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1 Running head: Nutrients homogenize leaf microbiome

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3 **Nitrogen enrichment suppresses other environmental drivers and homogenizes salt marsh**

4 **leaf microbiome**

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20

Abstract:

Microbial community assembly is affected by a combination of forces that act simultaneously, but the mechanisms underpinning their relative influences remain elusive. This gap strongly limits our ability to predict human impacts on microbial communities and the processes they regulate. Here, we experimentally demonstrate that increased salinity stress, food web alteration and nutrient loading interact to drive outcomes in salt marsh fungal leaf communities. Both salinity stress and food web alterations drove communities to deterministically diverge, resulting in distinct fungal communities. Increased nutrient loads, nevertheless, partially suppressed the influence of other factors as determinants of fungal assembly. Using a null model approach, we found that increased nutrient loads enhanced the relative importance of stochastic over deterministic divergent processes; without increased nutrient loads, samples from different treatments showed a relatively (deterministic) divergent community assembly whereas increased nutrient loads drove the system to more stochastic assemblies, suppressing the effect of other treatments. These results demonstrate that common anthropogenic modifications can interact to control fungal community assembly. Furthermore, our results suggest that when the environmental conditions are spatially heterogeneous (as in our case, caused by specific combinations of experimental treatments), increased stochasticity caused by greater nutrient inputs can reduce the importance of deterministic filters that otherwise caused divergence, thus driving to microbial community homogenization.

KEYWORDS: *Microbial community assembly, leaf fungal communities, nutrient loading, deterministic vs neutral processes, salt marshes, Spartina*

43 **Introduction:**

44 There is increasing acceptance that the assembly of species into natural communities is
45 affected by a combination of both deterministic and stochastic forces that act simultaneously
46 (Leibold and McPeek 2006, Vellend 2010). The current challenge is to understand the
47 mechanisms underpinning their relative influences (see Chase 2007, 2010, Vellend et al. 2014).
48 Understanding community assembly processes that maintain biological diversity is especially
49 challenging for microbial systems where communities are often overwhelmingly diverse (Sogin
50 et al. 2006, Allison and Martiny 2008) and comprised of a high number of competing yet co-
51 occurring species (Foster 2012, Coyte et al. 2015, Widder et al. 2016). For example, next-
52 generation sequencing technologies reveal tremendous microbial diversity existing over small
53 spatial scales in a wide range of environments, including an astonishing number of ~20,000-
54 50,000 species per gram of soil (Roesch et al. 2007), ~1,000-3,000 species per liter of open ocean
55 water (Sogin et al. 2006, Walsh et al. 2016) and ~100-1,000 species in the gut of each human
56 (Browne et al. 2016). These patterns of high diversity, and the widespread presence of apparently
57 redundant species, suggest potential limitations in extrapolation of species coexistence theory to
58 microbial communities (Prosser et al. 2007).

59 Historically, determinants of microbial diversity were interpreted almost exclusively under
60 the paradigm of niche theory (see Dini-Andreote et al. 2015), a position immortalized in Baas
61 Becking and Beijerinck's famous and highly cited phrase "Everything is everywhere, but the
62 environment selects" (see De Wit and Bouvier 2006). In short, given their tremendous dispersal
63 capabilities, microbes had been thought to be unlimited in their dispersal and therefore
64 ubiquitous. According to this view, environmental conditions alone should determine the

