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 (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 (Tab. 2, 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).

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

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

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

190 environmental microbiomes and systems. Previous studies have found that invasive species
191 increased environmental microbial diversity [13,19], while others reported a decrease
192 [15,20,29]. However, the majority of studies did not analyze the microbial diversity, as they
193 used techniques that did not allow for such analysis, or reported no changes [9,10,23–
194 28,30,11,12,14,16–18,21,22]. Within the studies included in our analysis, invasion by feral pigs
195 decreased soil microbial diversity, while invasion by *Artemisia rothrockii* increased soil microbial
196 diversity. The remaining three studies in our analysis reported no effects of biological invasions
197 on environmental microbiome diversity. Our analysis was constrained in terms of sampled
198 environment (soil) and invasive organism (plants), and an expanded dataset would be beneficial
199 to generalize our results. Microbial diversity is tied to the function of microbiomes, and changes
200 in diversity can reflect changes in function [66–68]. Changes in microbial diversity and function
201 do not always have the same direction [69], which might explain the discrepancy between our
202 results and other studies. While we observed a relatively small reduction of the Shannon
203 diversity index in invaded environments compared to non-invaded environment, this matches
204 with the changes we observed in terms of community composition.

205 Indeed, our report of changes in community composition was relatively consistent with
206 the published literature and the individual results of the studies we analyzed. Most studies of
207 the influence of biological invasions on environmental microbiomes found that biological
208 invasions alter environmental microbial community composition. However, some previous
209 reports did not report changes [24–30], including the study by Gibbons et al. [28] considered in
210 our analysis. This variation may be due to individual effects of organisms on the environment.
211 For example, invasive plants may alter soil microbiome composition through root exudates [5],

212 and invasive mussels may alter water microbiome composition via bacterial removal through
213 their feeding activity [22]. Thus, reported influences on community composition are more
214 consistent. Alternatively changes in community composition might be due to the response of
215 some bacterial groups to environmental disturbance. The bacterial families that we found to be
216 differentially abundant between the invaded and control environments have diverse ecological
217 functions ranging from nitrogen fixation and carbohydrate metabolism to antimicrobial
218 properties. Although the differences in relative abundance we found might be relatively small,
219 they can have an important impact on the functions of the environmental microbiome [70].
220 Many of the families that showed a significant difference in abundance between invaded and
221 control environments include taxa that play important roles at various points during nitrogen
222 and carbon cycling (i.e. Nitrosomonadaceae, Acetobacteraceae, Chitinophagaceae,
223 Micromonosporaceae, Gemmataceae, Beijerinckiaceae, Pirellulaceae) [71,72,81,73–80].
224 However, nitrogen-fixing and carbohydrate-degrading bacteria did not have a unified response
225 to invaded environments as some increased and others decreased in abundance in invaded
226 environments. Many nitrogen-fixing bacteria have been shown to respond to environmental
227 disturbance, such as Acidobacteria abundances during forest to pasture conversions or
228 Pirellulaceae's response to the presence of microplastics [82,83]. Thus, changes in
229 environmental microbiome community composition appear to be linked to changes in
230 ecosystem functions, although this pattern is not yet predictable across functions and taxa.

231 Few previous studies on biological invasions have reported details on the differential
232 abundance of taxa, and among these we found limited general consensus. For example, some
233 studies report a decrease in abundance of bacteria associated with nitrogen cycling (e.g.

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

242 Meta-analyses are also useful to highlight gaps in the literature, and here we highlight
243 some aspects that warrant further investigation. First, we identified a large gap in the
244 availability of sequencing data from multiple types of environments and types of invasive
245 species. For our analysis almost all available data came from two environments: four sets of
246 data came from soil and one came from freshwater. Greater effort is needed for sample
247 collection from invasions in both freshwater and marine environments. Without sufficient
248 diversity of sample environments, it is impossible to tell whether microbial shifts following an
249 invasion are unique to an invaded environment. Second, in our analysis the majority of data
250 came from one type of invasive species: plants. Noticeably absent from our dataset were
251 invasions by insects, fish, and amphibians. Sequencing data is needed from a larger number of
252 invasive species to allow us to broadly assess shifts in microbial community structure. A third
253 gap we identified was the lack of spatial and temporal resolution. Almost all of the initially
254 identified 22 studies we assessed were also restricted to one season of sampling and were
255 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.

