

Colonization and succession as drivers of small-scale spatial variability in epibionts on mangrove roots in the Southern Caribbean

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ABSTRACT: High variability in species diversity among samples from the same site is a consistent pattern described for several benthic marine assemblages. In general, small-scale spatial variability has been shown to be as significant as regional-scale variability in species diversity, indicating that the processes which generate such patterns are equally important in both cases. However, the focus of most ecological studies is to understand species diversity at a regional scale. Using epibenthic assemblages associated with mangrove roots, we experimentally evaluated the effects of colonization timing, succession and species–area relationships as factors that might explain the high occurrence of small-scale variability observed in nature. Assemblages were evaluated on artificial mangrove roots deployed at different sites and times, and under different environmental conditions, along 500 km of the Southern Caribbean coast. Residual variation obtained in this experiment was compared with variation among natural neighboring roots in order to test the hypothesis that the former would be lower. The identities and abundances of both colonizing and late-successional species varied considerably among sites and colonization timing; nevertheless, changes in assemblage structure always occurred in the same direction. No relationship among richness and structure of assemblages was associated with root area. Residual variation was considerably lower (40%) than variation measured in natural neighboring roots, indicating that colonization and succession explained a significant component of the variation in assemblages among neighboring roots. Understanding the mechanics of these processes could be the key to understanding small-scale spatial variability of benthic assemblages.

KEY WORDS: Supply-side ecology · Benthic assemblages · Colonization · Succession · *Rhizophora* mangrove roots

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INTRODUCTION

High variability in species composition among samples from the same site is an important and common pattern described for benthic marine assemblages around the world (Fraschetti et al. 2005, Chapman et al. 2010, Barnes & Ellwood 2012). Generally, small-scale spatial variability in species diversity has been

shown to be as significant as variability at a regional scale. This implies that processes responsible for variation among samples are as important, for example, as oceanographic processes that generate bioregions (Hewitt et al. 2010). These small-scale processes operate at the level of the interactions among different species at a particular site and, consequently, have an effect on species richness at that site (Hewitt et al.

2005). It has also been shown that the effects of small-scale processes can be scaled up and can be partially responsible for large-scale patterns of spatial distribution (Benedetti-Cecchi et al. 2012). Even though some early efforts were directed at understanding patterns and processes operating at small spatial scales, the last few decades have seen an increase in studies focusing on large-scale processes, very likely due to important developments in technology, access to electronic data bases and novel statistical methods (Fei et al. 2016). This discussion is particularly relevant in the ecology of epibenthic assemblages associated with Caribbean mangrove roots, a system in which large variations in species diversity among neighboring roots (i.e. smallest spatial scale) have always been recognized but not experimentally assessed (Farnsworth & Ellison 1996, Hunting et al. 2008, Díaz & Rützler 2009, Guerra-Castro et al. 2016).

Various processes can be responsible for small-scale variability: (1) disturbances generated by predators (Mook 1981, Menge & Sutherland 1987, but see Oricchio et al. 2016), (2) processes related to the species–area relationship (Simberloff 1976), (3) successional changes (Connell & Slatyer 1977, Sutherland & Karlson 1977) and (4) variability in the supply of recruits and new habitats (Connell 1985, Underwood & Fairweather 1989). For mangrove root assemblages, disturbances generated by predators, like sea stars of the genus *Echinaster* and the gastropod *Melongena melongena*, tend to modify epibenthic assemblages of grounded (accessible) and hanging (inaccessible) neighboring roots (Ellison & Farnsworth 1992, Schutte & Byers 2017). However, it is precisely between hanging neighboring roots where much of the high spatial variation in species diversity has been described (Farnsworth & Ellison 1996, Hunting et al. 2008, Guerra-Castro et al. 2011, 2016). Differences in species diversity among neighboring roots might reflect variations in the size of the root, i.e. larger roots will host more species than smaller roots. In general, the species–area relationship is regarded as an axiom, although it has been demonstrated that other factors, such as succession and colonization timing, could be more important in structuring natural assemblages than the size of the habitat (Anderson 1999).

In addition to succession (i.e. directional temporal changes), temporal changes could be a response to pulses of species recruitment rather than to the biological interactions which typically shape succession, like competition (Bingham & Young 1991a, Bingham 1992). Under this model, in the absence of predators, roots are constantly trapping species, and it can be hypothesized that younger roots will harbor fewer

species than long-lived and unperturbed roots. However, succession in benthic marine systems is neither linear nor predictable (Sutherland & Karlson 1977). Colonizing species might tolerate, induce or inhibit the incorporation of other species in the assemblage, and can therefore influence the direction of changes in species richness and composition (Connell & Slatyer 1977, Maggi et al. 2011). Succession, mediated by variability in the supply of colonizing species, has been proposed several times as a key element for understanding the structure of benthic marine communities at very small spatial scales (Underwood & Fairweather 1989, Anderson & Underwood 1994, Underwood & Chapman 2006). It can therefore be postulated that 2 adjacent roots may differ in species composition because they were colonized at different times. This would mean that assemblage development would depend on the identity and abundance of larvae present at the moment of substrate availability, and consequently, on their competitive strategies to tolerate, induce or inhibit later species.

In order to elucidate the potential effects of temporal variation in the supply of recruits, succession, and root area as factors that contribute to explaining small-scale variation in species composition among neighboring roots in Caribbean mangroves, manipulative experiments were done using artificial mangrove roots (AMRs) at sites with different communities and environmental conditions. It was of particular interest to compare the magnitude of variability among assemblages of the same age and colonization time with the magnitude of variability measured among natural, non-manipulated neighboring roots. As a general prediction, if succession and/or temporal variation in larval availability cause the small-scale variability measured in nature, it can be expected that variability among AMRs should be considerably lower than variability among natural roots. When the above prediction was supported, and based on these 3 models (i.e. succession, availability of larvae and species–area), specific hypotheses about the magnitude and directions of changes of species richness and assemblage structures were tested (see hypotheses below).

