Native and invading yellow starthistle (*Centaurea solstitialis*) microbiomes differ in composition and diversity of bacteria

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SUMMARY

- Invasive species could benefit from introduction to locations with favorable species interactions. Microbiomes are an important source of interactions that vary across regions. We examine whether bacterial communities could explain more favorable microbial interactions in highly invasive populations of yellow starthistle.

- We sequenced amplicons of prokaryotic 16S rRNA genes to characterize bacterial community composition in the phyllosphere, ectorhizosphere, and endorhizosphere of plants from seven invading populations in California, USA and eight native populations in Europe. We tested for differentiation of microbiomes by geography, plant compartment, and plant genotype.

- Bacterial communities differed significantly between native and invaded ranges within plant compartments, with consistently lower diversity in plants from the invaded range. Genera containing known plant pathogens also showed lower diversity in invaded range plants. The diversity of bacteria in roots was positively correlated with plant genotype diversity within both ranges, but this relationship did not explain microbial differences between ranges.

- Our findings reveal changes in the composition and diversity of bacterial interactions in invading plants, consistent with observations of altered soil interactions in this invasion. These results call for further study of the sources of variation in microbiomes and the potential for bacteria to facilitate invasion success.

KEYWORDS

species introductions, invasive species, microbial communities, phyllosphere, rhizosphere, endophytes, genetic diversity

INTRODUCTION

Humans continue to transport plant species around the globe, and increasing numbers of these translocations result in the invasive expansion of non-native species into recipient communities (Lonsdale, 1999; Butchart *et al.*, 2010; Essl *et al.*, 2011; Ellis *et al.*, 2012). While there are undoubtedly many reasons that species introductions lead to invasions, there is growing evidence that novel species interactions may facilitate the invasive spread of populations (Mitchell *et al.*, 2006; Pearson *et al.*, 2018). Initially, hypotheses about the contribution of species interactions to invasions focused on the potential for non-native species to escape from above-ground herbivores, which are easily observed (Keane & Crawley, 2002), though it is not clear that herbivore escape is a frequent mechanism of invasion (Maron & Vila, 2001; Levine *et al.*, 2004; Agrawal *et al.*, 2005; Felker-Quinn *et al.*, 2013). More recently, there has been increasing recognition that microbial taxa above- and below-ground can have large effects on plant fitness, both positive and negative, and could thus determine whether invasive plants benefit from novel species interactions (Callaway *et al.*, 2004; Colautti *et al.*, 2004; Torchin & Mitchell, 2004; Agrawal *et al.*, 2005; Mitchell *et al.*, 2006; Kulmatiski *et al.*, 2008; van der Putten *et al.*, 2016; Faillace *et al.*, 2017). Plant-associated microbial communities have been historically difficult to observe, however, and studies that leverage newly-available tools to quantify differences in these interactions across native and invading populations are needed to test hypotheses for invasion success (Dawson & Schrama, 2016).

Microbial communities have emerged as particularly likely candidates for facilitating invasions. Although many interactions between plants and microbes can be beneficial, soil microbial communities often appear to have negative net effects on plant fitness which may become more negative over time, e.g., via plant-soil feedbacks (Bever, 2003; Reinhart & Callaway, 2006; Kulmatiski *et al.*, 2008; Petermann *et al.*, 2008). These interactions between plants and their microbiomes can vary over space and environment (Nemergut *et al.*, 2013; van der Putten *et al.*, 2013; terHorst & Zee, 2016; Lankau & Keymer, 2018), creating opportunities for introduced plants to escape negative interactions that might characterize their native ranges. Moreover, reductions in microbial diversity are occurring in response to environmental change and human disturbances, and lowered microbial diversity may reduce the resistance of ecosystems to invasion (Schnitzer *et al.*, 2011; Wagg *et al.*, 2014; Dawson & Schrama, 2016; van der Putten *et*

al., 2016).

Invasive plant species have provided some of the best evidence to date that microbial interactions can be locally evolved, and can vary considerably over geographic regions (Rout & Callaway, 2012). Introduced plants have been shown to vary in their response to soil communities from their native and invaded ranges, and there are now many examples of more favorable interactions between plants and soil from their invaded range, consistent with escape from enemies or gain of mutualists during invasion (Reinhart *et al.*, 2003; Callaway *et al.*, 2004; Mitchell *et al.*, 2006; Engelkes *et al.*, 2008; Kulmatiski *et al.*, 2008; Maron *et al.*, 2014; van der Putten *et al.*, 2016). Plant-microbe interactions which provide relative benefits to invasive species can be explained by reduced negative effects of key microbial pathogens, increased direct beneficial effects of mutualistic taxa, or increased indirect benefits from taxa that affect competitors more negatively than they do the invader (Dawson & Schrama, 2016). It is also possible that invaders could benefit from a reduced diversity of enemy interactions, as a result of an associated reduction in ecological costs that derive from simultaneously deploying different defense responses against many different enemies (Agrawal, 2007; Colautti & Lau, 2015; Smakowska *et al.*, 2016). These hypotheses all require that there are differences in the microbial communities associated with invading vs. native plants, specifically changes in taxonomic composition, and the loss or gain of groups with pathogenic or mutualistic effects (Dawson & Schrama, 2016).

Here we conduct one of the first comparisons of plant microbiomes between invading populations and populations in their native source region. We survey plant-associated microbial communities in the highly invasive forb yellow starthistle (*Centaurea solstitialis*). Yellow starthistle is native to a wide region of Eurasia and was introduced from western Europe to South America in the 1600's and North America in the 1800's as a contaminant of alfalfa seed (Gerlach, 1997; Barker *et al.*, 2017). This herbaceous annual is a colonizer of grassland ecosystems, and is often cited as one of the '10 Worst Weeds of the West' in North America (DiTomaso & Healy, 2007). Its extensive invasion of California in the USA (>14 million acres; Pitcairn *et al.*, 2006) is particularly well-studied, and invading genotypes in this region have evolved to grow larger and produce more flowers than plants in the native range, suggesting a shift in resource allocation that has favored invasiveness (Widmer *et al.*, 2007; Eriksen *et al.*,

2012; Dlugosch *et al.*, 2015). Previous research has demonstrated that yellow starthistle throughout all of its native and invaded ranges experiences net fitness reductions when grown with its local soil communities (Andonian *et al.*, 2011, 2012; Andonian & Hierro, 2011). These studies have also indicated that this negative interaction is weaker (more favorable) in California, raising the possibility that changes in the microbial community have promoted an aggressive invasion.

We sample microbial communities associated with leaves (phyllosphere and endosphere) and roots (ectorhizosphere and endorhizosphere) of yellow starthistle plants in both the California invasion and native regions in Europe. Previous experiments with fungicide treatments have shown that plant-soil interactions between yellow starthistle and fungi in California are *more* negative (less favorable) than those in the native range, inconsistent with a role for fungi in beneficial species interactions in this invasion (Hierro *et al.*, 2017). Here, we focus on bacterial communities, using high-throughput sequencing of prokaryotic ribosomal 16S amplicon sequences to quantify taxonomic composition and diversity of bacteria in yellow starthistle microbiomes. Microbial communities are known to differ among plant compartments (Bulgarelli *et al.*, 2013) and to be influenced by individual plant genotype (Lundberg *et al.*, 2012; Peiffer *et al.*, 2013; Bodenhausen *et al.*, 2014), and so we explicitly test for differences in the microbiomes of native and invaded range plants relative to the influence of both plant compartment and plant genotype.

MATERIALS AND METHODS

Study species

Yellow starthistle (*Centaurea solstitialis* L., Asteraceae) is an obligately outcrossing annual plant, diploid throughout its range (Irmina *et al.*, 2017). Plants form a taproot and grow as a rosette through mild winter and/or spring conditions, bolting and producing flowering heads (capitula) throughout the summer. The species is native to Eurasia, where distinct genetic subpopulations have been identified in Mediterranean western Europe, central-eastern Europe, Asia (including the Middle East), and the Balkan-Apennine peninsulas (Barker *et al.*, 2017). The invasion of California as well as invasions in South America appear to be derived almost entirely from western European genotypes (Fig. 1; Barker *et al.*, 2017).

142

143 **Sample collection**

144 Fifteen populations of yellow starthistle were sampled for microbial communities: seven
145 sampling sites across the invasion of California, six in western Europe, and two in eastern
146 Europe (Fig. 1; Supporting Information Table S1). At each location, plants were sampled every
147 meter (or to the nearest meter mark) along a 25 meter transect, to yield microbial samples from
148 25 individual plants per population. Individuals in rosette or early bolting stages were
149 preferentially selected. In one population (HU29), low plant density yielded 20 individuals along
150 the 25 meter transect. Using sterile technique, plants were manually pulled and each individual
151 sampled using modified versions of protocols by Lundberg *et al.* (2013) and Lebeis *et al.* (2015)
152 as described below. Plants were pressed and dried after sampling, and submitted to the
153 University of Arizona Herbarium (ARIZ; Supporting Information Table S1).

154

155 Phyllosphere and ectorhizosphere One to three basal, non-senescent leaves were collected from
156 each plant, as well as the upper 2-5 cm of the taproot, together with accompanying lateral roots
157 (excess soil was brushed or shaken off). Leaf and root samples were placed in individual 50 ml
158 tubes containing 25 ml of sterile wash solution (45.9 mM NaH_2PO_4, 61.6 mM Na_2HPO_4, 0.1%
159 Tween 20). Tubes were shaken by hand for one minute (timed). Leaf and root samples were then
160 removed and stored on ice in separate tubes (leaves in empty tubes, roots in tubes containing 10
161 ml of wash solution) until further processing. Wash samples were stored on ice during transport,
162 then refrigerated at 4°C. Phyllosphere and ectorhizosphere washes were pooled across all (20 or
163 25) plants at a location, then centrifuged at 2,200 g at 4°C for 15 minutes. Supernatants were
164 discarded, and pellets were air-dried and stored at -20°C until DNA extraction.

165

166 Leaf endosphere Leaves were surface sterilized by submerging in bleach solution (10%
167 commercial bleach, 0.1% tween 20) for two minutes. Leaves were then rinsed in distilled water,
168 patted dry using a kimwipe, and sealed in individual sterile surgical envelopes (Fisherbrand #01-
169 812-50). Envelopes were kept in silica gel desiccant until leaf tissue was completely dry, then
170 stored at room temperature until DNA extraction.

171

172 Endorhizosphere Roots were further washed by shaking in 10 ml of wash solution until visible

173 residual soil was removed. Washed roots were stored and dried as described above for leaves.
174
175 Controls At each collection site, a tube of sterile wash solution was left uncapped while
176 sampling plants. Disinfected tools were periodically agitated in the blank wash tube before
177 sterilization and use for the next sample collection. For each population, rinse water and wipes
178 used to process tissue samples were represented in controls by rinsing and wiping flame-
179 sterilized forceps, then agitating the forceps in the blank wash tube. Controls were stored and
180 processed in the same manner as phyllosphere and ectorhizosphere samples.
181
182 **DNA extraction**
183 Extractions were carried out using sterile technique in a laminar flow hood. Leaf and root DNA
184 were extracted from tissue pooled by sampling site (15 total populations), and as individual plant
185 samples from 8 plants from each of 10 populations (80 total individuals). For pooled tissue
186 extractions, equal sections of leaf tissue (50 mm^2) and root tissue (12.5 mm^3 plus 10 mm of
187 lateral roots) were collected from each individual sample per location and pooled prior to
188 extraction. Control (blank) samples were collected for each batch of extractions by swabbing
189 tools and surfaces, then extracting DNA from the swab head.
190
191 All DNA samples were extracted using the MO BIO PowerSoil kit (MO BIO Laboratories, Inc.).
192 Phyllosphere and ectorhizosphere DNA was extracted from up to 0.25 g of wash pellets
193 following the standard kit protocol. Leaf and root tissues were ground to powder or sawdust
194 consistency in liquid nitrogen using sterile mortars and pestles. Leaf and root DNA was extracted
195 from 20 mg (leaf) or 100 mg (root) of ground tissue with the following modification to the
196 standard protocol: tissue was incubated at 65°C for 10 minutes in extraction buffer, then vortexed
197 for 1 minute, followed by a second 10 minute incubation (as described under "alternative lysis
198 methods" in the kit protocol). Control DNA was extracted by placing whole swab heads directly
199 into extraction tubes. Extracted DNA was eluted in PCR-grade water and stored at -20°C
200 pending library preparation.
201
202 **Library preparation and sequencing**
203 To remove secondary compounds inhibiting PCR, DNA extracted from root and leaf tissue