Acknowledgements

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.

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.

297 **S4 Table.** Results from two different the linear mixed-effects model testing the
298 abundance of each bacterial family against *sample type* (invaded or control), *organism* (plant,
299 mammal, mussel), and their interactions. In Model 1 we included *studyID* as random factor,
300 while in Model 2 we included both *studyID* and *environment* (soil or water) as random factors.
301
302 **S5 Table.** Comparison of the relative proportion of each bacterial family between
303 control and invaded environments. Differences are assessed using a linear mixed-effects model
304 testing the normalized proportions of each bacterial family against *sample type* (Model 1 in S4
305 Table).

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. doi:10.1038/35012241
2. Pejchar L, Mooney HA. Invasive species, ecosystem services and human well-being. Trends Ecol Evol. 2009;24: 497–504. doi:10.1016/j.tree.2009.03.016
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. doi:10.1016/j.jenvman.2018.05.007
4. Charles H, Dukes JS. Impacts of Invasive Species on Ecosystem Services. Biological Invasions. Berlin, Heidelberg: Springer Berlin Heidelberg; 2008. pp. 217–237. doi: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. doi:10.1007/s10886-009-9735-0
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. doi:10.1111/nph.14115
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. doi:10.1007/s11258-012-0112-7
8. Hall Jr RO, Tank JL, Dybdahl MF. Exotic snails dominate nitrogen and carbon cycling in a

328 highly productive stream. Front Ecol Environ. 2003;1: 407–411. doi:10.1890/1540-
329 9295(2003)001[0407:ESDNAC]2.0.CO;2
330 9. Lazzaro L, Giuliani C, Fabiani A, Agnelli AE, Pastorelli R, Lagomarsino A, et al. Soil and
331 plant changing after invasion: The case of Acacia dealbata in a Mediterranean ecosystem.
332 Sci Total Environ. 2014;497–498: 491–498. doi:10.1016/j.scitotenv.2014.08.014
333 10. Malinich E, Lynn-Bell N, Kourtev PS. The effect of the invasive Elaeagnus umbellata on
334 soil microbial communities depends on proximity of soils to plants. Ecosphere. 2017;8:
335 e01827. doi:10.1002/ecs2.1827
336 11. Lorenzo P, Pereira CS, Rodríguez-Echeverría S. Differential impact on soil microbes of
337 allelopathic compounds released by the invasive Acacia dealbata Link. Soil Biol Biochem.
338 2013;57: 156–163. doi:10.1016/j.soilbio.2012.08.018
339 12. Parsons LS, Sayre J, Ender C, Rodrigues JLM, Barberán A. Soil microbial communities in
340 restored and unrestored coastal dune ecosystems in California. Restor Ecol. 2020;
341 rec.13101. doi:10.1111/rec.13101
342 13. Piper CL, Siciliano SD, Winsley T, Lamb EG. Smooth brome invasion increases rare soil
343 bacterial species prevalence, bacterial species richness and evenness. De Deyn G, editor.
344 J Ecol. 2015;103: 386–396. doi:10.1111/1365-2745.12356
345 14. Zhang H-Y, Goncalves P, Copeland E, Qi S-S, Dai Z-C, Li G-L, et al. Invasion by the weed
346 Conyza canadensis alters soil nutrient supply and shifts microbiota structure. Soil Biol
347 Biochem. 2020;143: 107739. doi:10.1016/j.soilbio.2020.107739
348 15. Le Roux JJ, Ellis AG, van Zyl L-M, Hosking ND, Keet J-H, Yannelli FA. Importance of soil
349 legacy effects and successful mutualistic interactions during Australian acacia invasions in