MATERIALS AND METHODS

Study area

Fringe mangrove forests from 2 marine reserves along the Venezuelan coast were selected for the experiments: (1) Morrocoy National Park (MNP) and (2) La Restinga National Park (LRNP) (Fig. 1). These

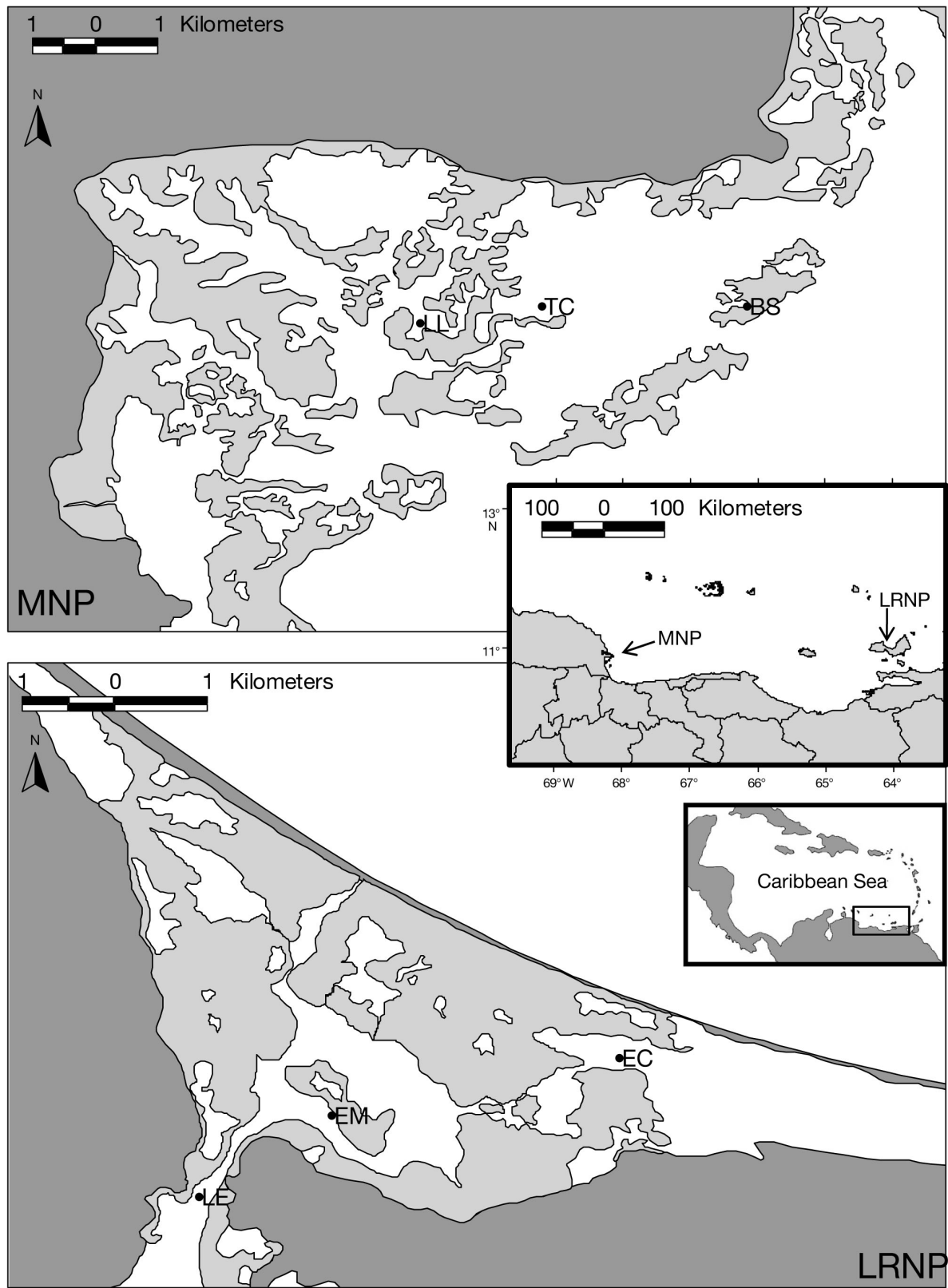


Fig. 1. Sites in (a) Morrocoy National Park (MNP) and (b) La Restinga National Park (LRNP) in Venezuela where artificial mangrove roots were deployed. Light grey = mangrove areas, dark grey = mainland. BS: Boca Seca; TC: Tumba Cuatro; LL: Las Luisas; LE: La Entrada; EM: El Mánamo; EC: El Conchal

forests are dominated by *Rhizophora mangle*, which grows on the shore of the mainland, sand cays and in shallow waters where it forms 'mangrove cays.' MNP stretches over 177 km² of marine habitats (coral reefs, biogenic sandy bottoms, seagrass beds and mangrove cays), of which 28 km² are occupied by mangroves, surrounding a system of interconnected lagoons with oligotrophic waters. This marine reserve opens to the sea through several channels, but is also affected by freshwater inflow during seasonal rainfall (Bone et al. 2001). In contrast, LRNP is a complex of hypersaline and eutrophic lagoons surrounded by well developed mangroves that occupy an area of 30 km². This system is connected to the sea by a single channel measuring 1.3 km long, 70 m wide and 6 m deep, and it is not affected by freshwater runoff. This mangrove system does not have coral reefs, and most of the area consists of muddy bottoms and marginal seagrass beds.

Hypotheses and experimental design

The fundamental factors proposed here that would explain the high variability among neighboring roots were (1) variability in temporal supply of recruits at the start of colonization (i.e. initiation time), (2) successional changes/stages (i.e. sampling time) and (3) variations in the species–area relationship. First, we evaluated if variation in species diversity among neighboring roots could be reduced once these 3 factors were controlled, given that AMRs can be used to (1) control timing of experiment initiation (i.e. all replicate units introduced at the same site and time were exposed to the same pool of larvae to start the colonization), (2) follow successional changes (i.e. the development of the assemblages can be described and compared in fixed time periods) and (3) control the length of the submerged portion of AMRs (i.e. all AMRs have the same diameter, so any variation in the submerged portion of the root will directly change the area that can be colonized). Subsequently, to assess the relative importance of these 3 factors, the following hypotheses were proposed:

(1) If timing of initial colonization explains variation among neighboring roots, we expected that the structure and composition of assemblages associated with AMRs with different colonization times would be different, independent of post-recruitment processes (e.g. competition among early and late species).