204 (together with corresponding blanks) was purified using a ZR-96 genomic DNA clean-up kit
205 (Zymo Research). All DNA concentrations were quantified using a Qubit fluorometer high-
206 sensitivity assay for double-stranded DNA (Invitrogen), and standardized to equimolar amounts.
207
208 Library preparation followed a dual-index approach (Kozich *et al.*, 2013) using a two-step PCR
209 protocol as follows. In the first step (target-specific PCR), the V4 region of the 16S rRNA gene
210 was amplified using target specific primers (515F and 806R; Caporaso *et al.*, 2011) appended
211 with common sequence (CS) tags through a linker sequence which varied from two to five
212 nucleotides in length. Target-specific PCR was carried out using Phusion Flash master mix
213 (Thermo Scientific) in 25 µl reaction volume in a Mastercycler pro thermocycler (Eppendorf)
214 under the following conditions: 25 cycles of 1 s at 98°C, 5 s at 78°C, 5 s at 57°C, 15 s at 72°C.
215 Products were visualized on an agarose gel and diluted by up to 1:15 (depending on yield); 1 µl
216 of diluted product was then used as template in the second step (barcode-adapter attachment
217 PCR). Using reagents and equipment as described above, barcoded primer pairs incorporating
218 Illumina P5 and P7 adapters were used to amplify products from target-specific PCR in 25 µl
219 reaction volumes under the following conditions: 10 cycles of 1 s at 98°C, 5 s at 78°C, 5 s at
220 51°C, 15 s at 72°C. Barcoded amplicons were quantified by fluorometry, pooled in equimolar
221 amounts, cleaned, and submitted to the University of Idaho's IBEST Genomic Resources Core
222 for QC and sequencing. Amplicons were multiplexed to use half the capacity of one 2 × 300 bp
223 run on an Illumina MiSeq platform. Raw sequence data are deposited in the NCBI Short Read
224 Archive under accession number XXXXXX [pending submission].
225
226 Peptide nucleic acid clamps (PNAs) were included in both PCR steps of library preparation to
227 block amplification of plant chloroplast and mitochondrial 16S as recommended by Lundberg *et*
228 *al.* (2013). Clamp sequences published by Lundberg *et al.* (2013) were compared with
229 chloroplast and mitochondrial 16S sequences from yellow starthistle and three other species of
230 Asteraceae with published organellar genomes (*Centaurea diffusa*, *Helianthus annuus*, *Lactuca*
231 *sativa*). We found a single nucleotide mismatch between the Asteraceae chloroplast 16S and the
232 plastid PNA sequences, and designed an alternative plastid PNA specific to the Asteraceae
233 sequence (5'—GGCTCAACTCTGGACAG—3') (Fitzpatrick *et al.*, in revision). All samples for
234 this study were amplified using the plastid PNA of our design, together with the mitochondrial

235 PNA published by Lundberg *et al.* (2013). To gauge the effectiveness of our alternative PNA,
236 two duplicate samples were processed using both PNAs published by Lundberg *et al.* (2013).
237
238 **Identification of operational taxa and potential plant pathogens**
239 Demultiplexed paired reads were merged and quality filtered using tools from the USEARCH
240 package version 9.0.2132 (Edgar & Flyvbjerg, 2015). Merged reads were truncated to uniform
241 length and primer sequences were removed using a combination of the seqtk toolkit version 1.2
242 (github.com/lh3/seqtk) and a custom script. The UPARSE pipeline (Edgar, 2013) implemented
243 in the USEARCH package was used for further data processing and analysis: unique sequences
244 were identified, and those represented only once or twice in the processed read set were
245 discarded as likely PCR or sequencing errors. Remaining sequences were clustered into
246 operational taxonomic units (OTUs) at a 97% threshold, chimeras were filtered out, and per-
247 sample OTU read counts were tabulated using the UPARSE-OTU algorithm. Assignment of
248 OTUs to nearest taxonomic match in the Greengenes database (McDonald *et al.*, 2012) was
249 carried out using the UCLUST algorithm implemented in QIIME version 1.9.1 (Caporaso *et al.*,
250 2010; Edgar, 2010). Data were further processed using tools from the QIIME package: reads
251 mapping to chloroplast and mitochondrial OTUs were removed, and samples were rarefied by
252 plant compartment. Rarefaction levels were chosen to reflect the distribution of read counts per
253 sample within plant compartments, subsampling to the minimum number of reads necessary to
254 include all samples except those that were outliers for low read count.
255
256 Taxa known to contain plant pathogens were identified using the FAPROTAX database (version
257 1.1, (Louca *et al.*, 2016). A list of all genera included under the "plant pathogen" functional
258 category was used to filter our OTU tables by taxonomic assignment. While pathogen-containing
259 genera are likely to include both pathogenic and non-pathogenic strains (Newton et al., 2010),
260 our pathogen-containing OTU dataset should be enriched for potential plant pathogens, relative
261 to the full dataset.
262
263 **Microbial community analyses**
264 All statistical analyses were performed in R (R Core Team, 2015). We evaluated differences in
265 bacterial community composition between plant compartments, and between native and invaded

266 ranges within plant compartments, by performing non-metric multidimensional scaling (NMDS)
267 using the R packages vegan (Oksanen *et al.*, 2016) and MASS (Venables & Ripley, 2002).
268 Individual plant samples or samples pooled within sampling site provided replicates in these
269 comparisons. Ordinations were based on Bray-Curtis distances, and were performed using a two-
270 dimensional configuration to minimize stress, using Wisconsin double standardized and square
271 root transformed data, with expanded weighted averages of species scores added to the final
272 NMDS solution. Significant differences among plant compartments and between native and
273 invaded samples were assessed using the envfit function in vegan. Ellipses were drawn on
274 NMDS plots using the vegan function ordiellipse, representing 95% confidence limits of the
275 standard error of the weighted average of scores.
276
277 We further explored the underlying correlates of bacterial community variation using Principal
278 Components Analysis (PCA; using R function prcomp) for samples from native and invaded
279 ranges within each plant compartment. Prior to performing the PCA, we performed Hellinger's
280 transformation to minimize the influence of OTUs with low counts or many zeros (Legendre &
281 Legendre, 1998; Legendre & Gallagher, 2001; Ramette, 2007). We then identified the OTUs
282 with the highest loading on the dominant PC axis of variation by examining the matrix of
283 variable loadings produced by prcomp. The OTU composition of samples pooled by sampling
284 site (phyllosphere, ectorhizosphere, and endorhizosphere samples; hereafter 'pooled samples')
285 was visualized using a heatmap generated in ggplot2 (Wickham, 2009), and samples were
286 hierarchically clustered by Bray-Curtis dissimilarity (hclust function in R) using McQuitty's
287 method (McQuitty, 1966).
288
289 We compared the diversity of OTUs between the native and invaded range for each plant
290 compartment using richness (R), evenness (Pielou's J; Pielou, 1966), and their combined effects
291 via the Hill series exponent $e^{H'}$ (Hill, 1973) of the Shannon diversity index (H'; Shannon, 1948).
292 Again, individual plant samples or samples pooled by sampling site provided replicates in these
293 comparisons. Diversity values were calculated using the packages vegan and iNEXT (Hsiegh *et*
294 *al.*, 2016), and compared between native and invaded ranges using a nonparametric Kruskal-
295 Wallis rank sum test on rarefied read counts. For plant tissue samples that included multiple
296 individuals per site, we compared diversity between regions using a nested ANOVA with fixed

297 effects of region and population nested within region, and among sites using a post hoc Kruskal-
298 Wallis test within regions. We conducted these comparisons on a dataset including all OTUs, and
299 a reduced dataset including only OTUs assigned to genera with known plant pathogens
300 according to the FAPROTAX database, as described above.
301
302 Finally, we examined the influence of plant genotype on microbial composition. Our geographic
303 regions correspond to genetically differentiated subpopulations, and within these regions, our
304 study sites are also known to vary in plant genetic diversity (Barker *et al.*, 2017). Microbial
305 diversity estimates ($e^{H'}$) for each plant compartment were predicted using linear models that
306 included fixed effects of plant genetic diversity, region (native vs. invaded), and the interaction
307 between these two effects. Measurements of plant genetic diversity at each of our sampling sites
308 were obtained from previously published genome-wide marker analyses by Barker and
309 colleagues (Barker *et al.*, 2017), calculated as the average proportion of pairwise nucleotide
310 differences between alleles (π) at variable positions across the yellow starthistle genome.
311
312 **RESULTS**
313 **Sequencing and data processing**
314 Sequencing yielded 9,672,898 read pairs, of which 6,217,852 remained after merging and quality
315 control; these were 253 bp in length after removing artificial and primer sequences. The number
316 of raw read counts per sample ranged from 16 to 306,200 with a median of 21,964. Analysis of
317 the merged and processed reads resulted in 4,014 OTUs, of which 60 were identified as plastid or
318 mitochondrial, and 428 were unidentifiable (11%). Of the remaining 3,526, 206 were identified
319 to species (6%), 1,084 to genus (27%), and 2,229 to family (56%). A total of 103 OTUs (3%)
320 were identified as members of the 49 genera with known plant pathogens in the FAPROTAX
321 v.1.1 database.
322
323 Sequence reads representing yellow starthistle chloroplast and mitochondrial 16S accounted for
324 40% and 1% of all reads, respectively. Amplification of host chloroplast in samples using the
325 Asteraceae-specific plastid PNA was reduced by up to 51% compared with the Lundberg *et al.*
326 (2013) PNA (Supporting Information Table S2). Despite PNA blocking activity, 83% of the total
327 reads from leaf endosphere samples were yellow starthistle chloroplast sequences. After removal

328 of chloroplast and mitochondrial reads, remaining read counts for most leaf endosphere samples
329 were low relative to controls (Supporting Information Fig. S1), so no further analysis of leaf
330 endosphere bacterial communities was performed.
331
332 Rarefaction levels (chosen to reflect the minimum number of reads per sample by compartment,
333 not including outliers) were 18,000 reads per sample for phyllosphere, 17,000 for
334 ectorhizosphere, and 5,000 for endorhizosphere samples (Supporting Information Fig. S1). These
335 levels were also higher than nearly all control samples. Rarefaction cutoffs resulted in the
336 exclusion of five non-control samples which were outliers for low read count: one phyllosphere
337 (DIA), one ectorhizosphere (SAZ), and three individual endorhizosphere samples (two from
338 SAZ; one from SIE). An NMDS ordination of all un-rarefied samples showed that the controls
339 clustered together and were clearly differentiated from all samples in all plant compartments
340 other than the leaf endosphere (Supporting Information Fig. S2).
341
342 **Microbial community analyses**
343 Results from NMDS ordinations indicated that bacterial communities differed overall among the
344 phyllosphere, ectorhizosphere, and endorhizosphere compartments (Fig. 2a; stress = 0.14, P =
345 0.001). Within compartments, NMDS further revealed significant differences between native and
346 invaded range endorhizosphere samples (Fig. 2b; stress = 0.16, P = 0.001) and ectorhizosphere
347 samples (P = 0.001). Native and invaded range phyllosphere samples differed with marginal
348 significance (P = 0.05). Clustering analyses within the phyllosphere and ectorhizosphere
349 compartments consistently grouped invaded range samples together, as well as samples from the
350 source region in western Europe (Supporting Information Fig. S3). Native range samples from
351 eastern Europe (HU01 and HU29) clustered together in these compartments but were variable in
352 their relationship to the other regions. Endorhizosphere samples pooled by location showed less
353 consistent clustering by range.
354
355 The dominant phyla among all bacterial communities were Proteobacteria, Actinobacteria,
356 Bacteroidetes, and Firmicutes (Fig. 3). Principal component analyses suggested that the strongest
357 contributions to changes in bacterial community composition between the native and invaded
358 ranges were made by shifts in the representation of *Bacillus* (Firmicutes), *Chryseobacterium*

(Bacteroidetes), and the Proteobacteria taxa *Erwinia*, *Pseudomonas*, and Xanthomonadaceae (Supporting Information Table S3; Fig. S4). All of these taxa other than *Chryseobacterium* include known plant pathogens in the FAPROTAX v.1.1 database (Louca *et al.*, 2016).

In general, bacterial OTUs showed a pattern of lower median richness (R), evenness (J), and diversity ($e^{H'}$) for plants from invaded range sites in all compartments, with the exception of richness in the phyllosphere (Figs 4, 5). Within the phyllosphere, invaders were not significantly different in richness ($\chi^2_1 = 1.67$, $P = 0.20$), were significantly lower in evenness ($\chi^2_1 = 8.07$, $P = 0.005$), and were marginally lower in Hill diversity ($\chi^2_1 = 3.75$, $P = 0.05$), relative to native range plants. Similarly, the ectorhizosphere of invaders was not significantly different in richness ($\chi^2_1 = 0.69$, $P = 0.41$), and was significantly lower in both evenness ($\chi^2_1 = 5.0$, $P = 0.03$) and diversity ($\chi^2_1 = 6.21$, $P = 0.01$). In contrast, endorhizosphere samples pooled by site did not differ significantly in evenness ($\chi^2_1 = 2.26$, $P = 0.13$), but were marginally significantly lower in richness ($\chi^2_1 = 3.43$, $P = 0.06$) and diversity ($\chi^2_1 = 3.01$, $P = 0.08$). Nested ANOVA of individual endorhizosphere samples indicated strongly significant reductions in richness, evenness, and diversity in invading plants (Fig 5; fixed effect of region: all $P < 0.001$). For individual endorhizosphere samples, populations did not differ significantly in any metrics within native/invaded regions, with the exception of significantly higher evenness in plants at site SIE relative to site TRI in the invaded range.