350 nutrient-poor environments. Aerts R, editor. J Ecol. 2018;106: 2071–2081.
351 doi:10.1111/1365-2745.12965
352 16. Brunel C, Beifen Y, Pouteau R, Li J, van Kleunen M. Responses of Rhizospheric Microbial
353 Communities of Native and Alien Plant Species to Cuscuta Parasitism. Microb Ecol.
354 2020;79: 617–630. doi:10.1007/s00248-019-01438-z
355 17. Pickett B, Irvine IC, Bullock E, Arogyaswamy K, Aronson E. Legacy effects of invasive grass
356 impact soil microbes and native shrub growth. Invasive Plant Sci Manag. 2019;12: 22–35.
357 doi:10.1017/inp.2018.32
358 18. Rodrigues RR, Pineda RP, Barney JN, Nilsen ET, Barrett JE, Williams MA. Plant Invasions
359 Associated with Change in Root-Zone Microbial Community Structure and Diversity. Liu J,
360 editor. PLoS One. 2015;10: e0141424. doi:10.1371/journal.pone.0141424
361 19. Collins CG, Carey CJ, Aronson EL, Kopp CW, Diez JM. Direct and indirect effects of native
362 range expansion on soil microbial community structure and function. Austin A, editor. J
363 Ecol. 2016;104: 1271–1283. doi:10.1111/1365-2745.12616
364 20. Wehr NH, Kinney KM, Nguyen NH, Giardina CP, Litton CM. Changes in soil bacterial
365 community diversity following the removal of invasive feral pigs from a Hawaiian tropical
366 montane wet forest. Sci Rep. 2019;9: 14681. doi:10.1038/s41598-019-48922-7
367 21. Boeker C, Geist J. Effects of invasive and indigenous amphipods on physico-chemical and
368 microbial properties in freshwater substrates. Aquat Ecol. 2015;49: 467–480.
369 doi:10.1007/s10452-015-9539-y
370 22. Denef VJ, Carrick HJ, Cavaletto J, Chiang E, Johengen TH, Vanderploeg HA. Lake Bacterial
371 Assemblage Composition Is Sensitive to Biological Disturbance Caused by an Invasive

372 Filter Feeder. Kent AD, editor. mSphere. 2017;2. doi:10.1128/mSphere.00189-17

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

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

381 25. Hernández-Gómez O, Byrne AQ, Gunderson AR, Jenkinson TS, Noss CF, Rothstein AP, et
382 al. Invasive vegetation affects amphibian skin microbiota and body condition. PeerJ.
383 2020;8: e8549. doi:10.7717/peerj.8549

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

387 27. Carey CJ, Beman JM, Eviner VT, Malmstrom CM, Hart SC. Soil microbial community
388 structure is unaltered by plant invasion, vegetation clipping, and nitrogen fertilization in
389 experimental semi-arid grasslands. Front Microbiol. 2015;6.
390 doi:10.3389/fmicb.2015.00466

391 28. Gibbons SM, Lekberg Y, Mummey DL, Sangwan N, Ramsey PW, Gilbert JA. Invasive Plants
392 Rapidly Reshape Soil Properties in a Grassland Ecosystem. Shade A, editor. mSystems.
393 2017;2. doi:10.1128/mSystems.00178-16

394 29. Gomes SIF, Merckx VSFT, Hynson NA. Biological invasions increase the richness of
395 arbuscular mycorrhizal fungi from a Hawaiian subtropical ecosystem. Biol Invasions.
396 2018;20: 2421–2437. doi:10.1007/s10530-018-1710-7
397 30. de Menezes AB, Prendergast-Miller MT, Macdonald LM, Toscas P, Baker G, Farrell M, et
398 al. Earthworm-induced shifts in microbial diversity in soils with rare versus established
399 invasive earthworm populations. FEMS Microbiol Ecol. 2018;94.
400 doi:10.1093/femsec/fiy051
401 31. Bier RL, Bernhardt ES, Boot CM, Graham EB, Hall EK, Lennon JT, et al. Linking microbial
402 community structure and microbial processes: an empirical and conceptual overview.
403 Muyzer G, editor. FEMS Microbiol Ecol. 2015;91: fiv113. doi:10.1093/femsec/fiv113
404 32. Gibbons SM, Gilbert JA. Microbial diversity — exploration of natural ecosystems and
405 microbiomes. Curr Opin Genet Dev. 2015;35: 66–72. doi:10.1016/j.gde.2015.10.003
406 33. Gurevitch J, Koricheva J, Nakagawa S, Stewart G. Meta-analysis and the science of
407 research synthesis. Nature. 2018;555: 175–182. doi:10.1038/nature25753
408 34. Nakagawa S, Poulin R. Meta-analytic insights into evolutionary ecology: an introduction
409 and synthesis. Evol Ecol. 2012;26: 1085–1099. doi:10.1007/s10682-012-9593-z
410 35. Nakagawa S, Santos ESA. Methodological issues and advances in biological meta-analysis.
411 Evol Ecol. 2012;26: 1253–1274. doi:10.1007/s10682-012-9555-5
412 36. Senior AM, Grueber CE, Kamiya T, Lagisz M, O'Dwyer K, Santos ESA, et al. Heterogeneity
413 in ecological and evolutionary meta-analyses: its magnitude and implications. Ecology.
414 2016;97: 3293–3299. doi:10.1002/ecy.1591
415 37. Lozupone CA, Stombaugh J, Gonzalez A, Ackermann G, Wendel D, Vazquez-Baeza Y, et al.

