Nitrogen enrichment suppresses other environmental drivers and homogenizes salt marsh leaf microbiome

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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.

Key words: deterministic vs. neutral processes; leaf fungal communities; microbial community assembly; nutrient loading; salt marshes; Spartina.

Introduction

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

limitations in extrapolation of species coexistence theory to microbial communities (Prosser et al. 2007).

Historically, determinants of microbial diversity were interpreted almost exclusively under the paradigm of niche theory (see Dini-Andreote et al. 2015), a position immortalized in Baas Becking and Beijerinck's famous and highly cited phrase "Everything is everywhere, but the environment selects" (see De Wit and Bouvier 2006). In short, given their tremendous dispersal capabilities, microbes had been thought to be unlimited in their dispersal and therefore ubiquitous. According to this view, environmental conditions alone should determine the presence of each species in a site (Fenchel and Finlay 2004), creating predictive and limited-membership communities with low site-to-site variability in species composition (community convergence) among sites with similar environmental conditions and high site-to-site variability in species composition (community divergence) among sites with different environmental conditions (i.e., ecological selection among species, see Vellend 2010, Chase and Myers 2011). Over the past decade, observational field work, fueled by next-generation sequencing of complex microbial communities, has shown that important environmental drivers/filters may exist and can affect microbial assemblies. For example, segregation of microbial community composition commonly occurs across environmental and ecological gradients, including salinity (Mohamed and Martiny 2011), soil pH (Fierer and Jackson 2006, Siciliano et al. 2014), water depth (Walsh et al. 2016) and

successional stage (Zhou et al. 2014, Dini-Andreote et al. 2015). In apparent contrast, many other studies have recently suggested that microbial systems are, instead, constrained by dispersal (see Ramirez et al. 2014, Albright and Martiny 2018) and strongly influenced by processes such as stochastic recruitment and ecological drift (Prosser et al. 2007, Woodcock et al. 2007, Ofiteru et al. 2010, Vellend 2010). These stochastic processes should constrain the deterministic ones leading to high site-to-site variability in species composition (community divergence) even among sites with similar environmental conditions (see Chase and Myers 2011). Despite the strong contrast in the underlying mechanism and predicted effects, it has recently been suggested that deterministic and stochastic processes simultaneously influence microbial communities and that their relative influence can vary across environmental conditions (Stegen et al. 2012, 2013, Zhou et al. 2014, Dini-Andreote et al. 2015). This more recent idea has been widely examined in lab settings or with observational data, and has just started to be experimentally tested in the field (e.g., Evans et al. 2017, Vannette and Fukami 2017, Albright and Martiny 2018), fueling the advance of our understanding of microbial community assembly processes and our capacity to potentially predict community dynamics.

Here, we present the response of leaf fungal communities to a factorial field experiment manipulating nutrient loading, salinity stress and food web structure in a coastal wetland. By applying an ecological null model approach (Chase et al. 2011) to changes in microbial species abundances, this experiment allowed us to evaluate not only the effect of these factors on leaf fungal community assembly, but also how these factors mediate the relative contribution of deterministic and stochastic processes as drivers of fungal assembly. We hypothesize that salinity stress and food web alterations will drive to distinctive fungal communities by acting as ecological filters that select among species (see Chase 2007, Vellend 2010, Zhou et al. 2014), thus increasing the importance of deterministic over stochastic processes. Increased nutrient loads, nevertheless, will exert weak selection among species (as greater availability of resources allow most species in the regional species pool to survive; see Chase 2010, Zhou et al. 2014), thus increasing the importance of stochastic over deterministic processes. We further hypothesize that, by decreasing the importance of divergent selection among species, increased nutrient loads will overcome salinity stress and food web alterations as deterministic filters, thus driving to community convergence.

Materials and Methods

Study site

This study was performed in a salt marsh located near a creek at the mouth of the Mar Chiquita coastal lagoon (Argentina, 37°32′ S; 57°19′ W). This lagoon is affected by semidiurnal microtides (<1 m) and is characterized by mudflats in the low zone followed by a *Spartina densiflora* monoculture at intermediate elevations and an extended salt marsh community at high elevations. The marsh is dominated by the intertidal burrowing crab *Neohelice granulata* that, through grazing, can exert strong control over marsh plant production by directly removing plant tissue as well as by facilitating fungal infection in crab-generated injuries (Daleo et al. 2009). As in other worldwide salt marshes, nutrient availability and soil salinity can also exert a strong control of primary production (Alberti et al. 2010).