(2) If successional changes/stages are responsible for variation among neighboring roots, we expected that the structure and composition of assemblages associated with AMRs would be different across suc-

cessional stages, independent of early colonization timing. Consistent successional changes would be observed across sites and colonization times.

(3) If variation among neighboring roots is related to area, we expected that richness of assemblages associated with AMRs would be positively related to AMR area. This result would be general and consistent across sites and colonization times.

The above hypotheses are stated as independent; however, the proposed ecological processes could be interdependent (Connell 1985, Underwood & Fairweather 1989, Underwood & Chapman 2006). Consequently, and given that temporal changes in species diversity could be defined by 3 types of post-recruitment species interactions, i.e. tolerance, facilitation or inhibition (Platt & Connell 2003, Maggi et al. 2011), we further tested the following hypotheses:

(4) If temporal changes in assemblage diversity are mediated by tolerance of early species, we expected that the number of species would increase, independent of initiation time, whereas the dissimilarity in diversity across initiation times (for the same successional stage) would decrease with time.

(5) If recruitment of later species is mediated by facilitation of early species, we predicted an ordered and consistent evolution of the assemblage. In particular, we predicted that species recruited in older assemblages would not appear in younger assemblages. A replacement of species would be observed by real changes in species composition with time, but the number of species would be constant.

(6) If earlier species inhibit the arrival of new species, we expected that the number of species and assemblage structure would be constant through time.

Considering that local adults could be the suppliers of recruits, experiments were deployed at 3 sites in each location. Sites were separated by 2–10 km, and were arranged along the sea–land gradient (Fig. 1). We used pine wood broomsticks without any chemical treatment as AMRs (Guerra-Castro & Cruz-Motta 2014). Each unit was 118 cm long and 2.2 cm in diameter, with a perimeter of 7.5 cm. In the field, AMRs were vertically fixed (around 90° and 75° inclination) to natural mangrove branches, always ensuring that 60–100 cm of the AMR was submerged in the water at low tide and that the distant tip of the unit was separated by at least 30 cm from the lagoon bottom. This prevented benthic predators like *Echinaster* sp. from reaching the AMR. Maximum separations between adjacent AMRs were about 1 m.

Two initiation times (separated by 2 mo) and 3 early stages of succession (2, 4 and 6 mo) were compared (Table 1). At each of the 6 sites (i.e. 3 sites at

Table 1. Starting dates of each experiment and dates of subsequent evaluations (every 2 mo) of artificial mangrove roots (AMRs) at La Restinga National Park (LRNP) and Morrocoy National Park (MNP), Venezuela. T: Initiation Time. Dates are given as dd/mm/yyyy

Experiment	Months				
	0	2 0	4 2	6 4	6
LRNP					
T1	27/05/2009	30/07/2009	01/10/2009	27/11/2009	
T2		30/07/2009	01/10/2009	27/11/2009	01/02/2010
MNP					
T1	29/04/2009	25/06/2009	26/08/2009	26/10/2009	
T2		25/06/2009	26/08/2009	26/10/2009	28/01/2010

each location), 24 AMRs corresponding to Initiation Time 1 were placed. Two months later, an additional 24 AMRs, for Initiation Time 2, were placed at each site, interspersed with AMRs from Initiation Time 1. Every 2 mo, 8 random AMRs from each initiation time were sampled without replacement, assuring independence among temporal treatments. The area available for colonization of each AMR was not manipulated *a priori*; instead it was treated as a random factor. On each occasion, the AMRs were measured linearly in cm (all AMRs had the same diameter) with a measuring tape, from the high tide mark to the tip of the AMR. Natural variation among neighboring roots for comparison with AMRs was obtained for each site at the beginning and end of experiments with AMRs, using the same fieldwork protocol as that described by Guerra-Castro et al. (2016).

Field work and image processing

For each AMR, species were identified to the highest taxonomic level possible using a photographic field guide for the most common taxa found in LRNP and MNP, which was previously developed by qualified taxonomists (see Supplement 1 in Guerra-Castro et al. 2016). To estimate the relative abundance of each species per sample unit, several digital photographs were taken of the entire AMR, using a measuring tape as a reference. These photographs were used to estimate coverage of all sessile species present, applying the point intercept technique (Díaz et al. 1992). A digital grid with 1 cm spacing was overlaid on each image to yield intercept counts using Coral Point Counter v.3.6 (Kohler & Gill 2006). The resulting data are in the form of a species \times AMR matrix, with each cell containing the number of intercept points for a given species on a given AMR.

Statistical analyses

Temporal changes of assemblages were compared using the Bray-Curtis coefficient of similarity on the square root abundances of each species after standardizing by total abundance of each sample (Guerra-Castro & Cruz-Motta 2014, Guerra-Castro et al. 2016). Standardization was necessary because the AMRs did not all have the same length im-

mersed in the water (between 60 and 100 cm), which could potentially bias estimations of abundances and ultimately estimations of the Bray-Curtis coefficient (Clarke 1993). The square root transformation was used to downweight the dominance of highly abundant species relative to the less common species in the calculation of similarities (Clarke 1993).

Variability among neighboring AMRs was estimated as residual variation in a 4-factor linear model of mixed effects using distance-based permutational multivariate analyses of variance (PERMANOVA) (Anderson 2001). Sources of variation in the model were (1) Location (L, random with 2 levels), (2) Site (Si[L], random, nested in Location with 3 levels), (3) Initiation Time (T, fixed with 2 levels), (4) Successional Stage (S, fixed with 3 levels of 2, 4 and 6 mo), and (5) Residual Variation. These factors generate interactions of the first and second order. The second-order interaction Si[L] \times T \times S indicates whether assemblage development depended on initiation time, and if effects varied between sites within each locality. The second-order interaction L \times T \times S indicates whether assemblage development was dependent on initiation time, and if effects varied between localities. In the absence of significant second-order interactions, a significant T \times S indicates that assemblage development was dependent on initiation time, but that such interaction was constant among sites and localities, regardless of the differences in species composition between sites and locations. The terms Si[L] \times T and L \times T indicate whether the effect of colonization time was not constant between sites or localities, respectively, while Si[L] \times S and L \times S indicate whether assemblage development varied between sites and localities. Variability among natural neighboring roots was measured as residual variation in a 3-factor model and PERMANOVA, with Location, Site and Time

(fixed effect with 2 levels) being the main factors. For this analysis, interest was in the residuals rather than the main factors. Data in both analyses had the same pretreatments, so magnitudes of residuals were comparable.