416 Meta-analyses of studies of the human microbiota. Genome Res. 2013;23: 1704–1714.
417 doi:10.1101/gr.151803.112
418 38. Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with
419 obesity and IBD. FEBS Lett. 2014;588: 4223–4233. doi:10.1016/j.febslet.2014.09.039
420 39. Duvallet C, Gibbons SM, Gurry T, Irizarry RA, Alm EJ. Meta-analysis of gut microbiome
421 studies identifies disease-specific and shared responses. Nat Commun. 2017;8: 1784.
422 doi:10.1038/s41467-017-01973-8
423 40. Koren O, Knights D, Gonzalez A, Waldron L, Segata N, Knight R, et al. A Guide to
424 Enterotypes across the Human Body: Meta-Analysis of Microbial Community Structures
425 in Human Microbiome Datasets. Eisen JA, editor. PLoS Comput Biol. 2013;9: e1002863.
426 doi:10.1371/journal.pcbi.1002863
427 41. Krych L, Hansen CHF, Hansen AK, van den Berg FWJ, Nielsen DS. Quantitatively Different,
428 yet Qualitatively Alike: A Meta-Analysis of the Mouse Core Gut Microbiome with a View
429 towards the Human Gut Microbiome. Bereswill S, editor. PLoS One. 2013;8: e62578.
430 doi:10.1371/journal.pone.0062578
431 42. Wang J, Kurilshikov A, Radjabzadeh D, Turpin W, Croitoru K, Bonder MJ, et al. Meta-
432 analysis of human genome-microbiome association studies: the MiBioGen consortium
433 initiative. Microbiome. 2018;6: 101. doi:10.1186/s40168-018-0479-3
434 43. Jiao N, Baker SS, Nugent CA, Tsompana M, Cai L, Wang Y, et al. Gut microbiome may
435 contribute to insulin resistance and systemic inflammation in obese rodents: a meta-
436 analysis. Physiol Genomics. 2018;50: 244–254. doi:10.1152/physiolgenomics.00114.2017
437 44. Wirbel J, Pyl PT, Kartal E, Zych K, Kashani A, Milanese A, et al. Meta-analysis of fecal