65 presence of each species in a site (Fenchel and Finlay 2004), creating predictive and limited-
66 membership communities with low site-to-site variability in species composition (community
67 convergence) among sites with similar environmental conditions and high site-to-site variability
68 in species composition (community divergence) among sites with different environmental
69 conditions (i.e. ecological selection among species, see Vellend 2010; Chase and Myers 2011).
70 Over the past decade, observational field work, fueled by next-generation sequencing of complex
71 microbial communities, has shown that important environmental drivers/filters may exist and can
72 affect microbial assemblies. For example, segregation of microbial community composition
73 commonly occurs across environmental and ecological gradients, including salinity (Mohamed
74 and Martiny 2011), soil pH (Fierer and Jackson 2006, Siciliano et al. 2014), water depth (Walsh
75 et al. 2016) and successional stage (Zhou et al. 2014, Dini-Andreote et al. 2015). In apparent
76 contrast, many other studies have recently suggested that microbial systems are, instead,
77 constrained by dispersal (see Ramirez et al. 2014, Albright and Martiny 2018) and strongly
78 influenced by processes such as stochastic recruitment and ecological drift (Prosser et al. 2007,
79 Woodcock et al. 2007, Ofiteru et al. 2010, Vellend 2010). These stochastic processes should
80 constrain the deterministic ones leading to high site-to-site variability in species composition
81 (community divergence) even among sites with similar environmental conditions (see Chase and
82 Myers 2011). Despite the strong contrast in the underlying mechanism and predicted effects, it
83 has recently been suggested that deterministic and stochastic processes simultaneously influence
84 microbial communities and that their relative influence can vary across environmental conditions
85 (Stegen et al. 2012, 2013, Zhou et al. 2014, Dini-Andreote et al. 2015). This more recent idea has
86 been widely examined in lab settings or with observational data, and has just started to be

87 experimentally tested in the field (e.g. Evans et al. 2017, Vannette et al. 2017, Albright and
88 Martiny 2018), fueling the advance of our understanding of microbial community assembly
89 processes and our capacity to potentially predict community dynamics.
90 Here, we present the response of leaf fungal communities to a factorial field experiment
91 manipulating nutrient loading, salinity stress and food web structure in a coastal wetland. By
92 applying an ecological null model approach (Chase et al. 2011) to changes in microbial species
93 abundances, this experiment allowed us to evaluate not only the effect of these factors on leaf
94 fungal community assembly, but also how these factors mediate the relative contribution of
95 deterministic and stochastic processes as drivers of fungal assembly. We hypothesize that salinity
96 stress and food web alterations will drive to distinctive fungal communities by acting as
97 ecological filters that select among species (see Chase 2007, Vellend 2010, Zhou et al. 2014),
98 thus increasing the importance of deterministic over stochastic processes. Increased nutrient
99 loads, nevertheless, will exert weak selection among species (as greater availability of resources
100 allow most species in the regional species pool to survive; see Chase 2010, Zhou et al. 2014),
101 thus increasing the importance of stochastic over deterministic processes. We further hypothesize
102 that, by decreasing the importance of divergent selection among species, increased nutrient loads
103 will overcome salinity stress and food web alterations as deterministic filters, thus driving to
104 community convergence.
105

106 Materials and Methods

107 *Study site*

108 This study was performed in a salt marsh located near a creek at the mouth of the Mar

109 Chiquita coastal lagoon (Argentina, 37º 32´ S; 57º 19´ W). This lagoon is affected by semidiurnal
110 microtides (< 1 m) and is characterized by mudflats in the low zone followed by a *Spartina*
111 *densiflora* monoculture at intermediate elevations and an extended salt marsh community at high
112 elevations. The marsh is dominated by the intertidal burrowing crab *Neohelice granulata* that,
113 through grazing, can exert strong control over marsh plant production by directly removing plant
114 tissue as well as by facilitating fungal infection in crab-generated injuries (Daleo et al. 2009). As
115 in other worldwide salt marshes, nutrient availability and soil salinity can also exert a strong
116 control of primary production (Alberti et al. 2010).
117
118 *Experimental set-up*
119 A fully-factorial experiment was conducted in the *S. densiflora* monoculture zone. The
120 factorial design included: salinity stress (with and without salt addition), food web structure (with
121 and without herbivorous crabs) and nutrients loads (with and without nutrient addition)
122 implemented in 0.7 x 0.7 m plots. Each treatment combination was replicated 6 times (for a total
123 of 48 plots). Crab-exclusion plots were surrounded using a plastic mesh (10 mm opening) fence
124 0.6 m high and supported by iron stakes. Crab exclosures have been widely used in this system
125 and the use of cage controls revealed that there are no associated cage artifacts (Daleo et al.
126 2015). Salt addition plots received 20 g (~40 g m^{-2}) of commercial pelletized salt spread
127 superficially every 2 weeks. This salt loading increased plant tissue salinity at least up to 35%,
128 leaf surface salinity by near 400% (Canepuccia et al. 2010) and decreased plant growth by 50%
129 (Daleo et al. 2015). Nutrient addition treatments received 60 g (~120 g m^{-2}) of a slow-release
130 pelletized fertilizer (NPK: 29:5:5) monthly. This fertilization rate increased biomass production