Experimental set-up

A fully-factorial experiment was conducted in the *S. densiflora* monoculture zone. The factorial design included: salinity stress (with and without salt addition), food web structure (with and without herbivorous crabs) and nutrients loads (with and without nutrient addition) implemented in 0.7×0.7 m plots. Each treatment combination was replicated 6 times (for a total of 48 plots). Crab-exclusion plots were surrounded using a plastic mesh (10 mm opening) fence 0.6 m high and supported by iron stakes. Crab exclosures have been widely used in this system and the use of cage controls revealed that there are no associated cage artifacts (Daleo et al. 2015). Salt addition plots received 20 g (~40 g/m²) of commercial pelletized salt spread superficially every 2 weeks. This salt loading increased plant tissue salinity at least up to 35%, leaf surface salinity by near 400% (Canepuccia et al. 2010) and decreased plant growth by 50% (Daleo et al. 2015). Nutrient addition treatments received 60 g (~120 g/m²) of a slow-release pelletized fertilizer (NPK: 29:5:5) monthly. This fertilization rate increased biomass production by more than 400%, increased sediment nitrates by more than an order of magnitude (i.e., from 1.37 ± 0.14 μmol/L to 85.24 ± 24.28 μmol/L) and doubled *S. densiflora* leaf N content (Alberti et al. 2011, Daleo et al. 2015). Fertilizer was spread into six artificial holes (5 cm deep, 1 cm diameter) evenly distributed in each plot that were then filled with mud. The experiment started on March 2010 and after 1 yr (i.e., March 2011), three leaves were sampled from each plot. The number of sampled leaves per plot were constrained by practical issues but similar sampling designs have been shown to be adequate for leaf fungal community estimations (e.g., Jumpponen and Jones 2009, 2010). For the herbivory treatment plots, leaves with crab-induced injuries were sampled (Daleo et al. 2009). Leaf samples where transported to the laboratory, rinsed in sterile H_2O to remove non-adhering fungal spores and other adhered particles before extraction. A section of leaf laminae of 10 mm length was taken from each leaf, avoiding necrotic areas, and the 3 sections from each plot were pooled for DNA extraction.

DNA extraction, ITS2 library preparation, and sequencing

Total genomic DNA (gDNA) was extracted from samples with UltraClean® Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) following manufacturer's instructions, and eluted in 50 μL of solution S5 (sterile elution buffer). The DNA yields were quantified with a Nanodrop ND2000 spectrometer (Thermo Scientific, Wilmington, Delaware) and adjusted to a final 1 ng/μL concentration. We targeted the Internal Transcribed Spacer region 2 (ITS2) for amplification. ITS2 has been proposed as the universal metabarcode marker for fungi (Schoch et al. 2012), because of its interspecific hypervariability. We

amplified the ITS2 region in a 2-step PCR. Primary PCRs included the forward primer ITS1F (Gardes and Bruns 1993) and the reverse primer ITS4 (White et al. 1990). Each primary PCR contained 1 μM of each primer, 10 ng of template gDNA, 200 μM of dNTPs, 1.5 mM MgCl2, 0.5 units of Phusion Green Host Start II High-Fidelity DNA polymerase, and 10 μL of 5× Phusion Green HF PCR buffer (Thermo Scientific, Waltham, Massachusetts, USA). Primary PCR conditions consisted of initial denaturation at 94°C for 10 s, and then 25 cycles of 94°C for 10 s, 53°C for 30 s, and 72°C for 2 min, followed by final extension at 72°C for 8 min. To minimize primer carryover, primary PCRs were purified with Diffinity RapidTips (Diffinity Genomics, West Henrietta, New York, USA). Five μL of each primary PCR was used as DNA template in secondary PCRs with a nested forward primer fITS7 (Ihrmark et al. 2012) and ITS4 with a unique molecular identifier tag. The reaction conditions of secondary PCRs were identical to the primary PCR reactions, but were carried out for ten cycles. Secondary PCRs were cleaned with the AMPure XP bead system (1:1 bead to PCR volume ratio; Beckman Coulter Inc., Brea, CA), quantified on a Nanodrop ND2000, and 100 ng for each experimental unit pooled. The ITS2 amplicon library was sequenced on the Illumina MiSeq platform (v. 2; 2 × 250) at the Integrated Genomics Facility at Kansas State University. Raw sequence data (.fastq files) are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject PRJNA378881 and BioSamples SAMN06563186-06563230.

