Biological invasions alter environmental microbiomes: a meta-analysis

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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], *Vincetoxicum rossicum* [26] did not alter the structure of soil microbial communities. Also, 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 still 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 (Fig S1). 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 (Tab. 1), summing up to a total of 356 samples. Three studies focused on invasive plants, and the remaining studies focused on a mammal and a mussel (Tab. 1). The invasive mussel study [22] was the only study performed in an aquatic environment, while the remaining studies examined soil environments.

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]

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]. Although there might be small differences between them, they overlap in the V4 region of 16S gene (Fig. S2), so 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 directly downloading files from the MG-RAST database.

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 with *studyID* and *environment* (soil or water) included as random factors. 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 stratified at level of *studyID*, *environment* and *identity of invasive species*). 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 single taxa, with *studyID*, *organism* (plant, mammal, mussel) and *environment* included as random factors.

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. Although the number of OTUs might seem high, it is important to consider that we are analyzing samples across multiple environments (soil and water) and from different geographical regions which we would expect to increase richness.

Biological invasions led to a reduction in Shannon diversity (χ^2= 3.85, df=1, P=0.04, Fig 1A). We also found biological invasions altered microbiome community composition in the invaded environment compared to the control (Tab. 2, Fig 1B). 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 (Fig. 1C, Table S2) 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) and *environment* (soil or water) were 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	0.02

Fig 1. (A) Comparison of Shannon index between control and invaded environments. (B) RDA ordination using a Bray-Curtis distance matrix of samples. (C) Comparison of the relative abundance of microbial classes between control and invaded environments. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Discussion

We show biological invasions decrease the diversity of environmental microbiomes. Thus, understanding the impact of biological invasions on environmental microbiomes is of high priority if we want to preserve ecosystem functions [66]. 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]. But the majority of studies did not analyze the microbial diversity, because they used techniques that did not allow 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. Microbial diversity is tied to the function of microbiomes, and changes in diversity can reflect changes in function [67–69]. Changes in microbial diversity and function do not always have the same direction [70] and this might explain the discrepancy between our results and other studies.

In contrast to diversity, 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 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

207 have diverse ecological functions ranging from nitrogen fixation and carbohydrate metabolism
208 to antimicrobial properties. Many of the families that showed a significant difference between
209 invaded and control environments have species members that play important roles at various
210 points during nitrogen and carbon cycling (i.e. Nitrosomonadaceae, Acetobacteraceae,
211 Chitinophagaceae, Micromonosporaceae, Gemmataceae, Beijerinckiaceae, Pirellulaceae) [71–
212 81]. However, nitrogen fixing and carbohydrate degrading bacteria did not have a unified
213 response to invaded environments as some increased and others decreased in abundance in
214 invaded environments. Many nitrogen fixing bacteria have been shown to respond to
215 environmental disturbance, such as Acidobacteria abundances during forest to pasture
216 conversions or Pirellulaceae's response to the presence of microplastics [82,83]. Thus, changes
217 in environmental microbiome community composition appear to be linked to changes in
218 ecosystem functions, although this pattern is not yet predictable for all functions and taxa.
219 Few previous studies on biological invasions have reported details on the differential
220 abundance of taxa, and among these we found limited general consensus. For example, some
221 studies report a decrease in abundance of bacteria associated with nitrogen cycling (e.g.
222 Nitrosphaeria, Nitrospira, Nitrosomonadales) [13,14,19], while others report an increase of
223 Nitrosomonadaceae following invasion [30]. In our study some groups associated with the
224 nitrogen cycle were positively associated with biological invasions (i.e. Nitrosomonadaceae,
225 Pirellulaceae, Chitinophagaceae) while others were negatively associated (Beijerinckiaceae,
226 Micromonosporaceae). Unfortunately, amplicon-based sequencing has a limited power to infer
227 changes in the functions of microbiomes. Future metagenomic and metatranscriptomic studies
228 are needed to investigate whether biological invasions alter gene content or gene expression of
229 environmental microbiomes, and whether this reflects changes in biogeochemical cycling.
230 Meta-analyses are also useful to highlight gaps in the literature, and here we highlight
231 some aspects that warrant further investigation. We identified a large gap in the availability of
232 sequencing data from multiple types of environments and types of invasive species. For our
233 analysis almost all available data came from two environments: four sets of data came from soil
234 and one came from freshwater. Greater effort is needed for sample collection from invasions in
235 both freshwater and marine environments. Without sufficient diversity of sample
236 environments, it is impossible to tell whether microbial shifts following an invasion are unique
237 to an invaded environment. Second, in our analysis the majority of data came from one type of
238 invasive species: plants. Noticeably absent from our dataset were invasions by insects, fish, and
239 amphibians. Sequencing data is needed from a larger number of invasive species to allow us to
240 broadly assess shifts in microbial community structure. A third gap we identified was the lack of
241 spatial and temporal resolution. Almost all of the initially identified 22 studies we assessed
242 were also restricted to one season of sampling and were conducted in the Northern
243 Hemisphere. Thus, it is impossible to validate the influence of latitude with existing datasets or
244 explore how seasonality and biological invasions interact to modulate microbial communities.
245 Thus, there are a number of opportunities for future research on how biological invasions alter
246 environmental microbial communities.
247 Here we analyzed 16S amplicon sequencing data from five studies and show that
248 biological invasions influence both the diversity and the structure of environmental
249 microbiomes. We identified a number of gaps in our knowledge, including the need to assess a
250 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.