131 by more than 400%, increased sediment nitrates by more than an order of magnitude (i.e. from
132 1.37± 0.14 µmol L^{-1} to 85.24± 24.28 µmol L^{-1}) and doubled *S. densiflora* leaf N content (Alberti
133 et al. 2011, Daleo et al. 2015). Fertilizer was spread into 6 artificial holes (5 cm deep, 1 cm
134 diameter) evenly distributed in each plot that were then filled with mud. The experiment started
135 on March 2010 and after one year (i.e. March 2011), 3 leaves were sampled from each plot. The
136 number of sampled leaves per plot were constrained by practical issues but similar sampling
137 designs have been shown to be adequate for leaf fungal community estimations (e.g. Jumpponen
138 and Jones 2009, 2010). For the herbivory treatment plots, leaves with crab-induced injuries were
139 sampled (Daleo et al. 2009). Leaf samples where transported to the laboratory, rinsed in sterile
140 H_2O to remove non-adhering fungal spores and other adhered particles before extraction. A
141 section of leaf laminae of 10 mm length was taken from each leaf, avoiding necrotic areas, and
142 the 3 sections from each plot were pooled for DNA extraction.
143
144 *DNA extraction, ITS2 library preparation, and sequencing*
145 Total genomic DNA (gDNA) was extracted from samples with UltraClean® Soil DNA
146 Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) following manufacturer's instructions,
147 and eluted in 50 µL of solution S5 (sterile elution buffer). The DNA yields were quantified with a
148 Nanodrop ND2000 spectrometer (Thermo Scientific, Wilmington, Delaware) and adjusted to a
149 final 1 ng/µL concentration. We targeted the Internal Transcribed Spacer region 2 (ITS2) for
150 amplification. ITS2 has been proposed as the universal metabarcode marker for fungi (Schoch et
151 al. 2012), because of its interspecific hypervariability. We amplified the ITS2 region in a 2-step
152 PCR. Primary PCRs included the forward primer ITS1F (Gardes and Bruns 1993) and the reverse

153 primer ITS4 (White et al. 1990). Each primary PCR contained 1 μM of each primer, 10 ng of
154 template gDNA, 200 μM of dNTPs, 1.5 mM MgCl2, 0.5 units of Phusion Green Host Start II
155 High-Fidelity DNA polymerase, and 10 uL of 5x Phusion Green HF PCR buffer (Thermo
156 Scientific, Waltham, MA). Primary PCR conditions consisted of initial denaturation at 94°C for
157 10 sec., and then 25 cycles of 94°C for 10 sec., 53°C for 30 sec., and 72°C for 2 min., followed
158 by final extension at 72°C for 8 min. To minimize primer carryover, primary PCRs were purified
159 with Diffinity RapidTips (Diffinity Genomics, West Henrietta, NY). Five uL of each primary
160 PCR was used as DNA template in secondary PCRs with a nested forward primer fITS7 (Ihrmark
161 et al. 2012) and ITS4 with a unique molecular identifier tag. The reaction conditions of secondary
162 PCRs were identical to the primary PCR reactions, but were carried out for ten cycles. Secondary
163 PCRs were cleaned with the AMPure XP bead system (1:1 bead to PCR volume ratio; Beckman
164 Coulter Inc., Brea, CA), quantified on a Nanodrop ND2000, and 100 ng for each experimental
165 unit pooled. The ITS2 amplicon library was sequenced on the Illumina MiSeq platform (v. 2;
166 2x250) at the Integrated Genomics Facility at Kansas State University. Raw sequence data (.fastq
167 files) are available in the National Center for Biotechnology Information (NCBI) Sequence Read
168 Archive under BioProject PRJNA378881 and BioSamples SAMN06563186-06563230.