438 metagenomes reveals global microbial signatures that are specific for colorectal cancer.
439 Nat Med. 2019;25: 679–689. doi:10.1038/s41591-019-0406-6
440 45. Bisanz JE, Upadhyay V, Turnbaugh JA, Ly K, Turnbaugh PJ. Meta-Analysis Reveals
441 Reproducible Gut Microbiome Alterations in Response to a High-Fat Diet. Cell Host
442 Microbe. 2019;26: 265-272.e4. doi:10.1016/j.chom.2019.06.013
443 46. Adams RI, Bateman AC, Bik HM, Meadow JF. Microbiota of the indoor environment: a
444 meta-analysis. Microbiome. 2015;3: 49. doi:10.1186/s40168-015-0108-3
445 47. Debroas D, Domaizon I, Humbert J-F, Jardillier L, Lepère C, Oudart A, et al. Overview of
446 freshwater microbial eukaryotes diversity: a first analysis of publicly available
447 metabarcoding data. FEMS Microbiol Ecol. 2017;93. doi:10.1093/femsec/fix023
448 48. Shade A, Gregory Caporaso J, Handelsman J, Knight R, Fierer N. A meta-analysis of
449 changes in bacterial and archaeal communities with time. ISME J. 2013;7: 1493–1506.
450 doi:10.1038/ismej.2013.54
451 49. Rocca JD, Simonin M, Blaszczak JR, Ernakovich JG, Gibbons SM, Midani FS, et al. The
452 Microbiome Stress Project: Toward a Global Meta-Analysis of Environmental Stressors
453 and Their Effects on Microbial Communities. Front Microbiol. 2019;9.
454 doi:10.3389/fmicb.2018.03272
455 50. Duvallet C. Meta-analysis generates and prioritizes hypotheses for translational
456 microbiome research. Microb Biotechnol. 2018;11: 273–276. doi:10.1111/1751-
457 7915.13047
458 51. Drewes JL, White JR, Dejea CM, Fathi P, Iyadorai T, Vadivelu J, et al. High-resolution
459 bacterial 16S rRNA gene profile meta-analysis and biofilm status reveal common

460 colorectal cancer consortia. npj Biofilms Microbiomes. 2017;3: 34. doi:10.1038/s41522-
461 017-0040-3
462 52. Ramirez KS, Knight CG, de Hollander M, Brearley FQ, Constantinides B, Cotton A, et al.
463 Detecting macroecological patterns in bacterial communities across independent studies
464 of global soils. Nat Microbiol. 2018;3: 189–196. doi:10.1038/s41564-017-0062-x
465 53. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al.
466 Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample.
467 Proc Natl Acad Sci. 2011;108: 4516–4522. doi:10.1073/pnas.1000080107
468 54. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of
469 general 16S ribosomal RNA gene PCR primers for classical and next-generation
470 sequencing-based diversity studies. Nucleic Acids Res. 2013;41: e1–e1.
471 doi:10.1093/nar/gks808
472 55. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome
473 assemblies. Bioinformatics. 2011;27: 2957–2963. doi:10.1093/bioinformatics/btr507
474 56. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-
475 throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms.
476 ISME J. 2012;6: 1621–1624. doi:10.1038/ismej.2012.8
477 57. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool
478 for metagenomics. PeerJ. 2016;4: e2584. doi:10.7717/peerj.2584
479 58. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal
480 RNA gene database project: improved data processing and web-based tools. Nucleic
481 Acids Res. 2012;41: D590–D596. doi:10.1093/nar/gks1219

482 59. Katoh K, Standley DM. MAFFT Multiple Sequence Alignment Software Version 7:
483 Improvements in Performance and Usability. Mol Biol Evol. 2013;30: 772–780.
484 doi:10.1093/molbev/mst010
485 60. Price MN, Dehal PS, Arkin AP. FastTree: Computing Large Minimum Evolution Trees with
486 Profiles instead of a Distance Matrix. Mol Biol Evol. 2009;26: 1641–1650.
487 doi:10.1093/molbev/msp077
488 61. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R
489 Foundation for Statistical Computing; 2016. 2017.
490 62. McMurdie PJ, Holmes S. phyloseq: An R package for reproducible interactive analysis and
491 graphics of microbiome census data. Watson M, editor. PLoS One. 2013;8: e61217.
492 doi:10.1371/journal.pone.0061217
493 63. Dixon P. VEGAN, a package of R functions for community ecology. J Veg Sci. 2003;14:
494 927–930. doi:10.1111/j.1654-1103.2003.tb02228.x
495 64. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for
496 RNA-seq data with DESeq2. Genome Biol. 2014;15: 550. doi:10.1186/s13059-014-0550-8
497 65. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J
498 Stat Softw. 2015;67: 1–48. doi:10.18637/jss.v067.i01
499 66. Delgado-Baquerizo M, Maestre FT, Reich PB, Jeffries TC, Gaitan JJ, Encinar D, et al.
500 Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat Commun.
501 2016;7: 10541. doi:10.1038/ncomms10541
502 67. Miki T, Yokokawa T, Matsui K. Biodiversity and multifunctionality in a microbial
503 community: a novel theoretical approach to quantify functional redundancy. Proc R Soc B