Exploratory analyses indicated that the colonizable area of each AMR (i.e. Hypothesis 3) was strongly correlated with depth at each site. This lack of independence implies that colonizable area could not be used as a covariate in an analysis with Site as a factor. Having already proven that species composition (i.e. Hypotheses 4, 5 and 6) varied significantly among sites (Guerra-Castro & Cruz-Motta 2014, Guerra-Castro et al. 2016), potential colonization strategies of early species could also vary across sites. Consequently, Hypotheses 3, 4, 5 and 6 were evaluated separately for each site with a 3-factor linear model of mixed-effects ANOVA (for richness in Hypothesis 3) and PERMANOVA (for the structure and composition of assemblages in Hypotheses 4, 5 and 6). Sources of variation in the model were (1) Length of colonizable area of the AMR (Area, covariate), (2) Initiation Time (T, fixed effect with 2 levels), (3) Successional Stage (S, fixed effects with 3 levels of 2, 4 and 6 mo), and (4) Residual Variation. Because of the covariate, Type I sequential sums of squares were used to partition variance in the linear model. The statistical significance of each term was obtained using a random subset of 9999 permutations of residuals under a reduced model. Potential species–area relationship was assessed by significance of the covariate and estimation of the slope in a linear model regression analysis. An interaction among succession (S) and colonization time (T) would indicate that assemblage development is dependent on the early species that colonize the AMR. Principal coordinate ordinations were used to illustrate patterns of temporal changes in species composition, calculating distances among centroids for the interaction term $S \times T$ for each site. Species responsible for significant differences among AMRs from different successional stages and colonization timing at each site were identified using similarity percentage breakdown (SIMPER) analysis (Clarke 1993). All multivariate statistical procedures were carried out with the software PRIMER v.7 and PERMANOVA add-on (PRIMER-e).

RESULTS

Variation in species diversity among neighboring roots

The multivariate residual variation of this experiment (RMS = 1648, Table 2) was considerably lower (40%) than residual variation measured in natural roots (RMS = 2820, Table 3). The square root of these values (interpretable as standard deviation in Bray-Curtis units) indicated that averaged dissimilarity between roots of the same combination of treatments (site, successional stage and colonization timing) and their respective centroid was around 41% in a Bray-Curtis space, but in natural neighboring roots was around 53%.

Hypotheses 1 and 2

The second-order interaction among sites, colonization timing and successional stage was statistically significant (Table 2, interaction term $Si[L] \times T \times S$, $p < 0.001$), indicating that temporal changes in assemblage structure are not independent of initiation time and site. Detailed analysis of the second-order interaction (Fig. 2) revealed that changes in assemblage structure occurred in the same direction at each locality, but the magnitude of changes differed among sites, and to a lesser extent among initiation times. It can also be noted that differences between initiation times were larger in MNP than in LRNP;

Table 2. Permutational multivariate ANOVA based on Bray-Curtis dissimilarities and standardized square root abundances of species, to test for the effect of Initiation Time (T) and Successional Stage (S) on epibiont assemblages growing on artificial mangrove roots (AMRs) at 3 Sites (Si) from 2 Locations (L) in Venezuela. The square root of the variance components (\sqrt{CV}) is shown

Source	df	MS	Pseudo- <i>F</i>	p(perm)	\sqrt{CV}
Location = L	1	227680.0	6.49	0.0453	38
Timing = T	1	9485.6	1.45	0.3263	5
Successional Stage = S	2	17687.0	1.80	0.2217	9
Site = Si[L]	4	35246.0	21.38	<0.0001	27
L \times T	1	6527.9	1.54	0.2182	6
L \times S	2	9850.9	2.08	0.0178	11
T \times S	2	5442.6	1.05	0.4799	2
Si[L] \times T	4	4255.8	2.58	<0.0001	11
Si[L] \times S	8	4743.6	2.88	<0.0001	14
L \times T \times S	2	5162.1	1.55	0.1212	9
Si[L] \times T \times S	8	3338.6	2.03	<0.0001	15
Residuals	241	1648.3			41
Total	276				

Table 3. Variability among natural neighboring roots (residuals) using permutational multivariate ANOVA based on Bray-Curtis dissimilarities and standardized square root abundances of species. The square root of the variance components (\sqrt{CV}) is shown

Source	df	MS	Pseudo- <i>F</i>	p(perm)	\sqrt{CV}
Location = L	1	56016	5.64	0.119	31
Timing = T	1	11484	1.37	0.282	8
Site = Si[L]	4	9940	3.53	0.001	21
L × T	1	11020	1.31	0.294	10
T × Si[L]	4	8396	2.98	0.001	26
Residuals	84	2820			53
Total	95				

however, the interactions L × T and L × T × S were not significant. Individual analyses per site were similar, as the interaction T × S was significant for all 6 sites (Table 4). In 4 out of 6 sites, the covariate was statistically significant; however, in all cases, the component of variation of the interaction T × S was higher (12–18% of dissimilarities in Bray-Curtis space) than the component of variation of the covariate (i.e. length of the colonizable area, 3–12%). Overall, these results are evidence against Hypotheses 1 and 2.

Hypothesis 3

In 4 out of 6 sites, there was no significant effect of the covariate (i.e. length of colonizable area) on species richness (Table 4). At sites La Entrada and El Mánamo (both located in LRNP), where the covariate was statistically significant (Table 4), a linear regression model indicated that richness increased with the colonizable area of the root ($b = 0.156$ for La Entrada, $b = 0.181$ for El Mánamo). However, the components of variations were very low (CV = 8% for La Entrada, 2% for El Mánamo), which strongly suggests that mechanisms related to the species–area relationship might not play an important role in explaining small-scale variability in these systems.