169 Sequence data were analyzed using mothur (v. 1.32.1) (Schloss et al. 2009, 2011). The
170 paired-end .fastq files were contiged with a 100 bp minimum overlap, and subsequently had
171 homopolymers (maximum of 8 allowed), and sequences with any mismatch to primer or barcode
172 filtered. Sequences were then truncated to 250 bp, > 99% similar sequences pre-clustered (Huse
173 et al. 2010), and potential chimeras removed (UCHIME) (Edgar et al. 2011). The quality
174 screened sequences were pairwise aligned to retrieve a distance matrix, assigned to Operational

175 Taxonomic Units (OTUs) at 97% similarity using average neighbor joining, and rare OTUs (n ≤
176 10) omitted (Tedersoo et al. 2010). OTUs were assigned to taxon affinities using a naïve
177 Bayesian classifier (Wang et al. 2007) and the UNITE-curated INSD (International Nucleotide
178 Sequence Databases) reference database (Abarenkov et al. 2010), and the complete taxonomic
179 affinity strings retrieved (Table S1). We did not detect any OTUs not classified to Kingdom
180 Fungi. All experimental units were subsampled to 10,000 sequences to minimize sample loss but
181 retain as many high quality sequences as possible to have even and adequate library coverage. We
182 found a total of 305 fungal OTUs (Appendix S1:Table S1) in the final dataset.
183
184 *Statistical analysis*
185 We used ANOVA to evaluate the separate and interactive effects of salinity stress, food web
186 structure and nutrient loading on OTUs richness (i.e. number of OTUs per sample), OTUs
187 diversity (Shannon diversity index) and OTUs evenness. Data was transformed if visual
188 inspection of residual plots revealed any obvious deviations from homoscedasticity or normality.
189 To evaluate the separate and interactive effects of salinity stress, food web structure and nutrient
190 loading on fungal community composition, we performed a permutational multivariate analysis
191 of variance (PERMANOVA) (Anderson 2001) based on the Bray-Curtis dissimilarity index
192 applied to fourth-root transformed data (to reduce the weight of the most abundant OTUs), with
193 9999 permutations. We previously performed an analysis of multivariate homogeneity of group
194 dispersions to evaluate if homogeneity of group dispersions is achieved (Anderson et al. 2006).
195 We performed pairwise comparisons after significant interactions of PERMANOVA with the
196 *pairwise.perm.manova* function of the *RVAideMemoire* package (Hervé 2018). We also looked at

197 the treatment effects on species assembly using non-metric multi-dimensional scaling ordination
198 (NMDS) based on the Bray-Curtis dissimilarity index (Warwick and Clarke 1991). The NMDS
199 ordinations were obtained using the *metaMDS* function of the *vegan* package in R (v 2.3-0)
200 (Oksanen et al. 2015). To evaluate if nutrient loads affected variability in community composition
201 by suppressing divergence caused by the other experimental factors, we performed an analysis of
202 multivariate group dispersions (Anderson et al. 2006) comparing dispersion of samples coming
203 from plots with and without increased nutrient loads, with 9999 permutations. To be able to
204 perform this analysis, we first evaluated the non-existence of interactive effects among factors on
205 multivariate group dispersion. We performed this analysis using the *betadisper* and *permutest*
206 functions of the *vegan* package (Oksanen et al. 2015).