Sequence data were analyzed using mothur (v. 1.32.1) (Schloss et al. 2009, 2011). The paired-end .fastq files were contiged with a 100 bp minimum overlap, and subsequently had homopolymers (maximum of eight allowed), and sequences with any mismatch to primer or barcode filtered. Sequences were then truncated to 250 bp, >99% similar sequences pre-clustered (Huse et al. 2010), and potential chimeras removed (UCHIME) (Edgar et al. 2011). The quality screened sequences were pairwise aligned to retrieve a distance matrix, assigned to Operational Taxonomic Units (OTUs) at 97% similarity using average neighbor joining, and rare OTUs ($n \leq 10$) omitted (Tedersoo et al. 2010). OTUs were assigned to taxon affinities using a naïve Bayesian classifier (Wang et al. 2007) and the UNITE-curated INSD (International Nucleotide Sequence Databases) reference database (Abarenkov et al. 2010), and the complete taxonomic affinity strings retrieved (Table S1). We did not detect any OTUs not classified to Kingdom Fungi. All experimental units were subsampled to 10,000 sequences to minimize sample loss but retain as many high quality sequences as possible to have even and adequate library coverage. We found a total of 305 fungal OTUs (Appendix S1: Table S1) in the final dataset.

Statistical analysis

We used ANOVA to evaluate the separate and interactive effects of salinity stress, food web structure and nutrient loading on OTUs richness (i.e., number of OTUs per sample), OTUs diversity (Shannon diversity index) and OTUs evenness. Data was transformed if visual inspection of residual plots revealed any obvious deviations from homoscedasticity or normality. To evaluate the separate and interactive effects of salinity stress, food web structure and nutrient loading on fungal community composition, we performed a permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) based on the Bray-Curtis dissimilarity index applied to fourth-root transformed data (to reduce the weight of the most abundant OTUs), with 9999 permutations. We previously performed an analysis of multivariate homogeneity of group dispersions to evaluate if homogeneity of group dispersions is achieved (Anderson et al. 2006). We performed pairwise comparisons after significant interactions of PERMANOVA with the *pairwise.perm.manova* function of the *RVAideMemoire* package (Hervé 2018). We also looked at the treatment effects on species assembly using non-metric multi-dimensional scaling ordination (NMDS) based on the Bray-Curtis dissimilarity index (Warwick and Clarke 1991). The NMDS ordinations were obtained using the *metaMDS* function of the *vegan* package in R (v 2.3-0) (Oksanen et al. 2015). To evaluate if nutrient loads affected variability in community composition by suppressing divergence caused by the other experimental factors, we performed an analysis of multivariate group dispersions (Anderson et al. 2006) comparing dispersion of samples coming from plots with and without increased nutrient loads, with 9999 permutations. To be able to perform this analysis, we first evaluated the non-existence of interactive effects among factors on multivariate group dispersion. We performed this analysis using the *betadisper* and *permutest* functions of the *vegan* package (Oksanen et al. 2015).

To be able to disentangle whether differences in variability were the result of differences in the underlying assembly mechanisms (i.e., the relative importance of stochastic and deterministic processes in community assembly), we used a null model approach that is a slight modification (to consider species abundances; see Stegen et al. 2013) of the null model proposed by Chase et al. (2011), which in turn is a slight modification of the Raup-Crick (RC) index (Raup and Crick 1979, Chase et al. 2011, Stegen et al. 2013). For more details about the rationale of using such null models to evaluate the relative role of different assembly processes in shaping ecological communities see Mori et al. (2015). The null model was constructed by performing a probability-based randomization, in which randomly generated OTUs composition and abundance were assembled for each sample by randomly sampling from the total OTU pool (estimated as the list of OTUs observed in all sampling units) under four constrains: (1) the number of OTUs of the randomly generated sample equals the number of OTUs actually observed in the sample, (2) the probability of occurrence (i.e., probability of being present in a sample) of a given OTU was proportional to its observed total occurrence frequency (i.e., the proportion of samples where this OTU was actually observed), (3) the total abundance of the randomly generated sample equals the total abundance actually observed in the sample and (4) the abundance probability of each OTU in the randomly generated sample was proportional to its observed total abundance. For all possible pairs of plots, OTU composition of each plot was probabilistically generated 9999 times. For each iteration, Bray-Curtis dissimilarity index between plots was calculated, and the

