Biological invasions alter environmental microbiomes: a meta-analysis

Antonino Malacrinò[1,*], Victoria A. Sadowski[1], Tvisha K. Martin[2], Nathalia Cavichiolli de Oliveira[3], Ian J. Brackett[1], James D. Feller[1], Kristian J. Harris[1], Orlando Combita Heredia[1], Rosa Vescio[1,4], Alison E. Bennett[1]

[1] Department of Evolution, Ecology and Organismal Biology, The Ohio State University, Columbus (OH)

[2] College of Food, Agricultural and Environmental Sciences, The Ohio State University, Columbus (OH)

[3] Department of Entomology, University of Sao Paulo, Piracicaba (SP), Brazil

[4] Dipartimento AGRARIA, Università degli Studi Mediterranea, Reggio Calabria, Italy

*Corresponding author.

Email: malacrino.1@osu.edu; antonino.malacrino@gmail.com (AM)

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

38 functioning, we are still learning the consequences of biological invasions on environmental
39 microbiomes.

40 Previous studies have shown biological invasions can impact the diversity and
41 taxonomical structure of environmental microbiomes. For example, we often see a shift in soil
42 microbiota following invasion by non-native plant species [9,10,19,11–18]. Removal of feral pigs
43 increased the diversity of soil bacterial communities and shifted their structure [20], and
44 invasive crustaceans [21], mussels [22] and jellyfish [23] produce changes in the structure of
45 water microbiomes. However, shifts in environmental microbiome as consequence of biological
46 invasions do not always occur. For example, invasion by the plants *Robinia pseudoacacia* [24],
47 *Eucalyptus* sp. [25], and *Vincetoxicum rossicum* [26] did not alter the structure of soil microbial
48 communities. Also, some microcosms exposed to the simultaneous invasion of multiple plant
49 species [27–29] did not alter soil microbiomes. Similarly, soil microbiome structure in
50 microcosms did not change with the addition of the invasive earthworm *Aporrectodea*
51 *trapezoides* [30]. Several of these studies used techniques (e.g. DGGE, PLFA, t-RFLP) that limit
52 fine scale investigations of biological invasions on environmental microbiome diversity and
53 taxonomical composition. Among the studies using high-throughput amplicon-sequencing
54 techniques, most did not find changes in microbiome diversity [12,14,17,18,21,25,27,28,30],
55 few reported a decrease of microbial diversity in response to invasion [15,20,29], and fewer still
56 reported an increase [13,19]. Thus, there is still little consensus on the effects of biological
57 invasions on the diversity and taxonomical structure of the environmental microbiomes, both
58 tied to the stability and function of microbial communities [31,32].

59 Our ability to draw broad conclusions from published studies is limited, because
60 individual studies have occurred within a limited geographical range or with a limited group of
61 species. Meta-analyses of published biological means have long enabled more robust
62 conclusions than individual studies [33–36]. However, the meta-analytic approach has less
63 frequently been applied to amplicon-sequencing data that represent environmental
64 microbiome community composition. The majority of meta-analytic metabarcoding studies
65 have occurred in the medical sciences [37–45]. This approach can be successfully used to
66 address ecological questions. For example, meta-analytic metabarcoding studies have found
67 common patterns in the structure of indoor microbiomes [46] and freshwater eukaryotes [47].
68 Shade et al. [48] also used a meta-analysis of metabarcoding datasets from different
69 environments highlighting a time-dependent structure of microbiomes. A meta-analytic
70 approach has also been used to test the effects of stressors (e.g. water availability,
71 temperature, heavy metals) on environmental microbiomes [49]. Thus meta-analyses on
72 microbiome data have a striking potential to address global-scale questions, generate new
73 hypotheses and model common patterns [50], because they provide across study comparisons
74 [39,51,52].

75 Here, we aim to test whether the effect of biological invasion on environmental
76 microbiomes can be generalized or is idiosyncratic. To do so, we collected publicly available
77 data and re-analyzed this data under a common framework. We tested the effect of invasive
78 species on the diversity and structure of environmental microbiomes, with the hypothesis that
79 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 Figure). 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. 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. [18] in field condition. They identified three locations invaded by three different exotic plant species

101 (*Microstegium vimineum*, *Rhamnus davurica* and *Ailanthus altissima*) and, within each location,
102 they sampled soil form an invaded area and a non-invaded area for comparisons. Similarly,
103 Collins et al. [19] compared the soil microbial community of field sites invaded by *Artemisia*
104 *rothrockii* to non-invaded sites. The study by Wehr et al. [20] focused on the effects of feral pig
105 (*Sus scrofa*) invasion on soil microbiome comparing invaded areas to those where pigs were
106 removed over a ~25 year chronosequence. Finally, the only study performed in an aquatic
107 environment [22] compared water samples collected in lake areas invaded by the exotic mussel
108 *Dreissena bugensis* to non-invaded sites. Three studies focused on invasive plants, and the
109 remaining studies focused on a mammal and a mussel (Tab. 1).