Filtering the rarefied datasets for genera containing known plant pathogens resulted in 49 phyllosphere OTUs, 69 ectorhizosphere OTUs, and 88 endorhizosphere OTUs enriched for potential plant pathogens. *Pseudomonas* and *Erwinia* were among the most common pathogen-containing genera encountered in each plant compartment in both ranges. The phyllosphere also included a high frequency of *Janthinobacterium*, with a relative increase in *Serratia* in the invaded range. In the ectorhizosphere, *Serratia* was common in both ranges, but invading plants showed a large relative decline in *Erwinia* and increase in *Pseudomonas*. In the endorhizosphere, invading plant harbored less *Pseudomonas* and more *Bacillus* and *Streptomyces* than native plants (Supporting Information Fig. S5). Diversity of these OTUs showed similar trends to total diversity, with lower median values in invaded range root compartments. No differences between regions were statistically significant for the phyllosphere ($\chi^2_1 = 0.60$, $P = 0.44$) or

390 ectorhizosphere ($\chi^2_1 = 0.49$, $P = 0.48$). For the endorhizosphere samples pooled by site,
391 significantly lower diversity was indicated in the invaded range ($\chi^2_1 = 4.34$, $P = 0.04$). For
392 individual endorhizosphere samples, nested ANOVA also indicated significantly lower diversity
393 in the invaded range ($P < 0.0001$), and no significant differences among populations within
394 regions.
395
396 The diversity of bacteria in root compartments showed evidence of associations with plant
397 genetic diversity at sampling sites. A linear model predicting microbial diversity ($e^{H'}$) from plant
398 genetic diversity was significant for endorhizosphere samples pooled by site (Fig. 6; $F_{(2,12)} =$
399 5.89; $P = 0.02$; $r^2_{adj} = 0.41$), with significant main effects of both plant genetic diversity ($P =$
400 0.03) and region (native vs. invaded; $P = 0.006$). The interaction between these two effects was
401 not significant ($P = 0.71$) and was removed from the final model. This same pattern was
402 marginally significant when using only OTUs from pathogen-containing genera in the
403 endorhizosphere samples (effect of plant genetic diversity: $P = 0.08$). Similar linear models did
404 not identify significant effects of plant genetic diversity when predicting the median diversity of
405 individual plant endorhizosphere samples ($P = 0.74$), or diversity in the phyllosphere ($P = 0.35$).
406 There was a marginally significant positive effect of plant genetic diversity on diversity in the
407 ectorhizosphere ($P = 0.08$) again in addition to the effect of region ($P = 0.002$).
408
409 **DISCUSSION**
410 Introduced plants will encounter a variety of novel species interactions as they establish across
411 biogeographic regions. For many plants, severe invasions are associated with more favorable
412 interactions with soil microbial communities (Andonian & Hierro, 2011; Dawson & Schrama,
413 2016). We found that bacterial microbiomes of invading yellow starthistle were unique in
414 composition and lower in diversity relative to bacterial microbiomes of native plants, differences
415 that persisted within plant compartments and across variation in plant genetic diversity.
416
417 As observed in other species, bacterial communities differed most among plant compartments
418 (Bulgarelli *et al.*, 2013; Vandenkoornhuyse *et al.*, 2015). The numbers and diversity of taxa
419 within each compartment were similar in magnitude to those reported in other studies of
420 prokaryotic 16S sequences, e.g. from Agavaceae (Coleman-Derr *et al.*, 2016), Brassicaceae

421 (Bodenhausen *et al.*, 2013), Cactaceae (Fonseca-García *et al.*, 2016), and other Asteraceae (Leff
422 *et al.*, 2016). The dominant phyla were Proteobacteria, Actinobacteria, Bacteroidetes, and
423 Firmicutes, which are also characteristic of plant-associated bacterial communities surveyed to
424 date (Bulgarelli *et al.*, 2013). The exception was the leaf endosphere, where a paucity of
425 sequences relative to controls suggests that persistent chloroplast contamination obscured low
426 frequency endophytes, despite our development of an Asteraceae-specific PNA (FitzPatrick *et*
427 *al.*, submitted). A targeted survey is needed to better characterize this compartment (e.g. qPCR,
428 (Fierer et al., 2005).
429
430 Notably, diversity was approximately twice as high in the endorhizosphere as in the
431 ectorhizosphere. Current reviews have concluded that root endosphere communities are typically
432 less diverse than those in the ectorhizosphere (Bulgarelli *et al.*, 2013; Vandenkoornhuyse *et al.*,
433 2015). Our root collections were washed but not surface sterilized and may represent some of the
434 rhizoplane/rhizosphere in addition to the endosphere, elevating our estimates of diversity. It is
435 also possible that yellow starthistle deviates from initially reported patterns, which have also
436 been challenged by other recent studies (Fonseca-García *et al.*, 2016; Leff *et al.*, 2016).
437
438 Within compartments, community composition was consistently different between samples from
439 native and invaded ranges and included shifts in taxa across all major groups. Our native range
440 samples represented a larger geographic area and spanned distinct genetic subpopulations of
441 yellow starthistle, but native range sites clustered together in overall community composition and
442 there was little evidence of individual site differences within ranges. Between ranges, the
443 diversity of OTUs was lower in the invaded range, a pattern that was dominated by lower
444 evenness of OTUs in both the phyllosphere and ectorhizosphere, and by lower richness of OTUs
445 in the endorhizosphere. Thus invading plants were more strongly dominated by a few taxa at
446 high relative abundance on root and leaf surfaces, and harbored fewer bacterial taxa in their root
447 endophytic communities.
448
449 We observed a significant positive association between root microbial diversity and genotypic
450 diversity among plants at the population scale. This association was strongest in the
451 endorhizosphere, the only endophytic compartment in our analysis, consistent with plant

genotype having the largest influence on microbial taxa colonizing within the plant itself (Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012; Lebeis *et al.*, 2015). Within-species plant genotype effects have been observed previously and may interact with the effect of environment to shape microbial communities (Peiffer *et al.*, 2013; terHorst & Zee, 2016). Interactions between plant and microbial diversity could be particularly important for invasive species, where genetic bottlenecks during establishment and range expansion can reduce genetic diversity among plants (Dlugosch & Parker, 2008; Excoffier *et al.*, 2009; Uller & Leimu 2011). Nevertheless, it appears that genotype effects are often minor relative to site effects (Lundberg *et al.*, 2012; Peiffer *et al.*, 2013; Bodenhausen *et al.*, 2014; Bulgarelli *et al.*, 2015), and we found that genotypic effects were evident only within region and did not explain microbial diversity differences between regions.

The microbial differences that we observed across ranges are similar to those observed at continental scales (Bodenhausen *et al.*, 2013; Peiffer *et al.*, 2013). A variety of factors may explain these patterns, particularly abiotic differences (Fierer & Jackson, 2006; Bulgarelli *et al.*, 2013; Nemergut *et al.*, 2013; Vandenkoornhuyse *et al.*, 2015). Soil type appears to have a strong influence on microbial communities (e.g. Bulgarelli *et al.*, 2012; Lundberg *et al.*, 2012), and is known to differ broadly across yellow starthistle's range (Hierro *et al.*, 2017). In addition, populations in California are at the warm and dry extreme of yellow starthistle's climatic niche (Dlugosch *et al.*, 2015), and our sampling was conducted at the end of a period of severe drought (Griffin & Anchukaitis, 2014; Diffenbaugh *et al.*, 2015), which could have amplified microbial differences related to climate (Schrama & Bardgett, 2016). Interestingly, a recent study of grassland plants found that microbial diversity increased under drought, whereas we found reduced diversity in the drought-affected range (terHorst *et al.*, 2014).

Importantly, yellow starthistle's invasion could also cause changes to microbial communities. Species invasionss have been shown to alter microbial composition over short timescales (Collins *et al.*, 2016; Gibbons *et al.*, 2017), though long term effects are less clear (Lankau, 2011; Day *et al.*, 2015). Yellow starthistle invasions are denser than populations in the native range by an order of magnitude or more (Uygur *et al.*, 2004; Andonian *et al.*, 2011), and include lower diversity of plant species overall (Seabloom *et al.*, 2003; Zavaleta & Hulvey, 2004;

483 D'Antonio *et al.*, 2007). Low plant diversity can depress the diversity of microbes in the
484 environment and in plants (Garbeva *et al.*, 2004; Schnitzer *et al.*, 2011; Coleman-Derr *et al.*,
485 2016). Invading plants in California also grow to larger size, and could allow particular strains to
486 proliferate if size increases were gained at a cost to defenses (Huot *et al.*, 2014; Dlugosch *et al.*,
487 2015). These possibilities mean that invasions could be a cause rather than an effect of weaker
488 plant-soil interactions for invasive species in their introduced ranges (Kulmatiski *et al.*, 2008;
489 Dawson & Schrama, 2016), reinforcing a growing need for explicit observational and
490 experimental tests that disentangle associations between microbial diversity and plant density,
491 plant community diversity, and environmental gradients (Dawson & Schrama, 2016).
492
493 To date, few studies have compared microbial community compositionl between the native and
494 introduced ranges of invasive plants. McGinn and colleagues (2016) reported no differences in
495 the diversity of mutualistic fungal taxa associated with the roots of multiple species of European
496 *Trifolium* introduced to New Zealand, despite more favorable soil interactions in the invasions
497 (McGinn et al., 2018; but see Parker & Gilbert, 2007. Johansen and colleagues (2017) found
498 *increased* diversity of fungal communities on the roots of European *Ammophila arenaria*
499 invading Australia and New Zealand, though there appear to be no differences in interactions
500 with soil microbial communities in its invasions (in North America; Beckstead & Parker, 2003).
501 Gundale and colleagues explored the potential contribution of fungal endophyte communities to
502 more favorable (negative) soil interactions observed in introduced plantations of lodgepole pine
503 (*Pinus contorta*) from North America (Gundale *et al.*, 2014; 2016). For lodgepole pine,
504 microbial communities differed among several global regions examined, but there was no
505 consistent pattern of loss of potential fungal pathogens or gain of mutualists in the introductions,
506 and it remains unclear what part of the soil community is responsible for observed differences in
507 interactions across ranges (Gundale *et al.*, 2016). Reinhart and colleagues (2010) focused
508 specifically on *Phythium* fungal pathogens and quantified their virulence on North American
509 *Prunus serotina* introduced to Europe. They found that the most virulent strains occurred only in
510 the native range, consistent with benefits to invading plants escaping components this specific
511 pathogenic group. In the only microbiome comparison that included bacterial taxa, Finkel and
512 colleagues (Finkel *et al.*, 2011; 2016) explored the phyllosphere community of multiple species
513 of *Tamarix* in native and introduced parts of their ranges, finding that microbial communities

514 were in general most strongly structured by geographic region.
515
516 Our study is the first to find consistent differences in the microbiomes of native and invading
517 plants which coincide with fitness differences in plant interactions with soil communities.
518 Among the few invader microbiome studies to date, ours is unusual in focusing on the bacterial
519 community. Fungi have historically received more attention for their fitness effects on plants, but
520 bacteria can also play a critical role both as pathogens and mutualists (Haney *et al.*, 2015;
521 Vandenkoornhuyse *et al.*, 2015; Herrera Paredes & Lebeis, 2016). For yellow starthistle,
522 previous experiments have demonstrated that fungal communities are not responsible for more
523 favorable conditions in the invaded range (Hierro *et al.*, 2017), and our findings indicate that
524 bacterial communities warrant further investigation as the potential source of these differences.
525 The fitness effects of our specific OTUs are unknown, however, and identifying the bacterial
526 OTUs that accumulate during interactions with plants would help to elucidate important
527 pathogenic or mutualistic taxa, and allow field surveys to explicitly test hypotheses that these
528 strains are lost or gained in the invaded environment.
529
530 We have previously argued that yellow starthistle has benefitted from the historical loss of plant
531 competitors in California (Dlugosch *et al.*, 2015). Disturbance is critical for yellow starthistle
532 establishment, and functionally similar native species compete well against it in experiments;
533 however, key competitors have been lost from the ecosystem due toperturbations prior to yellow
534 starthistle invasion (Zavaleta & Hulvey, 2004; Hooper & Dukes, 2010; Hierro *et al.*, 2011, 2017;
535 Hulvey & Zavaleta, 2012). Any benefits of altered bacterial communities could be independent
536 of competition with native plant species, but these factors might also interact. Increased density
537 due to a lack of competition could have reduced plant-associated microbial diversity, as
538 described above. Yellow starthistle experiences negative plant-soil feedbacks across generations
539 (Andonian *et al.*, 2011), however, and the build up of high plant densities is therefore unlikely to
540 generate more favorable soil interactions. Alternatively, the historical loss of native species
541 diversity in California (D'Antonio *et al.*, 2007) could have resulted in the loss of associated
542 microbial diversity (Garbeva *et al.*, 2004; Schnitzer *et al.*, 2011; Coleman-Derr *et al.*, 2016),
543 generating particularly strong opportunities for invasion into a system with both reduced plant
544 competition and reduced pathogen diversity. Microbial surveys of remnant native communities,

545 as well as across densities of yellow starthistle would facilitate tests of alternative hypotheses for
546 interacting effects of plant and microbial diversity, and it may be informative to explore
547 microbial communities preserved on native plant specimens pre-dating the extensive invasion of
548 yellow starthistle into this region.
549
550 **Conclusions**
551 We found consistent differences between native and invading yellow starthistle plants in their
552 bacterial microbiomes. These differences were robust to additional variation associated with
553 plant compartment and the diversity of plant genotypes. Invaded range microbiomes differed in
554 composition across major taxonomic groups, and harbored a lower diversity of bacteria,
555 including reduced evenness on the surface of leaves and roots and reduced richness of root
556 endophytes. We suggest that bacteria could be the source of more favorable microbial
557 interactions that have been observed in this invasion. Our findings also raise questions about 1)
558 whether lower bacterial diversity is a feature of the invaded environment or whether it is caused
559 by the invasion itself, and 2) how differences in the microbial community might have interacted
560 with other changes in plant competitive interactions and the abiotic environment to affect plant
561 fitness. These questions highlight the need for additional studies that compare microbial
562 communities (including bacteria) associated with native and invading populations, that couple
563 microbial community identification with plant-soil feedback and fitness experiments, and that
564 examine the interaction of environment, plant diversity, and plant density on microbial
565 communities and their fitness effects on plants.
566
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578

579 **AUTHOR CONTRIBUTIONS**

580 P.L-I., D.A.B., and K.M.D. designed the study. P.L-I. and J.H. collected the samples with
581 assistance from H.S., S.M.S., and S.R.W. P.L-I conducted the microbial sequencing and
582 bioinformatics. P.L-I, S.R.W., and K.M.D. analyzed the data. P.L-I and K.M.D. wrote the
583 manuscript, which was edited by all authors.
584