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Data accessibility

Raw data from single studies is publicly available. The code used to perform our analyses has been deposited on GitHub: https://github.com/amalacrino/Biol_Invasion_Microb_MA

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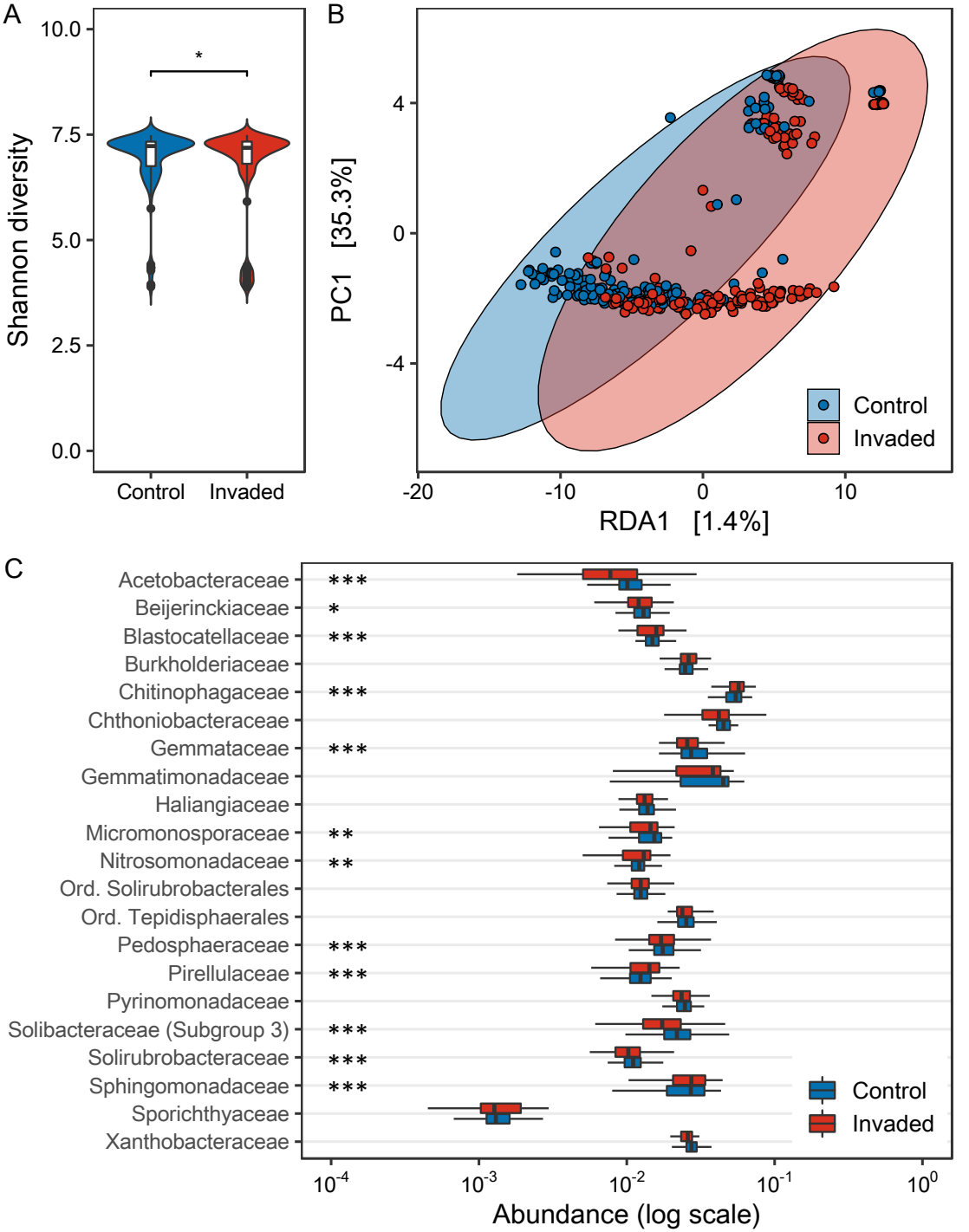
A
Shannon diversity
10.0
7.5
5.0
2.5
0.0
*
Control
Invaded
B
PC1 [35.3%]
4
0
-4
-20
-10
0
10
RDA1 [1.4%]
Control
Invaded
C
Acetobacteraceae ***
Beijerinckiaceae *
Blastocatellaceae ***
Burkholderiaceae
Chitinophagaceae ***
Chthoniobacteraceae
Gemmataceae ***
Gemmatimonadaceae
Haliangiaceae
Micromonosporaceae **
Nitrosomonadaceae **
Ord. Solirubrobacterales
Ord. Tepidisphaerales
Pedosphaeraceae ***
Pirellulaceae ***
Pyrinomonadaceae
Solibacteraceae (Subgroup 3) ***
Solirubrobacteraceae ***
Sphingomonadaceae ***
Sporichthyaceae
Xanthobacteraceae
Control
Invaded
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
Abundance (log scale)