110 **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. [18]
PRJNA320310	Plant	*Artemisia rothrockii*	Soil	Collins et al.

				[19]
PRJNA385848	Mussel	*Dreissena bugensis*	Water	Denef et al. [22]

111

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

126 Once the papers were selected, we assigned each a "Study ID" and collected meta-data
127 from each sample in each study (invasive species, type of organism, invaded environment). We
128 then downloaded data from repositories using SRA Toolkit 2.10.4 for data on the SRA
129 databases, or by manually 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. 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 obtaining similar results: 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 (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, S4 Table) revealed that some

173 taxonomic groups are significantly more abundant in invaded environments (Blastocatellaceae,
174 Chitinophagaceae, Nitrosomonadaceae, Pirellulaceae, Sphingomonadaceae), while others are
175 more abundant in non-invaded samples (Acetobacteraceae, Beijerinckiaceae, Gemmataceae,
176 Micromonosporaceae, Pedosphaeraceae, Solibacteraceae, Solirubrobacteraceae).
177
178 **Table 2**. Results from PERMANOVA analysis testing the effects of *sample type*
179 (invaded/control), *organism group* (plant, mammal, mussel) and their interaction on microbial
180 community composition. The factors *studyID* (unique for each study) was used as strata to
181 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

182
183
184 **Fig 1**. (A) Comparison of Shannon index between control and invaded environments.
185 Data is represented as violin plot (showing the probability distribution of Shannon index for
186 each group) with a boxplot inside each violin plot indicating median and interquartile range. (B)
187 RDA ordination using a Bray-Curtis distance matrix of samples. (C) Comparison of the relative
188 abundance of microbial classes between control and invaded environments. * $P<0.05$, **
189 $P<0.01$, *** $P<0.001$

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 [13,19], while others reported a decrease [15,20,29]. 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 [9,10,23–28,30,11,12,14,16–18,21,22]. 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.

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]

212 considered in our analysis. This variation may be due to individual effects of organisms on the
213 environment. For example, invasive plants may alter soil microbiome composition through root
214 exudates [5], and invasive mussels may alter water microbiome composition via bacterial
215 removal through their feeding activity [22]. Thus, reported influences on community
216 composition are more consistent. Alternatively changes in community composition might be
217 due to the response of some bacterial groups to environmental disturbance. The bacterial
218 families that we found to be differentially abundant between the invaded and control
219 environments have diverse ecological functions ranging from nitrogen fixation and
220 carbohydrate metabolism to antimicrobial properties. Many of the families that showed a
221 significant difference in abundance between invaded and control environments include taxa
222 that play important roles at various points during nitrogen and carbon cycling (i.e.
223 Nitrosomonadaceae, Acetobacteraceae, Chitinophagaceae, Micromonosporaceae,
224 Gemmataceae, Beijerinckiaceae, Pirellulaceae) [70,71,80,72–79]. However, nitrogen-fixing and
225 carbohydrate-degrading bacteria did not have a unified response to invaded environments as
226 some increased and others decreased in abundance in invaded environments. Many nitrogen-
227 fixing bacteria have been shown to respond to environmental disturbance, such as
228 Acidobacteria abundances during forest to pasture conversions or Pirellulaceae's response to
229 the presence of microplastics [81,82]. Thus, changes in environmental microbiome community
230 composition appear to be linked to changes in ecosystem functions, although this pattern is not
231 yet predictable across functions and taxa.

232 Few previous studies on biological invasions have reported details on the differential
233 abundance of taxa, and among these we found limited general consensus. For example, some

234 studies report a decrease in abundance of bacteria associated with nitrogen cycling (e.g.
235 Nitrosphaeria, Nitrospira, Nitrosomonadales) [12,13,18], while others report an increase of
236 Nitrosomonadaceae following invasion [30]. In our study some groups associated with the
237 nitrogen cycle were positively associated with biological invasions (i.e. Nitrosomonadaceae,
238 Pirellulaceae, Chitinophagaceae) while others were negatively associated (Beijerinckiaceae,
239 Micromonosporaceae). Unfortunately, amplicon-based sequencing has a limited power to infer
240 changes in the functions of microbiomes. Future metagenomic and metatranscriptomic studies
241 are needed to investigate whether biological invasions alter gene content or gene expression of
242 environmental microbiomes, and whether this reflects changes in biogeochemical cycling.

243 Meta-analyses are also useful to highlight gaps in the literature, and here we highlight
244 some aspects that warrant further investigation. First, we identified a large gap in the
245 availability of sequencing data from multiple types of environments and types of invasive
246 species. For our analysis almost all available data came from two environments: four sets of
247 data came from soil and one came from freshwater. Greater effort is needed for sample
248 collection from invasions in both freshwater and marine environments. Without sufficient
249 diversity of sample environments, it is impossible to tell whether microbial shifts following an
250 invasion are unique to an invaded environment. Second, in our analysis the majority of data
251 came from one type of invasive species: plants. Noticeably absent from our dataset were
252 invasions by insects, fish, and amphibians. Sequencing data is needed from a larger number of
253 invasive species to allow us to broadly assess shifts in microbial community structure. A third
254 gap we identified was the lack of spatial and temporal resolution. Almost all of the initially
255 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 [83]. 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.