resulting metric was the proportion of iterations in which the index was smaller than or equal to the actually observed Bray-Curtis dissimilarity index between those pair of plots (Chase et al. 2011, Stegen et al. 2013). Finally, we standardized the metric to range from −1 to 1 by subtracting 0.5 and multiplying by 2 (Chase et al. 2011), with negative values indicating that a pair of communities is more similar than expected at random (deterministic convergence), positive values indicating that a pair of communities is more dissimilar than expected at random (deterministic divergence), and zero indicating that a pair of communities is as similar as expected at random (purely neutral community assembly). The selection of species (OTUs in our case) pool plays a fundamental role in the calculation of this metric, and following others (e.g., Chase 2010, Chase et al. 2011, Stegen et al. 2013, Alberti et al. 2017) we defined the species pool as the list of species (OTUs) found in samples throughout the experiment. The R script of the used model can be found at https://github.com/stegen/Stegen_etal_ISME_2013. This metric can be used not only to calculate the probability of deviation from purely neutral expectation (Chase et al. 2011, Stegen et al. 2013) but also as a dissimilarity index that provides a quantitative estimation of the relative role of deterministic and stochastic processes in shaping community composition, and can be analyzed using statistical methods similar to those used for other pairwise dissimilarity indexes (Zhou et al. 2014). Thus, the metric allows to test if the relative contribution of deterministic and stochastic processes in community assembly differ among treatments (see Alberti et al. 2017). We performed the analysis of multivariate homogeneity of group dispersions (Anderson et al. 2006) based on the dissimilarity matrices constructed with these metrics. Significant results indicate that groups differ from another in its RC metric (i.e., differ in the relative importance of deterministic and neutral processes in community assembly). We started by evaluating multivariate homogeneity of group dispersions among samples from the individual treatment level (i.e., the levels of the three way interaction differ in its RC metric). If significant differences were not detected we moved to evaluate homogeneity among samples from the levels of the two way interactions and, finally, among samples from the levels of the main factors. This approach is not like classical factorial approaches where interactions and main effects are addressed at once because the RC metric is essentially a distance metric and, thus, is not a fixed value but a value that changes at different levels of the factorial design. However, it is the most reliable way to analyze homogeneity of group dispersion in such designs. We performed this analysis using the *betadisper* and *permutest* functions from *vegan* package in R (Oksanen et al. 2015).

RESULTS

Plants subjected to salinity stress had ~10% fewer OTUs (log transformed data, ANOVA: $F_{1,37} = 11.31$, $P = 0.0018$; Fig. 1), but we observed neither an effect of herbivore removal nor increased nutrient loads (Appendix S1: Table S2). As this pattern can be masked by the persistence of very low frequency OTUs, we re-analyzed data removing all OTUs that occurred at frequencies <1% in each sample.

We found that plants exposed to nutrient enrichment had ~16% higher number of frequent OTUs ($F_{1,37} = 11.12$, $P = 0.002$; Fig. 1). We also found that nutrient loads and the interaction between salinity stress and presence of herbivores affected OTU diversity and evenness (see Appendix S1: Fig. S1; Appendix S1: Table S2); plants exposed to nutrient enrichment presented higher OTU diversity and evenness (see Appendix S1: Fig. S1).

Salinity stress, presence of herbivores and nutrient loads interactively affected community composition (PERMANOVA: pseudo$F_{1,37} = 1.63$, $P = 0.02$; see Table S3 for specific individual and interactive effect of factors; see Appendix S1: Fig. S2 for changes in abundance of the 15 most abundant OTUs). Pairwise comparisons show four compositional groups; the first group included three treatments with nutrient addition (Nutrient addition; Herbivory and Nutrient addition; Herbivory, Salt and Nutrient addition) as well as the treatment with Salt addition and Herbivory. The second group included the treatment with Salt addition and the treatment with Salt and Nutrient addition (see Fig. 2). The third and fourth groups were formed by Control treatment and the treatment with Herbivory respectively (see Fig. 2). Regarding the variability in species composition, results of the analysis of multivariate group dispersions show that it was not affected by any of the potential interactions between factors. Moreover, it was only affected (reduced) by nutrient addition (pseudo$F_{1,43} = 6.09$, $P = 0.015$). This reduction in variability was driven by a smaller difference in composition between those treatments with nutrient addition (i.e., regardless of the other factor combinations, all treatments with nutrient addition were more similar in composition compared with treatments without nutrient addition; see Fig. 2, Appendix S1: Fig. S3). In other words, community composition from the different treatments were much more similar to each other when their nutrient loads were increased.