207 To be able to disentangle whether differences in variability were the result of differences in
208 the underlying assembly mechanisms (i.e. the relative importance of stochastic and deterministic
209 processes in community assembly), we used a null model approach that is a slight modification
210 (to consider species abundances; see Stegen et al. 2013) of the null model proposed by Chase et
211 al. (2011), which in turn is a slight modification of the Raup-Crick (RC) index (Raup and Crick
212 1979, Chase et al. 2011, Stegen et al. 2013). For more details about the rationale of using such
213 null models to evaluate the relative role of different assembly processes in shaping ecological
214 communities see Mori et al. (2015). The null model was constructed by performing a probability-
215 based randomization, in which randomly generated OTUs composition and abundance were
216 assembled for each sample by randomly sampling from the total OTU pool (estimated as the list
217 of OTUs observed in all sampling units) under four constrains: (1) the number of OTUs of the
218 randomly generated sample equals the number of OTUs actually observed in the sample, (2) the

219 probability of occurrence (i.e. probability of being present in a sample) of a given OTU was
220 proportional to its observed total occurrence frequency (i.e. the proportion of samples where this
221 OTU was actually observed), (3) the total abundance of the randomly generated sample equals
222 the total abundance actually observed in the sample and (4) the abundance probability of each
223 OTU in the randomly generated sample was proportional to its observed total abundance. For all
224 possible pairs of plots, OTU composition of each plot was probabilistically generated 9999 times.
225 For each iteration, Bray-Curtis dissimilarity index between plots was calculated, and the resulting
226 metric was the proportion of iterations in which the index was smaller than or equal to the
227 actually observed Bray-Curtis dissimilarity index between those pair of plots (Chase et al. 2011,
228 Stegen et al. 2013). Finally, we standardized the metric to range from -1 to 1 by subtracting 0.5
229 and multiplying by 2 (Chase et al. 2011), with negative values indicating that a pair of
230 communities is more similar than expected at random (deterministic convergence), positive
231 values indicating that a pair of communities is more dissimilar than expected at random
232 (deterministic divergence), and zero indicating that a pair of communities is as similar as
233 expected at random (purely neutral community assembly). The selection of species (OTUs in our
234 case) pool plays a fundamental role in the calculation of this metric, and following others (e.g.
235 Chase 2010, Chase et.al. 2011, Stegen et al. 2013, Alberti et al. 2017) we defined the species pool
236 as the list of species (OTUs) found in samples throughout the experiment. The R script of the
237 used model can be found at https://github.com/stegen/Stegen_etal_ISME_2013. This metric can
238 be used not only to calculate the probability of deviation from purely neutral expectation (Chase
239 et al. 2011, Stegen et al. 2013) but also as a dissimilarity index that provides a quantitative
240 estimation of the relative role of deterministic and stochastic processes in shaping community

241 composition, and can be analyzed using statistical methods similar to those used for other
242 pairwise dissimilarity indexes (Zhou et al. 2014). Thus, the metric allows to test if the relative
243 contribution of deterministic and stochastic processes in community assembly differ among
244 treatments (see Alberti et al. 2017). We performed the analysis of multivariate homogeneity of
245 group dispersions (Anderson et al. 2006) based on the dissimilarity matrices constructed with
246 these metrics. Significant results indicate that groups differ from another in its RC metric (i.e.
247 differ in the relative importance of deterministic and neutral processes in community assembly).
248 We started by evaluating multivariate homogeneity of group dispersions among samples from the
249 individual treatment level (i.e. the levels of the 3 way interaction differ in its RC metric). If
250 significant differences were not detected we moved to evaluate homogeneity among samples
251 from the levels of the 2 way interactions and, finally, among samples from the levels of the main
252 factors. This approach is not like classical factorial approaches where interactions and main
253 effects are addressed at once because the RC metric is essentially a distance metric and, thus, is
254 not a fixed value but a value that changes at different levels of the factorial design. However, it is
255 the most reliable way to analyze homogeneity of group dispersion in such designs. We performed
256 this analysis using the *betadisper* and *permutest* functions from *vegan* package in R (Oksanen et
257 al. 2015).
258
259 **Results:**
260 Plants subjected to salinity stress had ~10% fewer OTUs (log transformed data, ANOVA:
261 $F_{1,37} = 11.31$, $P = 0.0018$; Fig. 1A), but we observed neither an effect of herbivore removal nor
262 increased nutrient loads (Appendix S1:Table S2). As this pattern can be masked by the

