: 29323202

79. Dedysh SN, Ivanova AA. Planctomycetes in boreal and subarctic wetlands: diversity patterns and potential ecological functions. FEMS Microbiol Ecol. 2019;95. https://doi.org/10.1093/femsec/fiy227 PMID: 30476049

80. Wegner C-E, Gorniak L, Riedel S, Westermann M, Küsel K. Lanthanide-Dependent Methylotrophs of the Family Beijerinckiaceae: Physiological and Genomic Insights. Stams AJM, editor. Appl Environ Microbiol. 2019;86. https://doi.org/10.1128/AEM.01830-19 PMID: 31604774

81. Neufeld JD, Schäfer H, Cox MJ, Boden R, McDonald IR, Murrell JC. Stable-isotope probing implicates Methylophaga spp and novel Gammaproteobacteria in marine methanol and methylamine metabolism. ISME J. 2007; 1: 480–491. https://doi.org/10.1038/ismej.2007.65 PMID: 18043650

82. Miao L, Wang P, Hou J, Yao Y, Liu Z, Liu S, et al. Distinct community structure and microbial functions of biofilms colonizing microplastics. Sci Total Environ. 2019; 650: 2395–2402. https://doi.org/10.1016/j.scitotenv.2018.09.378 PMID: 30292995

83. Navarrete AA, Venturini AM, Meyer KM, Klein AM, Tiedje JM, Bohannan BJM, et al. Differential Response of Acidobacteria Subgroups to Forest-to-Pasture Conversion and Their Biogeographic Patterns in the Western Brazilian Amazon. Front Microbiol. 2015;6. https://doi.org/10.3389/fmicb.2015.00006 PMID: 25713560

84. Doblas-Miranda E, Martínez-Vilalta J, Lloret F, Álvarez A, Ávila A, Bonet FJ, et al. Reassessing global change research priorities in mediterranean terrestrial ecosystems: how far have we come and where do we go from here? Glob Ecol Biogeogr. 2015; 24: 25–43. https://doi.org/10.1111/geb.12224