504 Biol Sci. 2014;281: 20132498. doi:10.1098/rspb.2013.2498

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

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

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

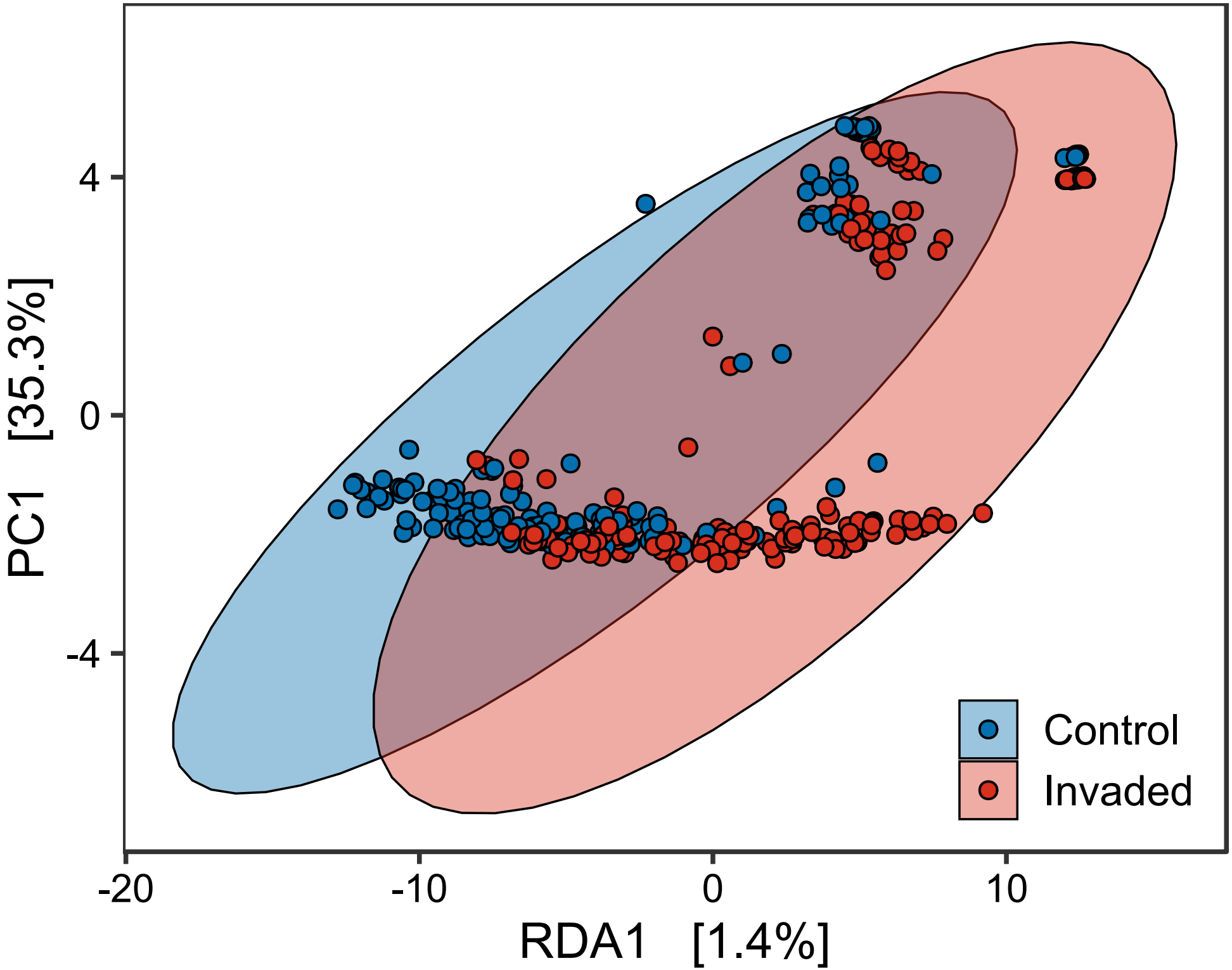
515 71. Chen J, Mo L, Zhang Z, Nan J, Xu D, Chao L, et al. Evaluation of the ecological restoration
516 of a coal mine dump by exploring the characteristics of microbial communities. Appl Soil
517 Ecol. 2020;147: 103430. doi:10.1016/j.apsoil.2019.103430