263 persistence of very low frequency OTUs, we re-analyzed data removing all OTUs that occurred
264 at frequencies < 1 % in each sample. We found that plants exposed to nutrient enrichment had
265 ~16% higher number of frequent OTUs ($F_{1,37}$ = 11.12, P = 0.002; Fig. 1B). We also found that
266 nutrient loads and the interaction between salinity stress and presence of herbivores affected OTU
267 diversity and evenness (see Appendix S1:Fig. S1; Appendix S1:Table S2); plants exposed to
268 nutrient enrichment presented higher OTU diversity and evenness (see Appendix S1:Fig. S1).
269 Salinity stress, presence of herbivores and nutrient loads interactively affected community
270 composition (PERMANOVA: pseudo$F_{1,37}$ = 1.63, P = 0.02; see Table S3 for specific individual
271 and interactive effect of factors; see Appendix S1:Fig. S2 for changes in abundance of the 15
272 most abundant OTUs). Pairwise comparisons show 4 compositional groups; the first group
273 included 3 treatments with nutrient addition (Nutrient addition; Herbivory and Nutrient addition;
274 Herbivory, Salt and Nutrient addition) as well as the treatment with Salt addition and Herbivory.
275 The second group included the treatment with Salt addition and the treatment with Salt and
276 Nutrient addition (see Fig. 2). The third and fourth groups were formed by Control treatment and
277 the treatment with Herbivory respectively (see Fig. 2). Regarding the variability in species
278 composition, results of the analysis of multivariate group dispersions show that it was not
279 affected by any of the potential interactions between factors. Moreover, it was only affected
280 (reduced) by nutrient addition (pseudo$F_{1,43}$= 6.09, P= 0.015). This reduction in variability was
281 driven by a smaller difference in composition between those treatments with nutrient addition
282 (i.e. regardless of the other factor combinations, all treatments with nutrient addition were more
283 similar in composition compared with treatments without nutrient addition; see Fig. 2, Appendix
284 S1:Fig. S3). In other words, community composition from the different treatments were much

285 more similar to each other when their nutrient loads were increased.

286 By using the null model approach based on the extended RC metric, we found that the

287 interactions among factors, as well as the main factors salinity stress and presence of herbivores,

288 did not affect the relative contribution of stochastic over deterministic processes (Appendix

289 S1:Fig. S4) but increased nutrient loads, as a main factor, significantly increased the importance

290 of neutral processes ($pseudoF_{1,43}$= 4.84, P= 0.034; Fig. 3). As different combinations of the

291 factors salinity stress and herbivory deterministically led to different community assemblies only

292 when applied without increased nutrient loads (see Fig. 2), samples without increased nutrient

293 loads showed a relatively (deterministic) divergent community assembly (see Fig. 3B) whereas

294 samples with increased nutrient loads showed values closer to stochastic assemblies (see Fig. 3B)

295 regardless of the level of combination of the other factors, thus counter-acting deterministic

296 divergence and leading to (inter-treatment) fungal community convergence.

297

298 **Discussion:**

299 Our experimental field study shows that despite the characteristic high levels of physical

300 stress in intertidal wetlands, and previous studies that have shown saltmarsh fungal leaf

301 communities are not diverse (Buchan et al. 2002), the salt marsh phyllosphere can harbor several

302 hundreds of different OTUs per cm of cordgrass leaf. This result corroborates others that pinpoint

303 leaf-associated microbial communities as diverse systems (Jumpponen and Jones 2009, 2010).