585 **REFERENCES**

586 **Agrawal AA**. **2007**. Macroevolution of plant defense strategies. *Trends in Ecology & Evolution*
587 **22**: 103–109.
588 **Agrawal AA, Kotanen PM, Mitchell CE, Power AG, Godsoe W, Klironomos J**. **2005**.
589 Enemy release? an experiment with congeneric plant pairs and diverse above- and
590 belowground enemies. *Ecology* **86**: 2979–2989.
591 **Andonian K, Hierro JL**. **2011**. Species interactions contribute to the success of a global plant
592 invader. *Biological Invasions* **13**: 2957–2965.
593 **Andonian K, Hierro JL, Khetsuriani L, Becerra PI, Janoyan G, Villareal D, Cavieres LA,**
594 **Fox LR, Callaway RM**. **2012**. Geographic mosaics of plant-soil microbe interactions in a
595 global plant invasion. *Journal of Biogeography* **39**: 600–608.
596 **Andonian K, Hierro JL, Khetsuriani L, Becerra P, Janoyan G, Villarreal D, Cavieres L,**
597 **Fox LR, Callaway RM**. **2011**. Range-expanding populations of a globally introduced weed
598 experience negative plant-soil feedbacks. *PLoS One* **6**: e20117.
599 **Barker BS, Andonian K, Swope SM, Luster DG, Dlugosch KM**. **2017**. Population genomic
600 analyses reveal a history of range expansion and trait evolution across the native and invaded
601 range of yellow starthistle (*Centaurea solstitialis*). *Molecular Ecology* **26**: 1131–1147.
602 **Beckstead J, Parker IM**. **2003**. Invasiveness of *Ammophila arenaria*: release from soil-borne
603 pathogens? *Ecology* **84**: 2824–2831.
604 **Bever JD**. **2003**. Soil community feedback and the coexistence of competitors: conceptual
605 frameworks and empirical tests. *The New Phytologist* **157**: 465–473.
606 **Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA**. **2014**. A synthetic

607 community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLoS*
608 *Genetics* **10**: e1004283.
609 **Bodenhausen N, Horton MW, Bergelson J**. **2013**. Bacterial communities associated with the
610 leaves and the roots of *Arabidopsis thaliana*. *PLoS One* **8**: e56329.
611 **Bulgarelli D, Garrido-Oter R, Münch PC, Weiman A, Dröge J, Pan Y, McHardy AC,**
612 **Schulze-Lefert P**. **2015**. Structure and function of the bacterial root microbiota in wild and
613 domesticated barley. *Cell Host & Microbe* **17**: 392–403.
614 **Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F,**
615 **Rauf P, Huettel B, Reinhardt R, Schmelzer E, *et al.* 2012**. Revealing structure and
616 assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* **488**: 91–95.
617 **Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P**. **2013**.
618 Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*
619 **64**: 807–838.
620 **Butchart SHM, Walpole M, Collen B, van Strien A, Scharlemann JPW, Almond REA,**
621 **Baillie JEM, Bomhard B, Brown C, Bruno J, *et al.* 2010**. Global biodiversity: indicators of
622 recent declines. *Science* **328**: 1164–1168.
623 **Callaway RM, Thelen GC, Rodriguez A, Holben WE**. **2004**. Soil biota and exotic plant
624 invasion. *Nature* **427**: 731–733.
625 **Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer**
626 **N, Peña AG, Goodrich JK, Gordon JI, *et al.* 2010**. QIIME allows analysis of high-
627 throughput community sequencing data. *Nature Methods* **7**: 335–336.
628 **Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ,**
629 **Fierer N, Knight R**. **2011**. Global patterns of 16S rRNA diversity at a depth of millions of
630 sequences per sample. *Proceedings of the National Academy of Sciences* **108 Suppl 1**: 4516–
631 4522.
632 **Colautti RI, Lau JA**. **2015**. Contemporary evolution during invasion: evidence for
633 differentiation, natural selection, and local adaptation. *Molecular Ecology* **24:** 1999-2017.
634 **Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ**. **2004**. Is invasion success explained
635 by the enemy release hypothesis? *Ecology Letters* **7**: 721–733.
636 **Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T,**
637 **North G, Visel A, Partida-Martinez LP, Tringe SG**. **2016**. Plant compartment and

638 biogeography affect microbiome composition in cultivated and native *Agave* species. *The*
639 *New Phytologist* **209**: 798–811.
640 **Collins CG, Carey CJ, Aronson EL, Kopp CW, Diez JM**. **2016**. Direct and indirect effects of
641 native range expansion on soil microbial community structure and function. *The Journal of*
642 *Ecology* **104**: 1271–1283.
643 **D'Antonio CM, Malmstrom C, Reynolds SA, Gerlach J**. **2007**. Ecology of invasive non-
644 native species in California grassland. In: Stromberg MR, Corbin JD, D'Antonio CM, eds.
645 *California grassland: ecology and management*. Berkeley, CA, USA: University of
646 California Press, 67–86.
647 **Dawson W, Schrama M**. **2016**. Identifying the role of soil microbes in plant invasions. *The*
648 *Journal of Ecology* **104**: 1211–1218.
649 **Day NJ, Dunfield KE, Antunes PM**. **2015**. Temporal dynamics of plant-soil feedback and root-
650 associated fungal communities over 100 years of invasion by a non-native plant. *The Journal*
651 *of Ecology* **103**: 1557–1569.
652 **Diffenbaugh NS, Swain DL, Touma D**. **2015**. Anthropogenic warming has increased drought
653 risk in California. *Proceedings of the National Academy of Sciences* **112**: 3931–3936.
654 **DiTomaso JM, Healy EA**. **2007**. *Weeds of California and other western states*. Oakland, CA,
655 USA: University of California Department of Agriculture and Natural Resources.
656 **Dlugosch KM, Cang FA, Barker BS, Andonian K, Swope SM, Rieseberg LH**. **2015**.
657 Evolution of invasiveness through increased resource use in a vacant niche. *Nature Plants* **1**:
658 15066.
659 **Dlugosch KM, Parker IM**. **2008**. Founding events in species invasions: genetic variation,
660 adaptive evolution, and the role of multiple introductions. *Molecular Ecology* **17**: 431–449.
661 **Edgar RC**. **2010**. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*
662 **26**: 2460–2461.
663 **Edgar RC**. **2013**. UPARSE: highly accurate OTU sequences from microbial amplicon reads.
664 *Nature Methods* **10**: 996–998.
665 **Edgar RC, Flyvbjerg H**. **2015**. Error filtering, pair assembly and error correction for next-
666 generation sequencing reads. *Bioinformatics* **31**: 3476–3482.
667 **Ellis EC, Antill EC, Kreft H**. **2012**. All is not loss: plant biodiversity in the anthropocene. *PLoS*
668 *One* **7**: e30535.

669 **Engelkes T, Morriën E, Verhoeven KJF, Bezemer TM, Biere A, Harvey JA, McIntyre LM,**
670 **Tamis WLM, van der Putten WH**. **2008**. Successful range-expanding plants experience
671 less above-ground and below-ground enemy impact. *Nature* **456**: 946–948.
672 **Eriksen RL, Desronvil T, Hierro JL, Kesseli R**. **2012**. Morphological differentiation in a
673 common garden experiment among native and non-native specimens of the invasive weed
674 yellow starthistle (*Centaurea solstitialis*). *Biological Invasions* **14**: 1459–1467.
675 **Essl F, Dullinger S, Rabitsch W, Hulme PE, Hülber K, Jarošík V, Kleinbauer I,**
676 **Krausmann F, Kühn I, Nentwig W, *et al.* 2011**. Socioeconomic legacy yields an invasion
677 debt. *Proceedings of the National Academy of Sciences* **108**: 203–207.
678 **Excoffier L, Foll M, Petit RJ**. **2009**. Genetic consequences of range expansions. *Annual Review*
679 *of Ecology, Evolution, and Systematics* **40**: 481–501.
680 **Faillace CA, Lorusso NS, Duffy S**. **2017**. Overlooking the smallest matter: viruses impact
681 biological invasions. *Ecology Letters* **EarlyView**.
682 **Felker-Quinn E, Schweitzer JA, Bailey JK**. **2013**. Meta-analysis reveals evolution in invasive
683 plant species but little support for Evolution of Increased Competitive Ability (EICA).
684 *Ecology and Evolution* **3**: 739–751.
685 **Fierer N, Jackson JA, Vilgalys R, Jackson RB**. **2005**. Assessment of soil microbial community
686 structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental*
687 *Microbiology* **71**: 4117–4120.
688 **Fierer N, Jackson RB**. **2006**. The diversity and biogeography of soil bacterial communities.
689 *Proceedings of the National Academy of Sciences* **103**: 626–631.
690 **Finkel OM, Burch AY, Lindow SE, Post AF, Belkin S**. **2011**. Geographical location
691 determines the population structure in phyllosphere microbial communities of a salt-
692 excreting desert tree. *Applied and Environmental Microbiology* **77**: 7647–7655.
693 **Finkel OM, Delmont TO, Post AF, Belkin S**. **2016**. Metagenomic signatures of bacterial
694 adaptation to life in the phyllosphere of a salt-secreting desert tree. *Applied and*
695 *Environmental Microbiology* **82**: 2854–2861.
696 **Fitzpatrick C; Lu-Irving P; Copeland J; Guttman DS; Wang PW; Baltrus DA; Dlugosch**
697 **KM; Johnson MTJ.** Chloroplast sequence variation and the efficacy of peptide nucleic acids
698 for blocking host amplification in plant microbiome studies. *Microbiome* **In minor revision**
699 **Fonseca-García C, Coleman-Derr D, Garrido E, Visel A, Tringe SG, Partida-Martínez LP**.

2016. The cacti microbiome: interplay between habitat-filtering and host-specificity. *Frontiers in Microbiology* **7**: 150.

Garbeva P, van Veen JA, van Elsas JD. **2004**. Microbial diversity in soil: selection microbial populations by plant and soil type and implications for disease suppressiveness. *Annual Review of Phytopathology* **42**: 243–270.

Gerlach JD. **1997**. How the West was lost: reconstructing the invasion dynamics of yellow starthistle and other plant invaders of western rangelands and natural areas. *California Exotic Pest Plant Council Symposium Proceedings* **3**: 67–72.

Gibbons SM, Lekberg Y, Mummey DL, Sangwan N, Ramsey PW, Gilbert JA, Shade A. **2017**. Invasive plants rapidly reshape soil properties in a grassland ecosystem. *mSystems* **2**: e00178–16.

Griffin D, Anchukaitis KJ. **2014**. How unusual is the 2012–2014 California drought? *Geophysical Research Letters* **41**: 2014GL062433.

Gundale MJ, Almeida JP, Wallander H, Wardle DA, Kardol P, Nilsson M-C, Fajardo A, Pauchard A, Peltzer DA, Ruotsalainen S, *et al.* 2016. Differences in endophyte communities of introduced trees depend on the phylogenetic relatedness of the receiving forest. *The Journal of Ecology* **104**: 1219–1232.

Gundale MJ, Kardol P, Nilsson M-C, Nilsson U, Lucas RW, Wardle DA. **2014**. Interactions with soil biota shift from negative to positive when a tree species is moved outside its native range. *The New Phytologist* **202**: 415–421.

Haney CH, Samuel BS, Bush J, Ausubel FM. **2015**. Associations with rhizosphere bacteria can confer an adaptive advantage to plants. *Nature Plants* **1**.

Herrera Paredes S, Lebeis SL. **2016**. Giving back to the community: microbial mechanisms of plant–soil interactions. *Functional Ecology* **30**: 1043–1052.

Hierro JL, Khetsuriani L, Andonian K, Eren Ö, Villarreal D, Janoian G, Reinhart KO, Callaway RM. **2017**. The importance of factors controlling species abundance and distribution varies in native and non-native ranges. *Ecography* **40:** 991-1002.

Hierro JL, Lortie CJ, Villarreal D, Estanga-Mollica ME, Callaway RM. **2011**. Resistance to *Centaurea solstitialis* invasion from annual and perennial grasses in California and Argentina. *Biological Invasions* **13**: 2249–2259.

Hill MO. **1973**. Diversity and evenness: a unifying notation and its consequences. *Ecology* **54**:

731 427–432.
732 **Hooper DU, Dukes JS**. **2010**. Functional composition controls invasion success in a California
733 serpentine grassland. *The Journal of Ecology* **98**: 764–777.
734 **Hulvey KB, Zavaleta ES**. **2012**. Abundance declines of a native forb have nonlinear impacts on
735 grassland invasion resistance. *Ecology* **93**: 378–388.
736 **Huot B, Yao J, Montgomery BL, He SY**. **2014**. Growth–defense tradeoffs in plants: a
737 balancing act to optimize fitness. *Molecular Plant* **7**: 1267–1287.
738 **Hsieh TC, Ma KH, Chao A**. **2016**. iNEXT: an R package for rarefaction and extrapolation of
739 species diversity (Hill numbers). *Methods in Ecology and Evolution* **7**: 1451–1456.
740 **Johansen RB, Johnston P, Mieczkowski P, Perry GLW, Robeson MS, Vilgalys R, Burns**
741 **BR**. **2017**. Scattered far and wide: A broadly distributed temperate dune grass finds familiar
742 fungal root associates in its invasive range. *Soil Biology & Biochemistry* **112**: 177–190.
743 **Keane R, Crawley MJ**. **2002**. Exotic plant invasions and the enemy release hypothesis. *Trends*
744 *in Ecology & Evolution* **17**: 164–170.
745 **Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD**. **2013**. Development of a
746 dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data
747 on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology* **79**:
748 5112–5120.
749 **Kulmatiski A, Beard KH, Stevens JR, Cobbold SM**. **2008**. Plant–soil feedbacks: a meta-
750 analytical review. *Ecology Letters* **11**: 980–992.
751 **Lankau RA**. **2011**. Resistance and recovery of soil microbial communities in the face of *Alliaria*
752 *petiolata* invasions. *The New Phytologist* **189**: 536–548.
753 **Lankau RA, Keymer DP**. **2018**. Simultaneous adaptation and maladaptation of tree populations
754 to local rhizosphere microbial communities at different taxonomic scales. *The New*
755 *Phytologist* **217**: 1267–1278.
756 **Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M, Malfatti S,**
757 **Glavina del Rio T, Jones CD, Tringe SG, *et al.* 2015**. Salicylic acid modulates colonization
758 of the root microbiome by specific bacterial taxa. *Science* **349**: 860–864.
759 **Leff JW, Lynch RC, Kane NC, Fierer N**. **2016**. Plant domestication and the assembly of
760 bacterial and fungal communities associated with strains of the common sunflower,
761 *Helianthus annuus*. *The New Phytologist* **214:** 412–423.