Hypotheses 4, 5 and 6

The identity and abundances of species that arrived as colonists, as well as the identity of species that recruited later, varied considerably among sites and colonization timing: this was particularly conspicuous for sites in MNP, but not in LRNP (Fig. 2). In all cases, most of the species that appeared in ‘older’ assemblages did not appear in younger ones, as indicated by SIMPER analyses (see Tables S1–S6 in the Supplement at www.int-res.com/articles/suppl/m588p015_supp.pdf). However, some early species remained on the AMRs throughout the duration of the experiment. In fact,

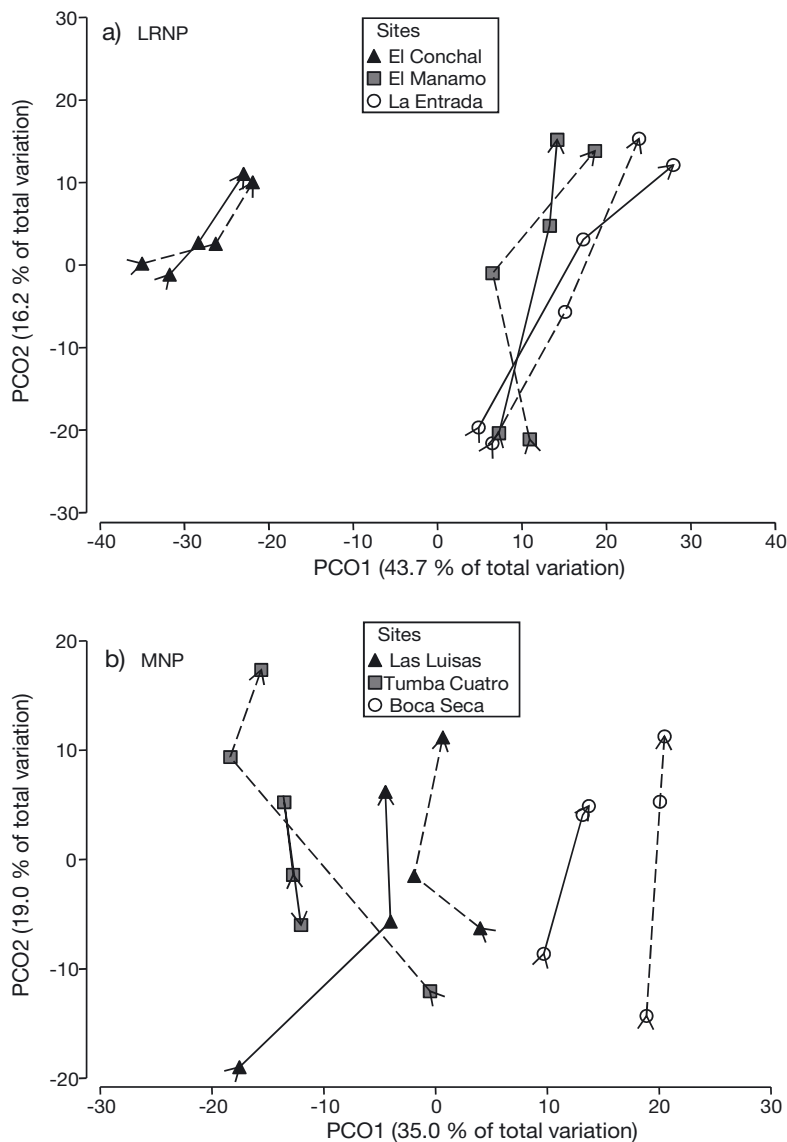


Fig. 2. Principal coordinate ordination (PCO) plots showing centroids for the interaction term Site × Initiation Time × Successional Stage for each sampled location in Venezuela: (a) La Restinga National Park (LRNP) and (b) Morrocoy National Park (MNP). Solid (dashed) arrows indicate the direction of changes at the first (second) initiation time

Table 4. Permutational multivariate and univariate ANOVA results for testing effects of length of colonizable area (Area, as covariate [covar.]), initiation time and successional (Succ.) changes in structure and richness of assemblages on artificial mangrove roots at 6 sites from La Restinga National Park (LRNP) and Morrocoy National Park (MNP), Venezuela

Source	df	Structure of assemblages				Richness of assemblages		
		MS	Pseudo-F	p(permutation)	√CV	MS	F-ratio	p(permutation)
Boca Seca (MNP)								
Area (covar.)	1	2591.3	3.28	0.0006	6	11.1	1.6	0.2151
Timing = T	1	3065.8	3.88	0.0002	10	21.2	3.1	0.0882
Succ. stage = S	2	3527.8	4.46	0.0001	14	48.1	6.9	0.0031
T × S	2	1816.0	2.30	0.0011	12	22.7	3.3	0.0563
Residuals	41	790.8			28	6.9		
Total	47							
Tumba Cuatro (MNP)								
Area	1	1612.5	1.51	0.1566	3	12.9	2.0	0.1642
T	1	2764.4	2.60	0.0098	9	74.9	11.7	0.0020
S	2	2931.9	2.75	0.0004	11	59.7	9.3	0.0001
T × S	2	3255.2	3.06	0.0002	18	18.1	2.8	0.0632
Residuals	41	1064.7			33	6.4		
Total	47							
Las Luisas (MNP)								
Area	1	2640.3	3.00	0.0047	6	1.7	0.5	0.4817
T	1	4308.5	4.89	0.0001	13	39.1	12.3	0.0021
S	2	2440.0	2.77	0.0005	11	22.7	7.1	0.0035
T × S	2	2225.9	2.53	0.0017	13	0.6	0.2	0.8482
Residuals	41	881.2			30	3.2		
Total	47							
La Entrada (LRNP)								
Area	1	2112.5	1.88	0.0310	10	147.4	6.4	0.0173
T	1	3449.4	3.07	0.0004	22	111.8	4.8	0.0424
S	2	8485.3	7.56	0.0001	15	78.3	3.4	0.0444
T × S	2	2778.4	2.48	0.0001	33	3.5	0.2	0.8591
Residuals	41	1122.2				23.1		
Total	47							
El Mánamo (LRNP)								
Area	1	6585.9	5.99	0.0001	12	241.3	7.8	0.0101
T	1	2312.7	2.10	0.0185	8	0.4	0.0	0.8962
S	2	3845.1	3.50	0.0001	16	17.1	0.6	0.5761
T × S	2	2022.8	1.84	0.0116	13	1.7	0.1	0.9497
Residuals	34	1099.3			33	30.8		
Total	40							
El Conchal (LRNP)								
Area	1	1577.3	1.83	0.0726	4	2.3	0.2	0.6194
T	1	2914.3	3.39	0.0003	10	0.0	0.0	0.9515
S	2	4910.9	5.71	0.0001	17	88.8	8.5	0.0012
T × S	2	2299.6	2.67	0.0004	14	15.0	1.4	0.2213
Residuals	37	860.47			29	10.4		
Total	43							