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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

Supporting information

S1 Figure. 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

S2 Figure. Primers pairs considered in our analysis mapped to the structure of the 16S rRNA gene that includes here just the hypervariable regions V1–V4.

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

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

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.

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

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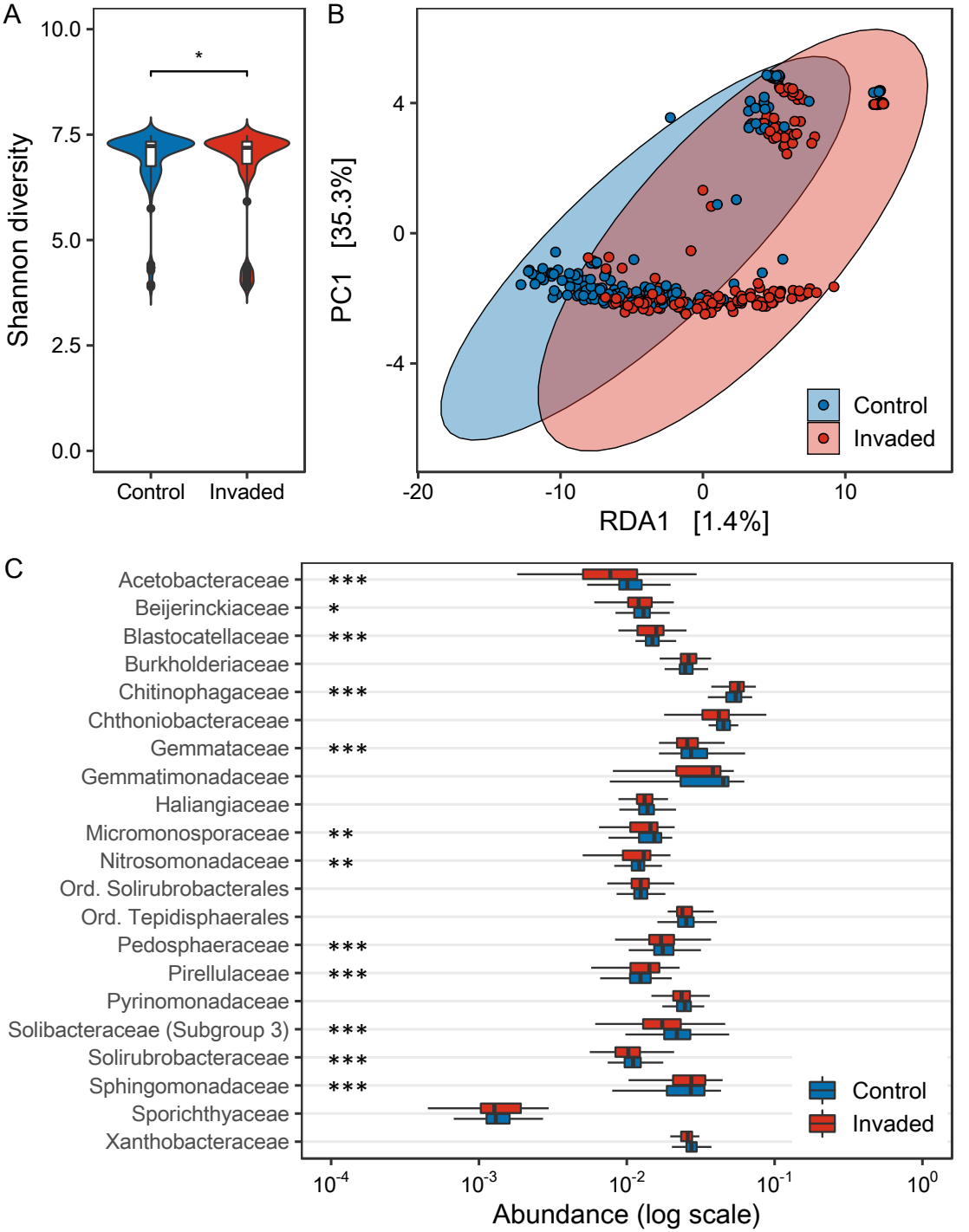
A
Shannon diversity
10.0
7.5
5.0
2.5
0.0
*
Control
Invaded
B
PC1 [35.3%]
4
0
-4
-20
-10
0
10
RDA1 [1.4%]
Control
Invaded
C
Acetobacteraceae ***
Beijerinckiaceae *
Blastocatellaceae ***
Burkholderiaceae
Chitinophagaceae ***
Chthoniobacteraceae
Gemmataceae ***
Gemmatimonadaceae
Haliangiaceae
Micromonosporaceae **
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)