Legendre P, Gallagher ED. **2001**. Ecologically meaningful transformations for ordination of species data. *Oecologia* **129**: 271–280.

Legendre P, Legendre L. **1998**. *Numerical Ecology*. Amsterdam: Elsevier Science.

Levine JM, Adler PB, Yelenik SG. **2004**. A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters* **7**: 975–989.

Lonsdale WM. **1999**. Global patterns of plant invasions and the concept of invasibility. *Ecology* **80**: 1522–1536.

Louca S, Jacques SMS, Pires APF, Leal JS, Srivastava DS, Parfrey LW, Farjalla VF, Doebeli M. **2016**. High taxonomic variability despite stable functional structure across microbial communities. *Nature Ecology & Evolution* **1**: s41559–016–0015.

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, del Rio TG, *et al.* 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488**: 86–90.

Lundberg DS, Yourstone S, Mieczkowski P, Jones CD, Dangl JL. **2013**. Practical innovations for high-throughput amplicon sequencing. *Nature Methods* **10**: 999–1002.

Maron JL, Klironomos J, Waller L, Callaway RM. **2014**. Invasive plants escape from suppressive soil biota at regional scales. *The Journal of Ecology* **102**: 19–27.

Maron JL, Vilà M. **2001**. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* **95**: 361–373.

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. **2012**. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal* **6**: 610–618.

McGinn KJ, van der Putten WH, Duncan RP, Shelby N, Weser C, Hulme PE. **2016**. *Trifolium* species associate with a similar richness of soil-borne mutualists in their introduced and native ranges. *Journal of Biogeography* **43**: 944–954.

McGinn KJ, van der Putten WH, Hulme PE, Shelby N, Weser C, Duncan RP. **2018**. The influence of residence time and geographic extent on the strength of plant-soil feedbacks for naturalised *Trifolium*. *The Journal of Ecology* **106**: 207–217.

McQuitty LL. **1966**. Similarity analysis by reciprocal pairs for discrete and continuous data. *Educational and Psychological Measurement* **26**: 825–831.

Mitchell CE, Agrawal AA, Bever JD, Gilbert GS, Hufbauer RA, Klironomos JN, Maron

JL, Morris WF, Parker IM, Power AG, *et al.* 2006. Biotic interactions and plant invasions. *Ecology Letters* **9**: 726–740.

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, *et al.* 2013. Patterns and processes of microbial community assembly. *Microbiology and Molecular Biology Reviews* **77**: 342–356.

Newton AC, Fitt BDL, Atkins SD, Walters DR, Daniell TJ. **2010**. Pathogenesis, parasitism and mutualism in the trophic space of microbe–plant interactions. *Trends in Microbiology* **18**: 365–373.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, *et al.* 2016. vegan: community ecology package. R package version 2.4-3.

Parker IM, Gilbert GS. **2007**. When there is no escape: the effects of natural enemies on native, invasive, and noninvasive plants. *Ecology* **88**: 1210–1224.

Pearson DE, Ortega YK, Eren Ö, Hierro JL. **2018**. Community assembly theory as a framework for biological invasions. *Trends in Ecology & Evolution* **33**: 313–325.

Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE. **2013**. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences* **110**: 6548–6553.

Petermann JS, Fergus AJF, Turnbull LA, Schmid B. **2008**. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* **89**: 2399–2406.

Pielou EC. **1966**. The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology* **13**: 131–144.

Pitcairn M, Schoenig S, Yacoub R, Gendron J. **2006**. Yellow starthistle continues its spread in California. *California Agriculture* **60**: 83–90.

R Core Team. **2015**. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ramette A. **2007**. Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology* **62**: 142–160.

Reinhart KO, Callaway RM. **2006**. Soil biota and invasive plants. *The New Phytologist* **170**: 445–457.

Reinhart KO, Packer A, Van der Putten WH, Clay K. **2003**. Plant–soil biota interactions and

spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters* **6**: 1046–1050.

Reinhart KO, Tytgat T, Van der Putten WH, Clay K. **2010**. Virulence of soil-borne pathogens and invasion by *Prunus serotina*. *The New Phytologist* **186**: 484–495.

Rout ME, Callaway RM. **2012**. Interactions between exotic invasive plants and soil microbes in the rhizosphere suggest that 'everything is not everywhere'. *Annals of Botany* **110**: 213–222.

Schnitzer SA, Klironomos JN, HilleRisLambers J, Kinkel LL, Reich PB, Xiao K, Rillig MC, Sikes BA, Callaway RM, Mangan SA, *et al.* 2011. Soil microbes drive the classic plant diversity–productivity pattern. *Ecology* **92**: 296–303.

Schrama M, Bardgett RD. **2016**. Grassland invasibility varies with drought effects on soil functioning. *The Journal of Ecology* **104**: 1250–1258.

Seabloom EW, Harpole WS, Reichman OJ, Tilman D. **2003**. Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academy of Sciences* **100**: 13384–13389.

Shannon CE. **1948**. A mathematical theory of communication. *Bell System Technical Journal* **27**: 379–423.

Smakowska E, Kong J, Busch W, Belkhadir Y. **2016**. Organ-specific regulation of growth-defense tradeoffs by plants. *Current Opinion in Plant Biology* **29**: 129–137.

terHorst CP, Lennon JT, Lau JA. **2014**. The relative importance of rapid evolution for plant-microbe interactions depends on ecological context. *Proceedings of the Royal Society: Biological Sciences* **281**: 20140028.

terHorst CP, Zee PC. **2016**. Eco-evolutionary dynamics in plant–soil feedbacks. *Functional Ecology* **30**: 1062–1072.

Torchin ME, Mitchell CE. **2004**. Parasites, pathogens, and invasions by plants and animals. *Frontiers in Ecology and the Environment* **2**: 183–190.

Uller T, Leimu R. **2011**. Founder events predict changes in genetic diversity during human-mediated range expansions. *Global Change Biology* **17**: 3478–3485.

Uygur S, Smith L, Uygur FN, Cristofaro M, Balciunas J. **2004**. Population densities of yellow starthistle (*Centaurea solstitialis*) in Turkey. *Weed Science* **52**: 746–753.

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. **2015**. The importance of the microbiome of the plant holobiont. *The New Phytologist* **206**: 1196–1206.

van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, *et al.* 2013. Plant–soil feedbacks: the past, the present and future challenges. *The Journal of Ecology* **101**: 265–276.

van der Putten WH, Bradford MA, Pernilla Brinkman E, van de Voorde TFJ, Veen GF. **2016**. Where, when and how plant–soil feedback matters in a changing world. *Functional Ecology* **30**: 1109–1121.

Venables WN, Ripley BD. **2002**. *Statistics and computing: modern applied statistics with S*. New York, NY, USA: Springer.

Wagg C, Bender SF, Widmer F, van der Heijden MGA. **2014**. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences* **111**: 5266–5270.

Wickham H. **2009**. *ggplot2: elegant graphics for data analysis*. New York: Springer-Verlag.

Widmer TL, Guermache F, Dolgovskaia MY, Reznik SY. **2007**. Enhanced growth and seed properties in introduced vs. native populations of yellow starthistle (*Centaurea solstitialis*). *Weed Science* **55**: 465–473.

Zavaleta ES, Hulvey KB. **2004**. Realistic species losses disproportionately reduce grassland resistance to biological invaders. *Science* **306**: 1175–1177.

SUPPORTING INFORMATION

Fig. S1. Frequency distributions of readcounts per sample.

Fig. S2. NMDS plot of OTU composition across all sample types.

Fig. S3. Clustering dendrograms for bulk samples from three plant compartments.

Fig. S4. Heat maps showing relative abundance of top 25 OTUs per bulk sample from three plant compartments.

Table S1. Collecting information for populations sampled in this study.

Table S2. Extent of host chloroplast amplification with different PNA clamps.

Table S3. Axis loadings from Principal Component Analysis of three plant compartments.

FIGURE LEGENDS

Fig. 1. The distribution (gray) of yellow starthistle and sampling sites (circles) for this study. Maps detail the native range in Eurasia (a) and the invasion of western North America (b).

886 Previous work has indicated that western Europe is the source for the severe invasion of
887 California, USA (both in dark shading; Barker et al. 2017). Sampling included seven locations in
888 California (b, filled circles), six locations in western Europe and an additional two locations in
889 eastern Europe (a, open circles).
890
891 **Fig 2.** NMDS plots of bacterial OTU composition in phyllosphere (green), endorhizosphere
892 (light blue), and ectorhizosphere (dark blue) samples from native (open symbols) and invaded
893 (closed symbols) ranges. Plotted are a) pooled samples for each sampling location, showing
894 overall separation by range within compartment (stress = 0.14), and b) whole root samples for
895 individual plants within native and invading populations (stress = 0.16). Ellipses indicate 95%
896 confidence intervals for samples grouped by range (native range: dashed lines; invaded range:
897 solid lines).
898
899 **Fig 3.** Relative abundance of (proportion of reads mapping to) phyla in yellow starthistle
900 phyllosphere, ectorhizosphere, and endorhizosphere samples from native and invaded ranges.
901
902 **Fig 4.** Distributions of OTU (a) richness, (b) evenness, and (c,d) diversity ($e^{H'}$) among samples
903 (pooled plants) from each location in the native and invaded ranges for phyllosphere,
904 ectorhizosphere, and endorhizosphere compartments. (a-c) show values for all OTUs and (d)
905 shows values based on OTUs from known pathogen-containing genera. Significant differences
906 from Kruskal-Wallis tests are indicated with asterisks: $^{*}P < 0.05$, $^{(*)}P < 0.1$.
907
908 **Fig 5.** Distributions of endorhizosphere OTU (a) richness, (b) evenness, and (c,d) diversity ($e^{H'}$)
909 among individual plants at each location in the native and invaded ranges. (a-c) show values for
910 all OTUs and (d) shows values based on OTUs from known pathogen-containing genera.
911 Significant differences from Kruskal-Wallis tests are indicated with asterisks: $^{*}P < 0.05$, $^{(*)}P <$
912 0.1.
913
914 **Fig 6.** Bacterial diversity ($e^{H'}$) in endorhizosphere samples pooled by sampling location, as a
915 function of the genetic diversity among plants at the same sites (calculated as the average
916 proportion of pairwise nucleotide differences between alleles (π) at variable positions in the

917 genome; from Barker *et al.*, 2017). Lines show significant positive relationships (linear model: P
918 = 0.02) between microbial and plant diversity at sampling locations in both the native range
919 (open symbols, dashed line) and the invaded range (closed symbols, solid line).
920

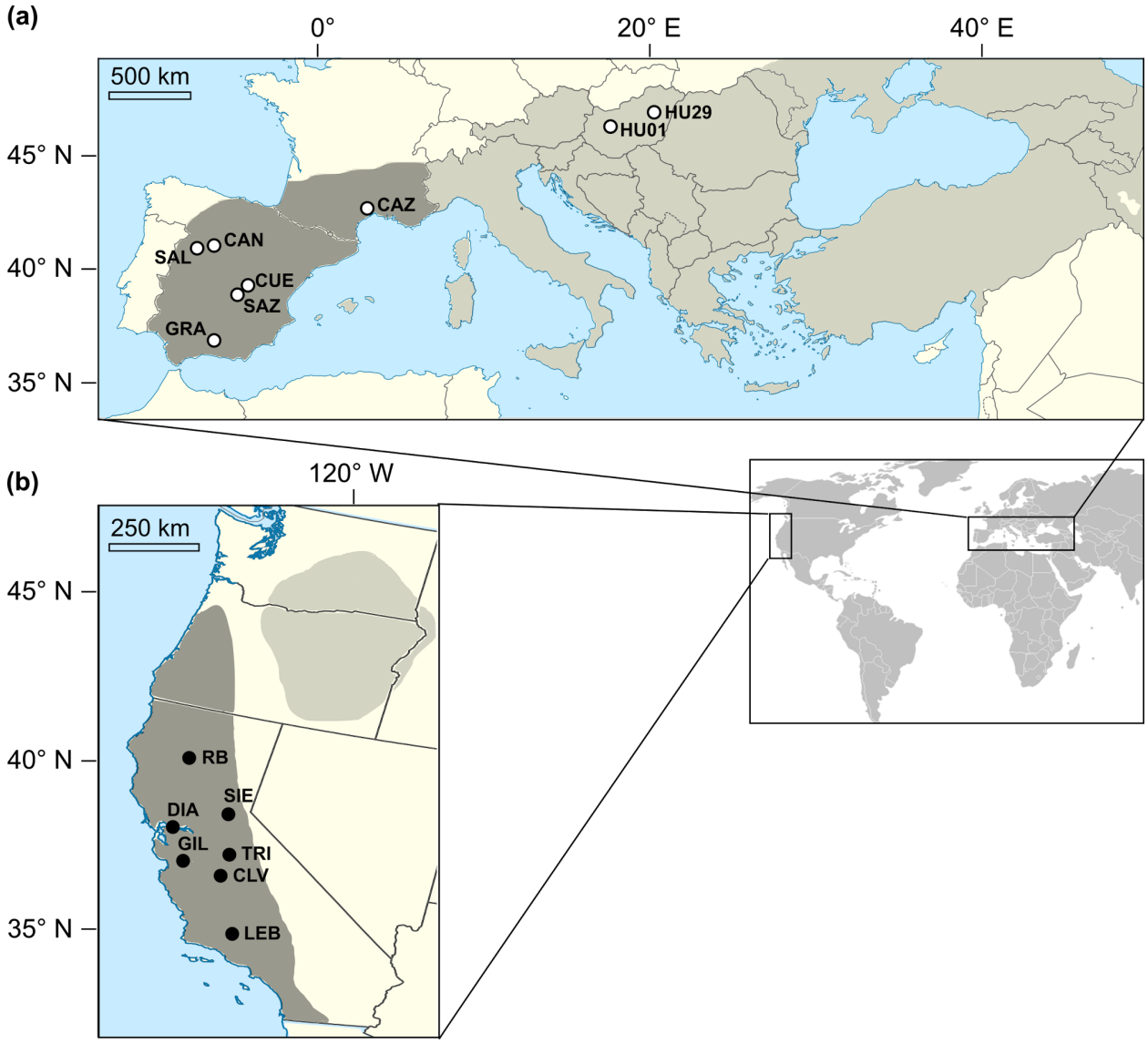

Fig. 1 The distribution (gray) of yellow starthistle and sampling sites (circles) for this study. Maps detail the native range in Eurasia (a) and the invasion of western North America (b). Previous work has indicated that western Europe is the source for the severe invasion of California, USA (both in dark shading; Barker et al. 2017). Sampling included seven locations in California (b, filled circles), six locations in western Europe and an additional two locations in eastern Europe (a, open circles).