richness did increase with time at all sites, but did not increase significantly after the fourth month at 4 of the 6 sites (Fig. 3). Despite this trend in the number of species, assemblage composition changed continuously, indicating a real replacement of species in almost all cases. These results are consistent with the predictions of Hypothesis 5 (i.e. succession mediated by facilitation of early species), but not with those of Hypothesis 4 (i.e. tolerance of early species) or 6 (i.e. inhibition of arrival of new species).

Detailed descriptions of species replacements showed that they varied among sites (see text in the Supplement); however, some general temporal patterns were identified in each locality. In LRNP, the modular bryozoans *Synnotum circinatum* and *Bugula neritina*, the hydroid *Nemalécium* sp., the barnacle *Balanus* sp., the polychaetes *Branchiommma nigromaculatum* and *Hydroïdes* sp. and the colonial tunicates *Botrylloides nigrum* and *Symplegma brakenhielmi* tended to dominate the substrates in the early stages of colonization. Progressively over time, other species such as solitary ascidians (e.g. *Styela canopus*, *Microcosmus exasperatus* and *Phallusia nigra*), the mangrove oyster *Crassostrea rhizophorae*, and encrusting sponge species of the genera *Haliclona* and *Mycale* were recruited on the substrates. Towards the end of the experiment, AMRs were dominated by oysters, encrusting sponges and solitary ascidians. In MNP, development was slower and varied more among sites and colonization timing; nevertheless, these changes always occurred in the same direction. Generally, the red alga *Acanthophora spicifera* and the bryozoans *S. circinatum* and *Scrupocellaria* sp. were the most frequent species during the first weeks. Then, although coverage of these species increased, other species like the green alga *Caulerpa verticillata* and the sponge

Mycale microsigmatosa appeared on almost all of the substrates. After 6 mo, the dominant species tended to be sponges like *Haliclona manglaris*, *Mycale carmi-gropila*, *M. magnirhaphidifera* and *Aplysilla glacialis*.

DISCUSSION

In this study, 3 model types that could explain high variability in species diversity among neighboring

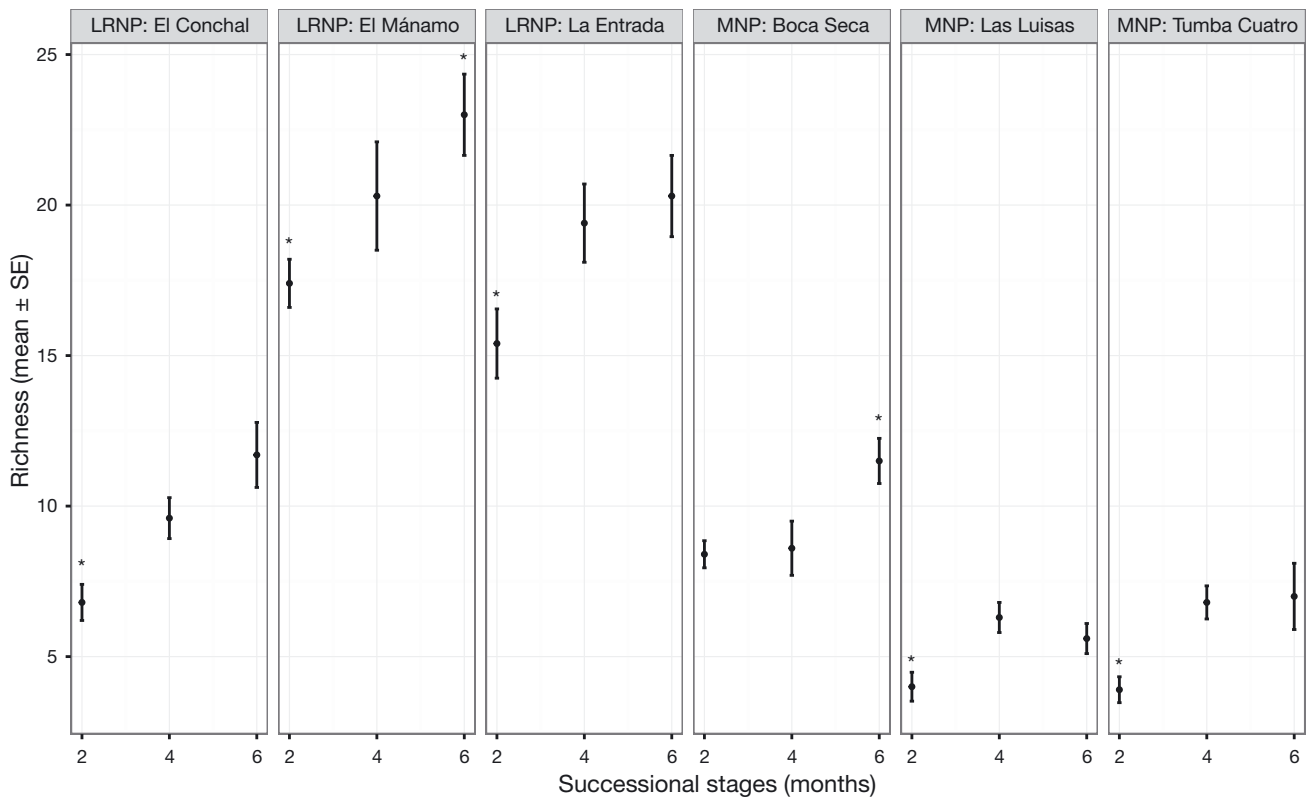


Fig. 3. Mean \pm SD numbers of epibiont species on artificial mangrove roots (AMRs) in each successional stage at 3 sites in La Restinga National Park (LRNP) and Morrocoy National Park (MNP), Venezuela. Data were pooled between initiation times because the interaction Initiation Time \times Successional Stage was not significant for all sites. Asterisks indicate statistical differences in pairwise comparisons using a *t*-test without α correction