By using the null model approach based on the extended RC metric, we found that the interactions among factors,
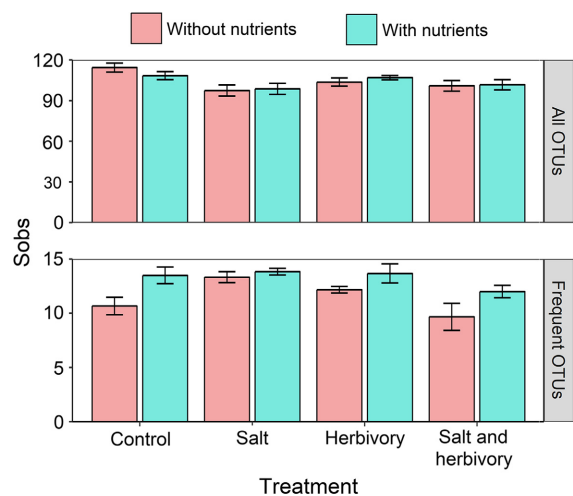

FIG. 1. Effect of salt, nutrients and herbivores on total number of OTUs (all OTUs) and the number of OTUs with an occurrence frequency >1% detected in leaves of the saltmarsh cordgrass *Spartina densiflora*. Bars represent means and standard errors.
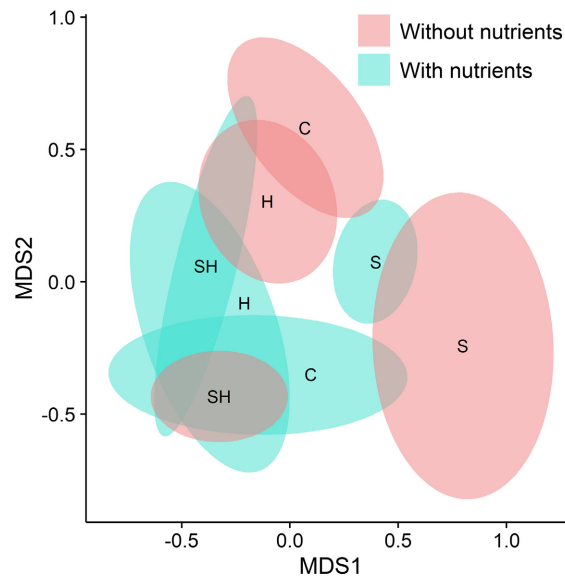
FIG. 2. Nonmetric multidimensional scaling (NMDS) ordination based on Bray Curtis dissimilarity. Ellipses depicting 95% confidence intervals of centroid positions of each treatment combination. Blue ellipses (corresponding to treatments with nutrient addition) are more similar in community composition, thus are close to each other, whereas red ellipses (corresponding to treatments without nutrient addition) are more dissimilar thus farther apart. Letters inside ellipses indicate Salinity and Herbivory treatment combination (C = Control, S = Increased salinity, H = Herbivory). Stress = 0.2.