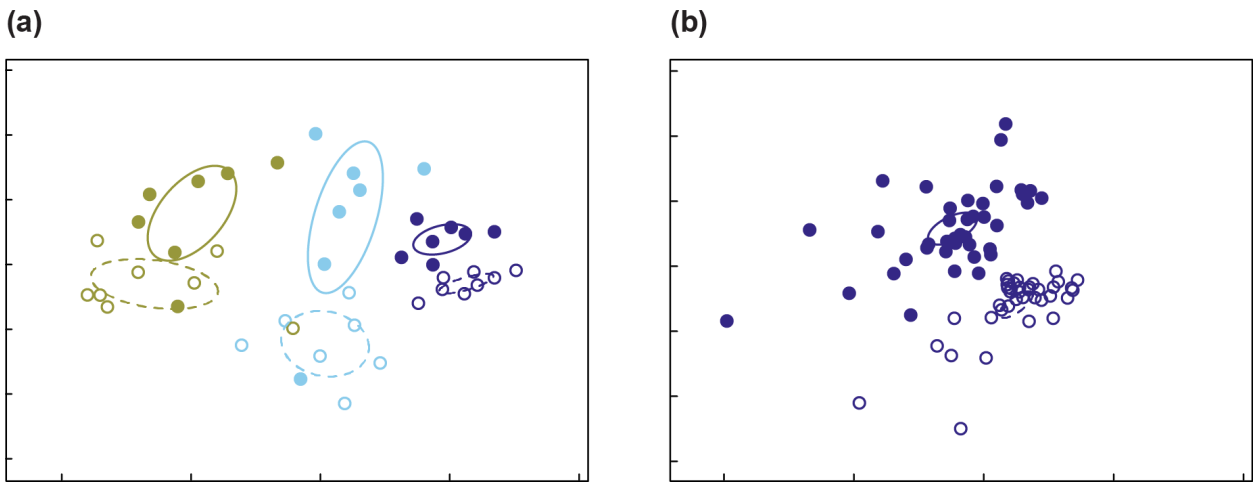

Fig 2. NMDS plots of bacterial OTU composition in phyllosphere (green), endorhizosphere (light blue), and ectorhizosphere (dark blue) samples from native (open symbols) and invaded (closed symbols) ranges. Plotted are a) pooled samples for each sampling location, showing overall separation by range within compartment (stress = 0.14), and b) whole root samples for individual plants within native and invading populations (stress = 0.16). Ellipses indicate 95% confidence intervals for samples grouped by range (native range: dashed lines; invaded range: solid lines).

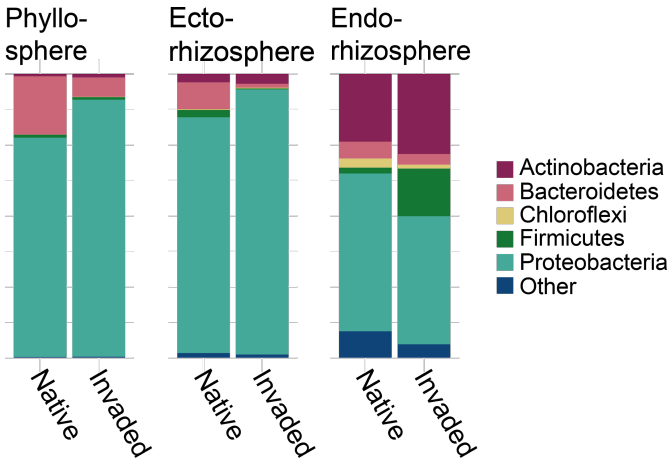

Fig 3. Relative abundance of (proportion of reads mapping to) phyla in yellow starthistle phyllosphere, ectorhizosphere, and endorhizosphere samples from native and invaded ranges.

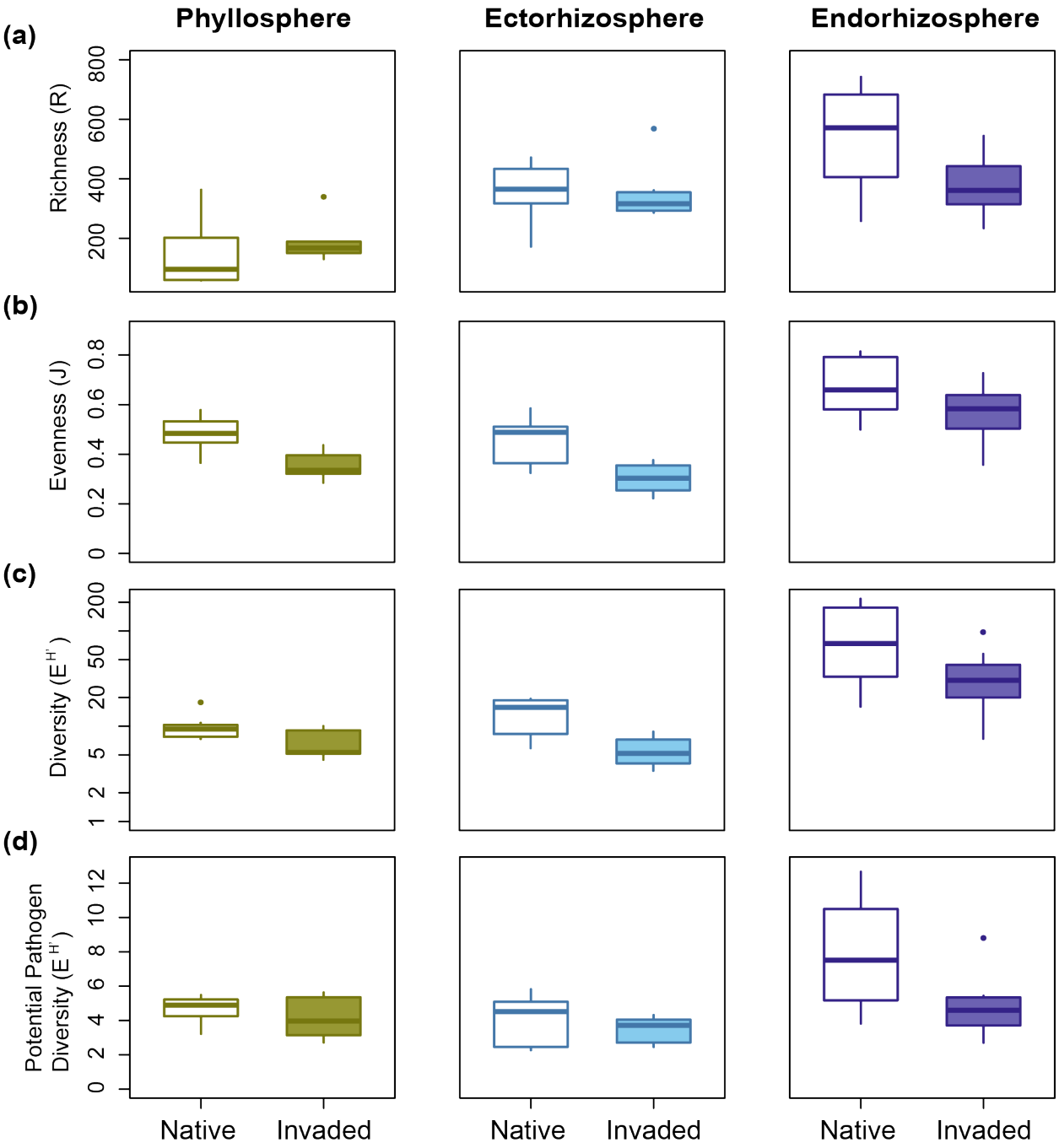

Fig 4. Distributions of OTU (a) richness, (b) evenness, and (c,d) diversity ($e^{H'}$) among samples (pooled plants) from each location in the native and invaded ranges for phyllosphere, ectorhizosphere, and endorhizosphere compartments. (a-c) show values for all OTUs and (d) shows values based on OTUs from known pathogen-containing genera. Significant differences from Kruskal-Wallis tests are indicated with asterisks: $^{*}P < 0.05$, $^{(*)}P < 0.1$.

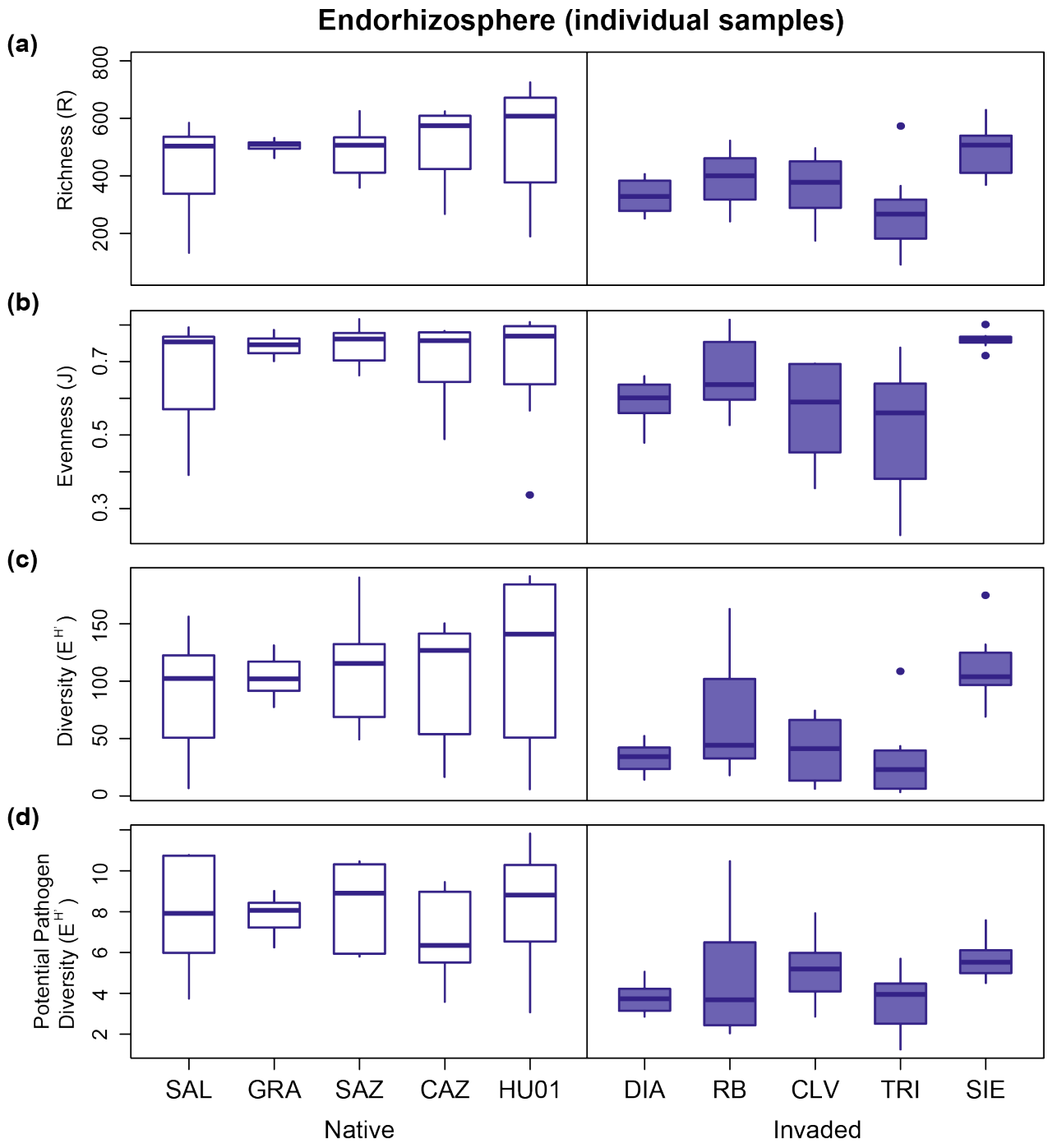

Fig 5: Distributions of endorhizosphere OTU (a) richness, (b) evenness, and (c,d) diversity ($e^{H'}$) among individual plants at each location in the native and invaded ranges. (a-c) show values for all OTUs and (d) shows values based on OTUs from known pathogen-containing genera. Significant differences from Kruskal-Wallis tests are indicated with asterisks: $^{*}P < 0.05$, $^{(*)}P < 0.1$.