roots were evaluated: initiation time (i.e. temporal variation in supply of recruits), successional development and the species–area relationship. Our results provided clear evidence on how successional changes, mediated by colonization timing, explained a large proportion of variability among neighboring roots in terms of richness, abundance and species composition. Even though area explained some variation (i.e. in richness) among neighboring roots at 2 sites, its relative contribution to that variation was a fraction of what was explained by the 2 other drivers (i.e. successional changes and initiation time). Despite differences among sites and between initiation times, similar general patterns of successional changes were described in this study, providing strong evidence towards the existence of an ordered community development over time (i.e. succession sensu Connell & Slatyer 1977). Simultaneously, it was shown that initiation time has far-reaching implications in defining assemblage development, which was much stronger in MNP than LRNP. This result confirms that variation in supply of recruits (i.e. identity and abundance) for colonization of the experimental substrates might deter-

mine initial community development and subsequent changes within that community (Underwood & Fairweather 1989, Anderson 1999). The identification of these processes allows us to understand the large differences among neighboring roots; adjacent mangrove roots differ largely in epibenthic species composition because they present different stages of succession and were colonized at different times.

Various studies have shown that recruitment rates in these systems (mangrove roots) are very low and larval composition is highly variable in time and space; consequently, no predictions on assemblage development could have been made (Sutherland 1980, Ellison & Farnsworth 1990, Bingham 1992). Similarly, a study done on mangrove roots from MNP (Álvarez 1989) showed that it was not possible to predict the order of species arrival, but it was possible to predict the arrival order of some taxonomic groups. Despite some variation in the identity of particular species across sites and initiation times, we were able to identify the existence of an earlier and a later group of species. Based on these results, we propose that colonization of mangrove roots initiates with

modular organisms like hydroids, bryozoans and algae, which are followed by solitary ascidians, oysters and encrusting sponges/tunicates, with the final stages of succession in these assemblages being dominated by massive sponges and squirt species (Fig. 4).

Despite these broad functional group patterns, differences in species composition and abundance in AMRs from the same stage but different colonization timing indicated that community development is not predictable in the manner stated by Paine (1984) (i.e. single-species monoculture at the final stage of succession). Sutherland (1974) suggested that the structure and composition of an encrusting community is strictly dependent on the identity and abundance of the larvae present when the substrate becomes available for colonization. This claim has been supported by various experiments done in coastal benthic systems (e.g. Underwood & Chapman 2006, Cifuentes et

al. 2010, Maggi et al. 2011). After a long discussion about determinism in rocky intertidal communities, Connell (1985) recognized that classical ecological models related to post-recruitment mortality tend to be evident only when the larval supply is high; however, this does not occur at sites where larval supply is low (Lewin 1986). In our study, recruitment to AMRs deployed in MNP was lower and more variable than in LRNP, which could explain why variation in succession from different colonization times was more marked at sites in MNP than LRNP. All of this implies that community development can occur in multiple directions and different climax communities can be reached. Although it is not possible to predict which specific species will dominate the final stages, the deterministic idea of successional development may be rescued if the broader taxonomic or functional groups are used.

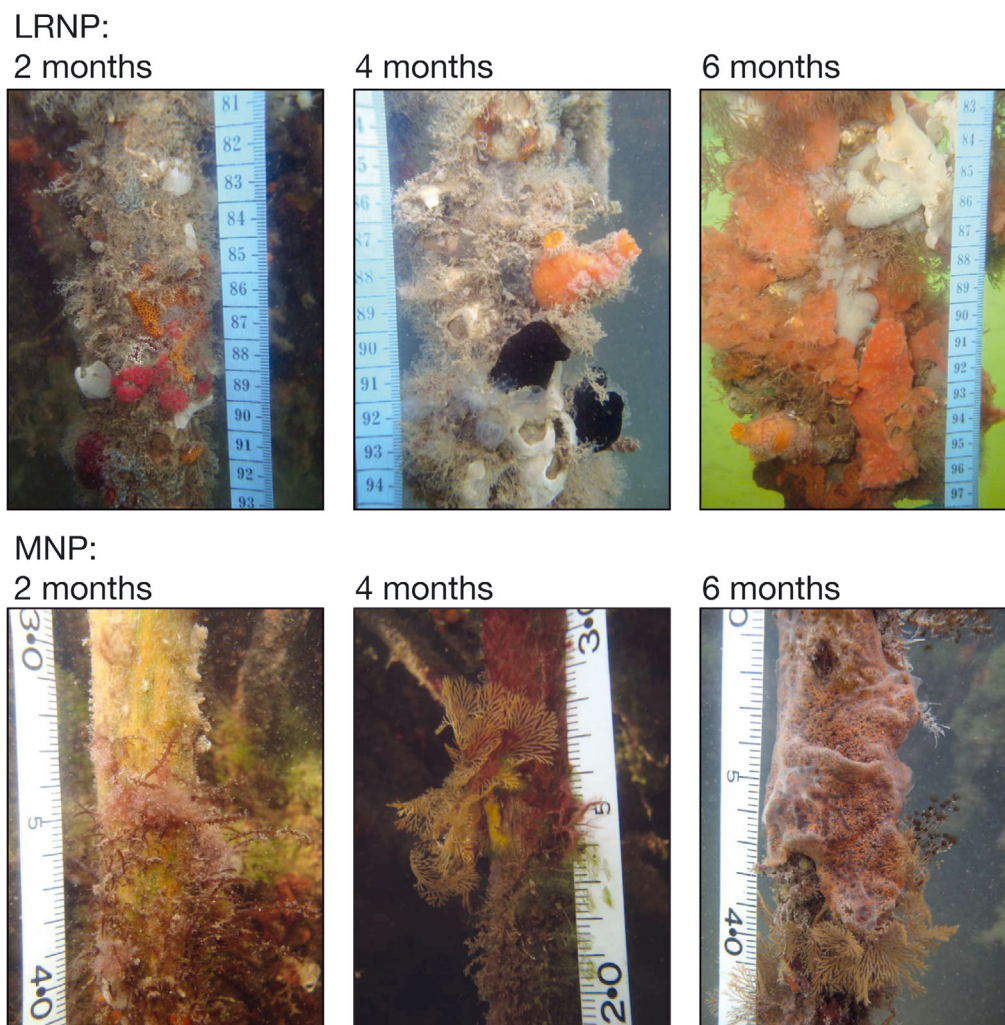


Fig. 4. Evolution of assemblage development on artificial mangrove roots (AMRs) from La Restinga National Park (LRNP) and Morrocoy National Park (MNP), Venezuela, over 6 mo