304 Our empirical findings suggest that anthropogenic environmental drivers, such as greater salinity

305 stress, herbivore/consumer presence and nutrient loading, can interact to drive outcomes in salt

306 marsh fungal leaf communities. Thus, both species interactions through a primary consumer (i.e.,

307 grazing or other herbivore related modification) and physical factors can drive microbial
308 community assembly (see Mohamed and Martiny 2011), a divergence from recent niche-theory
309 work in microbial systems suggesting that physical factors are the primary determinants of
310 community filtering. Our results, however, also show that stochastic processes, together with
311 these deterministic filters, drive microbial assembly - an experimental finding that fortifies recent
312 observational and experimental work (e.g. Stegen et al. 2012, 2013, Brown and Jumpponen 2014,
313 Zhou et al. 2014, Dini-Andreote et al. 2015, Evans et al. 2017, Vannette et al. 2017, Albright and
314 Martiny 2018) highlighting the relative importance of stochastic processes, as dispersal and drift,
315 in a variety of microbial communities.

316 Recent characterization of microbial communities has uncovered patterns of microbial
317 diversity across spatial and temporal scales (Fierer and Jackson 2006), promoting the attempts to
318 understand the mechanisms behind those patterns. Most of those attempts focus on how microbial
319 assemblies can be explained through correlation with physical stress gradients that change in time
320 and space (Mohamed and Martiny 2011, Zhou et al. 2014, Siciliano et al. 2014, Maestre et al.
321 2015) and with resource heterogeneity gradients (Zhou et al. 2002). In contrast, only a small
322 number of studies highlight small-scale processes, such as species interactions (i.e., herbivore –
323 microbe interactions), as important contributing factors to microbial assembly (but see Maherali
324 and Klironomos 2007, Saarenheimo et al. 2016). The results of the present study experimentally
325 demonstrate that microbial assemblies can be influenced by interactions between physical factors
326 (as nutrient availability and levels of salinity stress) and the food web structure (i.e. the presence
327 or not of herbivores).

328 In some systems (especially plant systems) increasing nutrient availability (or productivity)

329 can lead to community homogenization and diversity loss by deterministic processes such as light
330 competition (see Hautier et al. 2009, Borer et al. 2014). In other systems, however, greater
331 nutrient inputs are thought to increase community divergence by enhancing the relative
332 importance of stochastic processes as ecological drift (e.g. processes of birth, death, colonization,
333 and extinction, as well as random change in species relative abundance (Chase 2010)) and by
334 weakening niche selection (greater availability of resources allows more species in the regional
335 species pool to survive). In contrast to both cases, our results show that increased nutrient inputs
336 enhanced the relative importance of stochastic processes but driving to community convergence.
337 Without increased nutrient inputs, ecological selection determines what species of the regional
338 species pool can be present at each (different) environmental condition, creating distinctive and
339 limited-membership communities (community divergence). Increased nutrient inputs, by
340 removing the importance of deterministic filters (weakening ecological selection caused by
341 heterogeneous environmental conditions), increase the convergence of communities. Our results
342 indeed show that, without nutrients, different treatments (factor combinations) generate
343 distinctive communities, but increased nutrient inputs canceled this divergence, increasing
344 evenness by enhancing the frequency of otherwise less abundant OTUs, and driving communities
345 with nutrient addition to similar endpoints regardless of other factor combinations. Thus, when
346 the environmental conditions are spatially homogeneous, increased nutrient inputs can weaken
347 ecological selection and increase stochastic processes, driving communities to diverge instead of
348 converge (as has been seen in experimental ponds (Chase 2010), groundwater microbial
349 communities (Zhou et al. 2014) and bacterial communities in worm intestine (Vega and Gore
350 2017)). In contrast, we propose that, when the environmental conditions are spatially

351 heterogeneous (as in our case, caused by specific combinations of experimental treatments),
352 increased stochasticity reduces the importance of ecological selection that otherwise cause
353 divergence, thus driving communities to decreased divergence.