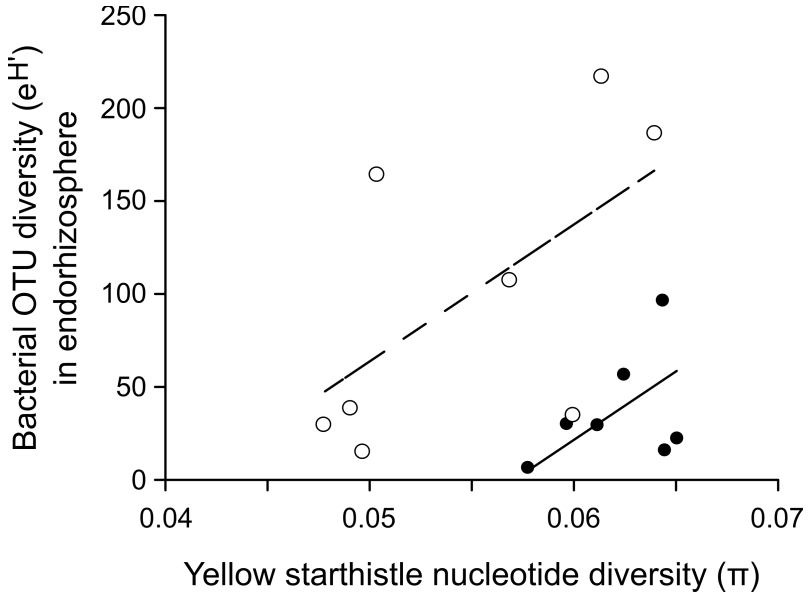

Fig 6. Bacterial diversity ($e^{H'}$) in endorhizosphere samples pooled by sampling location, as a function of the genetic diversity among plants at the same sites (calculated as the average proportion of pairwise nucleotide differences between alleles (□) at variable positions in the genome; from Barker *et al.*, 2017). Lines show significant positive relationships (linear model: $P = 0.02$) between microbial and plant diversity at sampling locations in both the native range (open symbols, dashed line) and the invaded range (closed symbols, solid line).

Supporting Information

Fig. S1 Frequency distributions of un-rarefied read counts for samples from all four plant compartments (native and invading population samples combined), as well as control (blank) samples. Vertical red lines indicate thresholds for rarefaction where relevant.

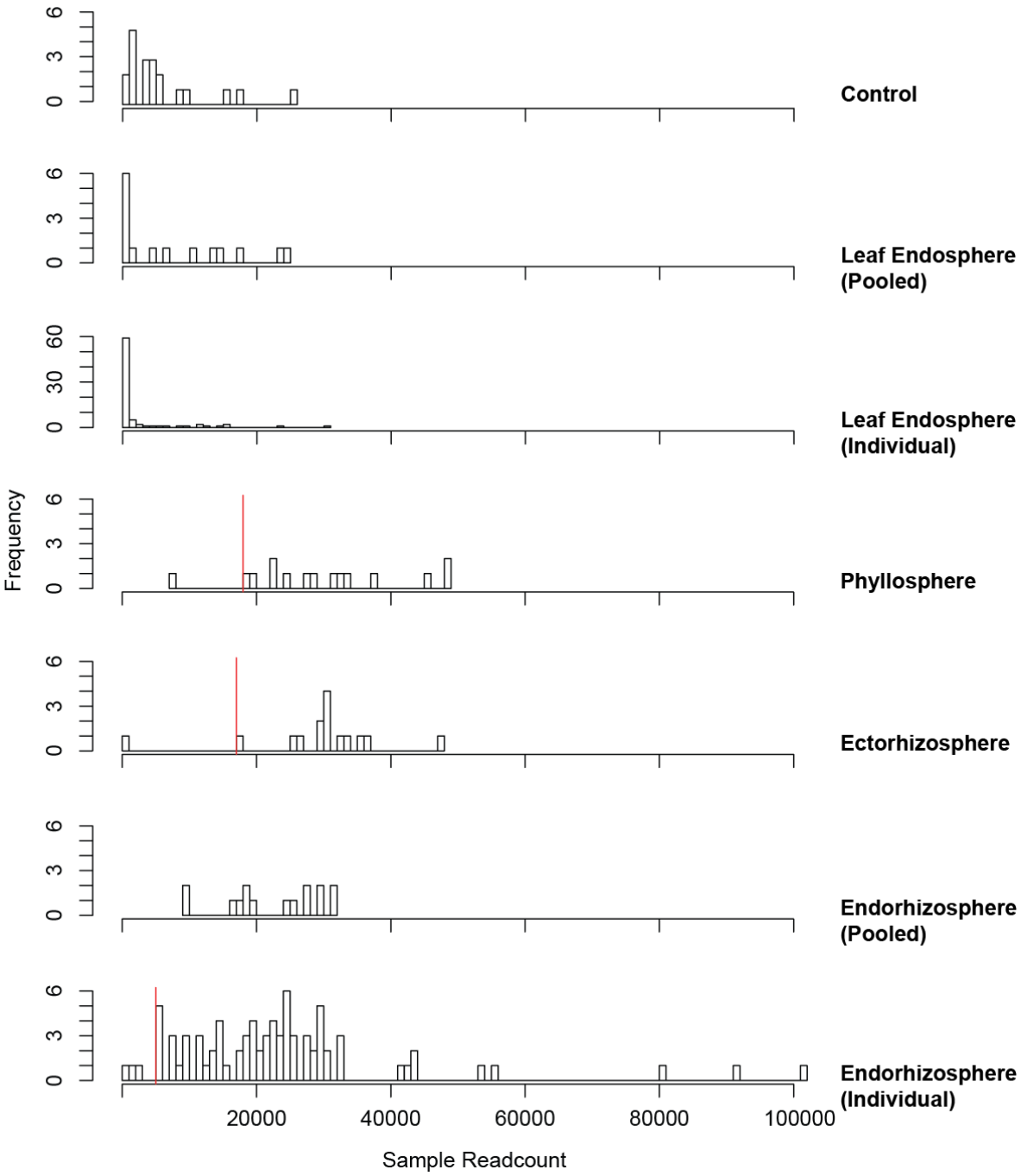

Fig. S2 NMDS plot of bacterial OTU composition in unrarefied control, phyllosphere, leaf endosphere, ectorhizosphere, and endorhizosphere samples (stress = 0.10).

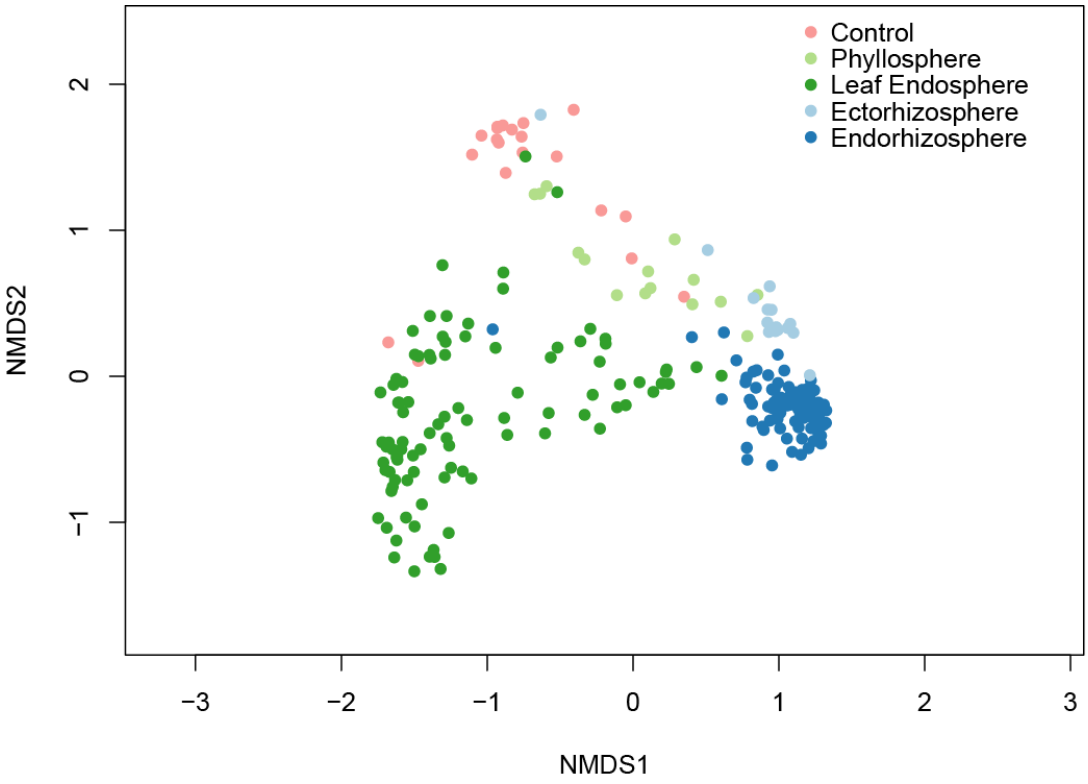

Fig. S3 Yellow starthistle populations clustered according to Bray-Curtis dissimilarity between rarefied, square-root transformed pooled samples from three plant compartments.

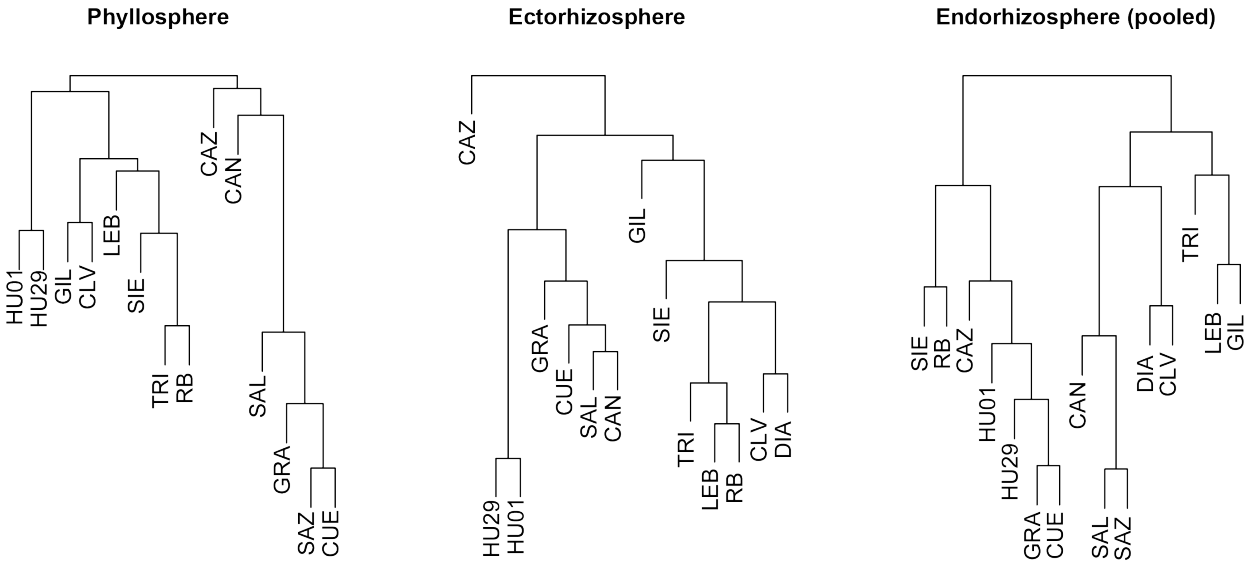

Fig. S4 Heat maps showing the fraction of reads mapping to each of the top 25 OTUs in rarefied bulk samples from three plant compartments. The top five OTUs contributing to the first principal component separating native and invaded samples in each compartment are indicated with an asterisk. Taxonomic assignments for each OTU are given to the lowest taxonomic rank identified.