The type of succession observed in this study, in which colonizing species were always displaced by later species, is defined as a succession mediated by facilitation (Connell & Slatyer 1977). In this case, the late arrival of sponges and solitary sea squirts may be associated with the chemical stimulation induced by earlier species already present in the area (Pawlik 1992, Rodríguez et al. 1993). However, a structure with chemoreceptor functions in larvae of sponges has not yet been described or identified (Maldonado 2006). To date, most studies based on the settlement of sponge larvae indicate that the main stimulus is phototaxis (Maldonado 2006), which has been shown for mangrove sponge species, like *Tedania ignis*, *Haliclona tubifera*, *H. caerulea* and *Halichondria magniconulosa* (Maldonado & Young 1996). Nevertheless, Avila & Carballo (2006) detected selectivity in the settlement by the sponge *H. caerulea*, induced by the presence of red algae *Jania adherens*. Similarly, Hunting et al. (2010) experimentally demonstrated that the tannins of mangroves stimulate the settlement of *T. ignis*. In both studies, chemotaxis was the model that explained high settlement rates in their experimental treatments. Similarly, for tunicates, phototaxis is the most important mechanism described as a settlement inducer (Svane & Young 1989). However, evidence indicates that high aggregations of tunicates are produced by the ability of larvae to chemically recognize adults that are already present on a given substrate (Osman & Whitlatch 1995). This has been shown for *Ecteinascidia turbinata*, a colonial ascidian with high aggregation rates in mangroves of the Florida Keys, USA (Bingham & Young 1991b), and was also found in MNP and LRNP. For other taxonomic groups, different biological mechanisms have been described as inducers of larval settlement, highlighting the effects of bacteria and benthic diatoms (Zardus et al. 2008), ciliated protozoans (Shimeta et al. 2012) or the presence of individuals of particular species (Toonen & Pawlik 1994). The potential effects of the colonizing species on the settlement of later species must be addressed experimentally to establish which specific mechanisms of facilitation are operating in these systems.

In addition to succession, temporal changes in species composition and abundance could also be a response to pulses of species recruitment, rather than the biological interactions that typically shape succession, like competition (Bingham & Young 1991a, Bingham 1992). Under this model, in the absence of predators, hanging roots are constantly trapping species, and it can be hypothesized that younger roots will host fewer species than long-lived and unper-

turbed roots. However, independent of this, succession might be affected by stochastic disturbances of a different nature (Platt & Connell 2003). For example, a sudden change in environmental conditions (e.g. drop in salinity after a heavy rainfall) can generate high mortality of epibenthic species (i.e. catastrophic disturbances; e.g. Orihuela et al. 1991, Bingham & Young 1995) or lead to assemblages whose survivors are mainly species able to tolerate those conditions (i.e. non-catastrophic disturbances). These kinds of events will equally affect all roots at the site, and will reduce the site β -diversity. Alternatively, catastrophic and non-catastrophic disturbances might occur in some, but not all, of the neighboring roots, for example, an aggressive colonial ascidian (e.g. *Aplidium accarensense*) or colonial bryozoan (e.g. *Schizoporella pungens*) overgrowing and killing the entire assemblage in the root, or a benthic sponge predator (e.g. *Echinaster paucispinus*) that reaches an unperturbed hanging root helped by debris of mangrove branches. In such cases, site β -diversity will increase. In this study, the potential effect of benthic predators was avoided by placing the AMRs 30 cm from the bottom. Although present in the area, aggressive overgrowing species did not affect the AMRs used in this experiment. Nonetheless, the effects of stochastic catastrophic and non-catastrophic disturbances on the direction of succession will be discussed with experimental data in a forthcoming paper.

Despite the fact that the species–area relationship is perhaps one of the most commonly identified patterns in ecology (Anderson 1999, Lomolino 2000), processes associated with root area did not play an important role in determining patterns of temporal variation in richness and structure of assemblages associated with mangrove roots. The absence of a species–area relationship has been discussed in detail for other marine assemblages and taxa (Anderson 1999, Hirst & Attrill 2008, Hachich et al. 2015). In those cases, the initial stages of colonization, habitat properties and age of each island/patch were considerably more important than the area per se. For epibenthic assemblages on mangrove roots, the variability in supply of recruits, as well as the life history of early and late species, seems to be more important than area as a driver for species richness and composition. However, even when a significant portion of variability among neighboring roots was experimentally explained, there was still an important amount of unexplained variability. Part of this variation might be stochastic; however, some other processes might be involved. Small differences in orientation, position

and shading of mangrove roots (Glasby 1999, Glasby & Connell 2001), as well as specific settlement strategies (Pawlik et al. 1991, Pawlik 1992) might be responsible for the remaining variability in species diversity.

In conclusion, our data showed that succession (sensu Connell & Slatyer 1977), mediated by colonization timing, explained an important portion of the variation in species diversity among neighboring roots. Assemblage development on AMRs was dependent on foundational species, which varied among localities and colonization times. Also, the empirical evidence presented here showed that the high variability among neighboring roots cannot be explained by root area. Finally, this is the first quantitative experimental research for assemblages associated with mangrove roots which accounts for an orderly development of community structure, where facilitation was the mechanism which best explained the observed pattern, although tolerance was also detected in some species. This study therefore suggests that new insights can be gained from considering mechanisms that underlie the succession of community assembly and by accounting for biotic and abiotic interactions influencing post-recruitment community dynamics.

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