in salt marsh fungal leaf communities. Thus, both species interactions through a primary consumer (i.e.,, grazing or other herbivore related modification) and physical factors can drive microbial community assembly (see Mohamed and
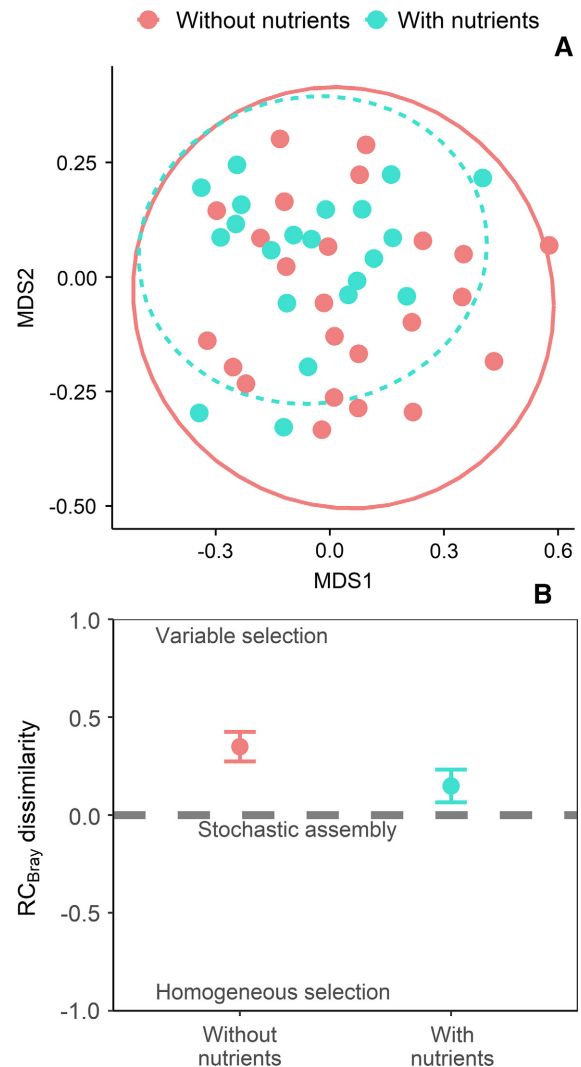

FIG. 3. The effect of nutrient additions on the balance between deterministic and stochastic processes. (A) Nonmetric multidimensional scaling (NMDS) ordination based on Raup-Crick metric (RC metric) of samples from treatments without and with nutrient addition. The distance between any two points represent the dissimilarity between those two community assemblies according to RC metric. Lines represent the confidence ellipses at 95% level. Stress = 0.18. (B) Mean (± SE) dissimilarity according to Raup-Crick metric (RC metric) of samples from treatments without and with nutrient additions. The RC metric ranges from −1 to 1 indicating whether a pair of plots are less dissimilar (approaching −1), as similar (approaching 0), or more dissimilar (approaching 1), than a pair of plots randomly assembled. As samples without nutrients deterministically diverge according to the other factors (i.e., salinity and herbivory), they have a positive RC dissimilarity value. Samples with nutrients, in contrast, have a lower RC dissimilarity, indicating a more stochastic community assembly. Differences between treatments are evaluated using the analysis of multivariate homogeneity of group dispersions, in which non-euclidean distances between objects and group centroids are derived from reduction of the original distances to principal coordinates.

as well as the main factors salinity stress and presence of herbivores, did not affect the relative contribution of stochastic over deterministic processes (Appendix S1: Fig. S4) but increased nutrient loads, as a main factor, significantly increased the importance of neutral processes (pseudo$F_{1,43}$ = 4.84, P = 0.034; Fig. 3). As different combinations of the factors salinity stress and herbivory deterministically led to different community assemblies only when applied without increased nutrient loads (see Fig. 2), samples without increased nutrient loads showed a relatively (deterministic) divergent community assembly (see Fig. 3B) whereas samples with increased nutrient loads showed values closer to stochastic assemblies (see Fig. 3B) regardless of the level of combination of the other factors, thus counter-acting deterministic divergence and leading to (inter-treatment) fungal community convergence.

DISCUSSION

Our experimental field study shows that despite the characteristic high levels of physical stress in intertidal wetlands, and previous studies that have shown saltmarsh fungal leaf communities are not diverse (Buchan et al. 2002), the salt marsh phyllosphere can harbor several hundreds of different OTUs per cm of cordgrass leaf. This result corroborates others that pinpoint leaf-associated microbial communities as diverse systems (Jumpponen and Jones 2009, 2010). Our empirical findings suggest that anthropogenic environmental drivers, such as greater salinity stress, herbivore/consumer presence and nutrient loading, can interact to drive outcomes

Martiny 2011), a divergence from recent niche-theory work in microbial systems suggesting that physical factors are the primary determinants of community filtering. Our results, however, also show that stochastic processes, together with these deterministic filters, drive microbial assembly - an experimental finding that fortifies recent observational and experimental work (e.g., Stegen et al. 2012, 2013, Brown and Jumpponen 2014, Zhou et al. 2014, Dini-Andreote et al. 2015, Evans et al. 2017, Vannette and Fukami 2017, Albright and Martiny 2018) highlighting the relative importance of stochastic processes, as dispersal and drift, in a variety of microbial communities.

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

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

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

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SUPPORTING INFORMATION