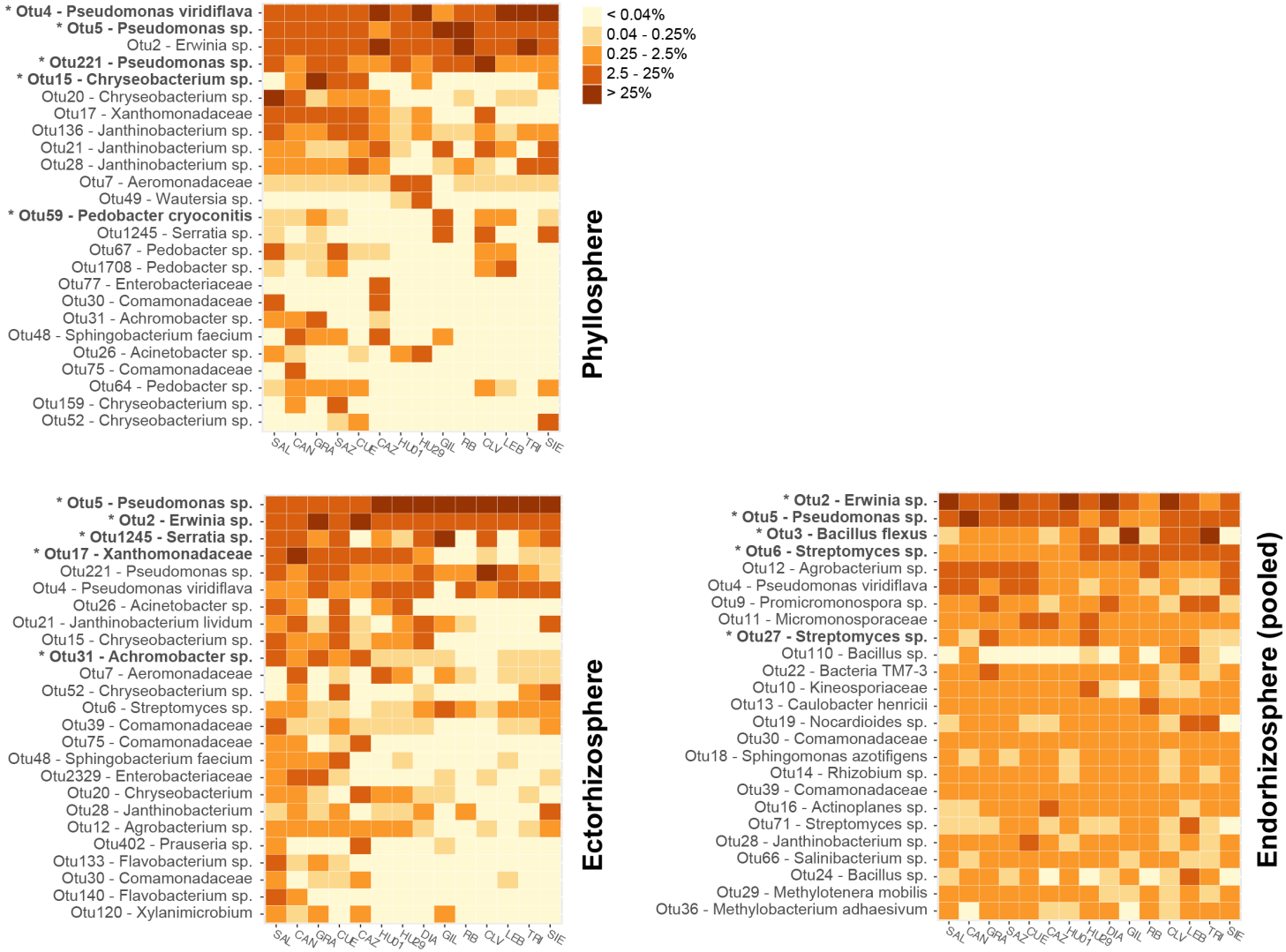

Fig. S5 Proportion of rarefied read counts assigned to pathogen-containing genera in all samples according to plant compartment and range.

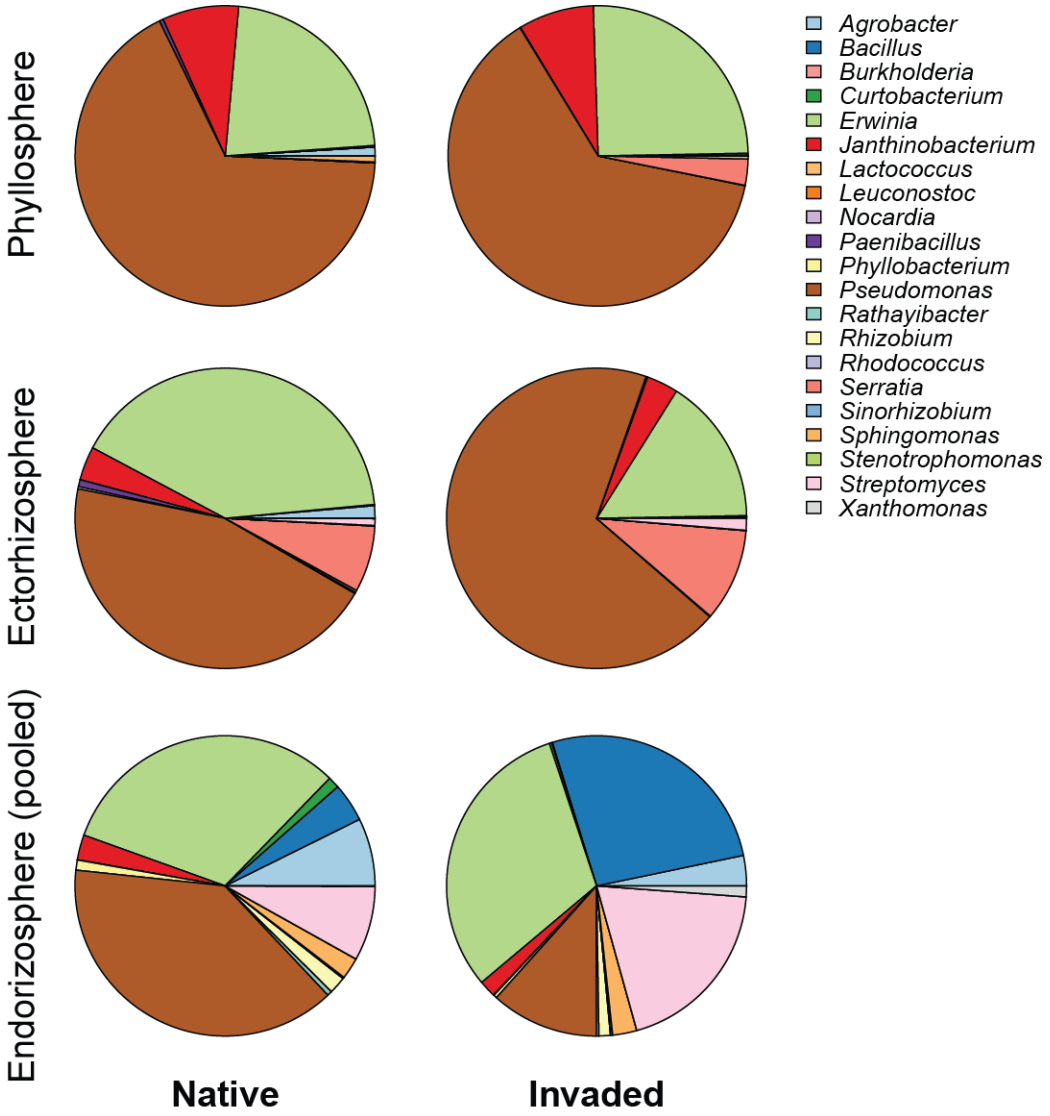

Table S1 Collecting information for populations sampled in this study, including designation codes, localities, coordinates, sampling dates, and specimen accession numbers of plant collections at ARIZ.

Code	Lat.	Long.	Locality	Date	ARIZ specimen numbers
SAL	N40.99112	W005.65831	Avenida de la Merced, ~1.5km N of Salamanca. Dirt path between road and field, on E side of road.	22-Jun-15	426137, 426032, 425636, 426125, 426034, 426033, 426035, 426126, 426140, 426068, 426127, 426128, 426129, 426130, 426131, 426132, 426133, 426134, 426135, 426136, 426138, 425635, 426139
CAN	N41.00085	W004.89715	Junction AV800 & road to Canales. On E side of Canales road, in the narrow strip between road and field edge.	22-Jun-15	425643, 426065, 426064, 426063, 426055, 426054, 425646, 426067, 426053, 426052, 425642, 426066, 426051, 425641, 426062, 426061, 426059, 426058, 426060, 426057, 426056
GRA	N37.26843	W003.66488	GR300 ~100m from junction w 213, ~20m up dirt path to the E side of the road. Weedy waste area near edge of olive orchard.	23-Jun-15	425716, 425715, 425714, 425713, 425739, 425738, 425737, 425736, 425735, 425734, 425733, 425732, 425728, 425740, 425729, 425711, 425702, 425703, 425704, 425705, 425706, 425707, 425708, 425709, 425710
SAZ	N39.83529	W002.50997	Gravel road by W side of CM3118 (road to Villares del Saz), just next to underpass under A3. Dirt farming path (for tractors) on S side of road, between sunflower fields.	24-Jun-15	425678, 425741, 425679, 425731, 425680, 425681, 425682, 425683, 425684, 425685, 425757, 425755, 425753, 425751, 425749, 425746, 425744, 425743, 425758, 425756, 425752, 425750, 425748, 425747, 425745, 425742
CUE	N40.12939	W002.13880	CM2105 between Cuenca & Tragacete ~8km W of Cuenca. Gravel road to the N of 2105, between field and river.	24-Jun-15	425424, 425421, 425420, 425423, 425425, 425426
CAZ	N43.74958	E003.77119	D113, ~1km from junction w 986, near Cazevielle. Flat, gravelly turnout & intersection w unpaved road, & gravelly roadsides.	20-Jun-15	425691, 425690, 425971, 425688, 425689, 425972, 425692, 425970, 425693
HU01	N47.18240	E18.08928	Unnamed dirt roads about 200m NW of E66/8, running parallel. Access by perpendicular unnamed paved road.	7-Jul-15	425655, 425660, 425661, 425653, 425652, 425658, 425657, 425656, 425654, 425631, 425687, 425634, 425633, 425686, 425630, 425628, 425629, 425627, 425662

HU29	N47.31785	E21.03385	Margin of irrigation ditch bordered by hay and sunflower fields on one side and service road parallel to main road (E60/4) on the other side. Site to the south of E60/4.	8-Jul-15	425730, 425677, 425676, 425640, 425675, 425674, 425673, 425726, 425725, 425724, 425723, 425722, 425721, 425720, 425719, 425718, 425717, 425727
DIA	N37.86275	W121.98003	Hillside to the West of parking lot at the end of Green Valley Road.	8-Jun-15	425394, 425400, 425401, 425402, 425403, 425404, 425405, 425406, 425407, 425408, 425409, 425115, 425381, 425410, 425382, 425383, 425384, 425385, 425386, 425387, 425388, 425389, 425390, 425391, 425392
GIL	N37.03389	W121.53611	Disturbed grassy area adjacent to turn out on South side of Leavesley Rd. near intersection with New Ave. Grassy patch between road and tilled field.	7-Jun-15	425374, 425373, 425380, 425379, 425378, 425377, 425376, 425375, 425124, 425123, 425122, 425121, 425119, 425120, 425118, 425114, 425113, 425399, 425112, 425395, 425111, 425398, 425397, 425396
RB	N40.27085	W122.27103	Lightly grassy patch to north-west of rest stop (Herbert S. Miles rest area) between Cottonwood and Red Bluff on southbound I-5.	9-Jun-15	425372, 425371, 425370, 425369, 425368, 425418, 425365, 425415, 425367, 425366, 425416, 425417, 425419
CLV	N36.91603	W119.79341	Gully on eastern side of the Yosemite fwy (41) adjacent to olive orchards.	6-Jun-15	426046, 426045, 426044, 426043, 426042, 426036, 426037, 425637, 426038, 426039, 426040, 426041, 425639, 426026, 426027, 426028, 426029, 426030, 426031, 425638
LEB	N34.82844	W118.87369	Cal trans maintained, weedy area adjacent to SW of rest stop off of south bound I-5.	5-Jun-15	425968, 425695, 425969, 425966, 425967, 426047, 425694, 426050, 426049, 426048
TRI	N37.46159	W119.79399	Strip of grassland along south side of Hwy 49 at intersection with Triangle road. Strip between road and fenced off rangeland.	11-Jun-15	425116, 425117, 425414, 425412, 425413, 425411, 425393
SIE	N38.78162	W120.41639	Site upslope of Weber Mill rd. about ¼ mile from the turnoff from Ice House road.	10-Jun-15	425648, 425647, 425649, 425644, 425645

Table S2 Comparison of host chloroplast blocking levels achieved by different PNA clamps in pooled endorhizosphere samples from two populations. Total read counts, number of reads mapping to host chloroplast, and host chloroplast reads as percentage of total reads are reported for each sample.

Sample	plastid PNA	total	chloroplast	% chloroplast
CAN pooled endorhizosphere	Lundberg *et al.* 2013 (GGCTCAACCCTGGACAG)	14,763	2,873	19
CAN pooled endorhizosphere	Asteraceae-specific (GGCTCAACTCTGGACAG)	12,579	91	1
CUE pooled endorhizosphere	Lundberg *et al.* 2013 (GGCTCAACCCTGGACAG)	11,962	6,318	53
CUE pooled endorhizosphere	Asteraceae-specific (GGCTCAACTCTGGACAG)	14,608	314	2

Table S3 Loading by top 10 individual OTUs along first principal component axis from analysis of Hellinger transformed data matrices for three plant compartments. Column A: individual OTU unique identification number; column B: individual OTU lowest taxonomic assignment; column C: axis loading per individual OTU.

Phyllosphere		
A	B	C
15	*Chryseobacterium*	0.51
4	*Pseudomonas viridiflava*	0.41
17	Xanthomonadaceae	0.32
221	*Pseudomonas*	0.27
2	*Erwinia*	0.26
20	*Chryseobacterium*	0.22
136	*Janthinobacterium*	0.20
7	Aeromonadaceae	0.18
49	*Wautersiella*	0.16
31	*Achromobacter*	0.16

Ectorhizosphere		
A	B	C
5	*Pseudomonas*	0.62
17	Xanthomonadaceae	0.44
31	*Achromobacter*	0.24
26	*Acinetobacter*	0.24
15	*Chryseobacterium*	0.20
48	*Sphingobacterium faecium*	0.15
75	Comamonadaceae	0.14
4	*Pseudomonas viridiflava*	0.13
221	*Pseudomonas*	0.13
2329	Enterobacteriaceae	0.12

Endorhizosphere		
A	B	C
2	*Erwinia*	0.56
3	*Bacillus flexus*	0.41
5	*Pseudomonas*	0.41
6	*Streptomyces*	0.17
10	Kineosporiaceae	0.15
11	Micromonosporaceae	0.13
4	*Pseudomonas viridiflava*	0.13
27	*Streptomyces*	0.11
13	*Caulobacter henricii*	0.10
23	Actinomycetales	0.10