354 The Raup-Crick metric (i.e. the metric that we used to estimate the relative contribution of
355 stochastic and deterministic processes) can be used not only to calculate the probability of
356 deviation from purely neutral expectation (Chase et al. 2011, Stegen et al. 2013) but also as an
357 index that provides a quantitative estimation of the relative role of those stochastic and
358 deterministic processes in shaping community composition (see Alberti et al. 2017). Thus,
359 observed values may indicate not only that both types of processes played important roles in
360 structuring saltmarsh leaf fungal communities but also that, in some conditions (i.e. increased
361 nutrient loads) stochastic processes can have a relatively large contribution. As our model does
362 discriminate whether variations in community composition are due to variations in environmental
363 conditions (i.e. detected variability in community composition can be related to undetected
364 environmental heterogeneity) or not (Chase et al. 2011), our results contribute to a growing body
365 of evidence showing that microbial communities can be highly influenced by stochastic processes
366 such as dispersal and drift (e.g. Stegen et al. 2013, Zhou et al. 2014, Dini-Andreote et al. 2015).
367 This common pattern may help to explain why microbial communities are extremely diverse
368 (Zhou et al. 2013), but the answer for this (and other important unanswered questions related to
369 microbial community composition and function) will require integration between theory and
370 experiments, an emerging frontier in microbial ecology.

371

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379

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381 **Figure Captions:**

382

383 **Figure 1.** Effect of salt, nutrients and herbivores on OTUs diversity. (**A**) total number of OTUs
384 (all OTUs) and (**B**)**,** the number of OTUs with an occurrence frequency greater than 1% detected
385 in leaves of the saltmarsh cordgrass *Spartina densiflora*. Bars represent means and standard
386 errors.

387

388 **Figure 2.** Nonmetric multidimensional scaling (NMDS) ordination based on Bray Curtis
389 dissimilarity. Ellipses depicting 95% confidence intervals of centroid positions of each treatment
390 combination. Blue ellipses (corresponding to treatments with nutrient addition) are more similar
391 in community composition, thus are close to each other, whereas red ellipses (corresponding to
392 treatments without nutrient addition) are more dissimilar thus farther apart. Letters inside ellipses
393 indicate Salinity and Herbivory treatment combination (C= Control, S= Increased salinity, H=
394 Herbivory). Stress= 0.2.

395

396 **Figure 3.** The effect of nutrient additions on the balance between deterministic and stochastic
397 processes. (**A**) Nonmetric multidimensional scaling (NMDS) ordination based on Raup-Crick
398 metric (RC metric) of samples from treatments without and with nutrient addition. The distance
399 between any two points represent the dissimilarity between those two community assemblies
400 according to RC metric. Lines represent the confidence ellipses at 95% level. Stress= 0.18. (**B**)
401 Mean (±SE) dissimilarity according to Raup-Crick metric (RC metric) of samples from
402 treatments without and with nutrient additions. The RC metric ranges from -1 to 1 indicating

403 whether a pair of plots are less dissimilar (approaching -1), as similar (approaching 0), or more
404 dissimilar (approaching 1), than a pair of plots randomly assembled. As samples without nutrients
405 deterministically diverge according to the other factors (i.e. salinity and herbivory), they have a
406 positive RC dissimilarity value. Samples with nutrients, in contrast, have a lower RC
407 dissimilarity, indicating a more stochastic community assembly. Differences between treatments
408 are evaluated using the analysis of multivariate homogeneity of group dispersions, in which non-
409 euclidean distances between objects and group centroids are derived from reduction of the
410 original distances to principal coordinates.
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