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

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

526 74. Madhaiyan M, Poonguzhali S, Senthilkumar M, Pragatheswari D, Lee J-S, Lee K-C.
527 Arachidicoccus rhizosphaerae gen. nov., sp. nov., a plant-growth-promoting bacterium in
528 the family Chitinophagaceae isolated from rhizosphere soil. Int J Syst Evol Microbiol.
529 2015;65: 578–586. doi:10.1099/ijs.0.069377-0
530 75. Reis VM, Teixeira KR dos S. Nitrogen fixing bacteria in the family Acetobacteraceae and
531 their role in agriculture. J Basic Microbiol. 2015;55: 931–949.
532 doi:10.1002/jobm.201400898
533 76. Trujillo ME, Bacigalupe R, Pujic P, Igarashi Y, Benito P, Riesco R, et al. Genome Features of
534 the Endophytic Actinobacterium Micromonospora lupini Strain Lupac 08: On the Process
535 of Adaptation to an Endophytic Life Style? Brüggemann H, editor. PLoS One. 2014;9:
536 e108522. doi:10.1371/journal.pone.0108522
537 77. Carro L, Nouioui I, Sangal V, Meier-Kolthoff JP, Trujillo ME, Montero-Calasanz M del C, et
538 al. Genome-based classification of micromonosporae with a focus on their
539 biotechnological and ecological potential. Sci Rep. 2018;8: 525. doi:10.1038/s41598-017-
540 17392-0
541 78. Dedysh SN, Ivanova AA. Planctomycetes in boreal and subarctic wetlands: diversity
542 patterns and potential ecological functions. FEMS Microbiol Ecol. 2019;95.
543 doi:10.1093/femsec/fiy227
544 79. Wegner C-E, Gorniak L, Riedel S, Westermann M, Küsel K. Lanthanide-Dependent
545 Methylotrophs of the Family Beijerinckiaceae: Physiological and Genomic Insights.
546 Stams AJM, editor. Appl Environ Microbiol. 2019;86. doi:10.1128/AEM.01830-19
547 80. Neufeld JD, Schäfer H, Cox MJ, Boden R, McDonald IR, Murrell JC. Stable-isotope probing

548 implicates Methylophaga spp and novel Gammaproteobacteria in marine methanol and
549 methylamine metabolism. ISME J. 2007;1: 480–491. doi:10.1038/ismej.2007.65
550 81. Warneke C, Karl T, Judmaier H, Hansel A, Jordan A, Lindinger W, et al. Acetone,
551 methanol, and other partially oxidized volatile organic emissions from dead plant matter
552 by abiological processes: Significance for atmospheric HO x chemistry. Global
553 Biogeochem Cycles. 1999;13: 9–17. doi:10.1029/98GB02428
554 82. Miao L, Wang P, Hou J, Yao Y, Liu Z, Liu S, et al. Distinct community structure and
555 microbial functions of biofilms colonizing microplastics. Sci Total Environ. 2019;650:
556 2395–2402. doi:10.1016/j.scitotenv.2018.09.378
557 83. Navarrete AA, Venturini AM, Meyer KM, Klein AM, Tiedje JM, Bohannan BJM, et al.
558 Differential Response of Acidobacteria Subgroups to Forest-to-Pasture Conversion and
559 Their Biogeographic Patterns in the Western Brazilian Amazon. Front Microbiol. 2015;6.
560 doi:10.3389/fmicb.2015.01443
561 84. Doblas-Miranda E, Martínez-Vilalta J, Lloret F, Álvarez A, Ávila A, Bonet FJ, et al.
562 Reassessing global change research priorities in mediterranean terrestrial ecosystems:
563 how far have we come and where do we go from here? Glob Ecol Biogeogr. 2015;24: 25–
564 43. doi:10.1111/geb.12224
565


4
0
-4
PC1 [35.3%]
-20
-10
0
10
RDA1 [1.4%]
Control
Invaded