Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession

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ABSTRACT

Ecological succession and the balance between stochastic and deterministic processes are two major themes within microbial ecology, but these conceptual domains have mostly developed independent of each other. Here we provide a framework that integrates shifts in community assembly processes with microbial primary succession to better understand mechanisms governing the stochastic/deterministic balance. Synthesizing previous work, we devised a conceptual model that links ecosystem development to alternative hypotheses related to shifts in ecological assembly processes. Conceptual model hypotheses were tested by coupling spatiotemporal data on soil bacterial communities with environmental conditions in a salt marsh chronosequence spanning 105 years of succession. Analyses within successional stages showed community composition to be initially governed by stochasticity, but as succession proceeded, there was a progressive increase in deterministic selection correlated with increasing sodium concentration. Analyses of community turnover among successional stages — which provide a larger spatiotemporal scale relative to within stage analyses — revealed that changes in the concentration of soil organic matter were the main predictor of the type and relative influence of determinism. Taken together, these results suggest scale-dependency in the mechanisms underlying selection. To better understand mechanisms governing these patterns, we developed an ecological simulation model that revealed how changes in selective environments cause shifts in the stochastic/deterministic balance. Finally, we propose an extended — and experimentally testable — conceptual model integrating ecological assembly processes with primary and secondary succession. This framework provides a priori hypotheses for future experiments, thereby facilitating a systematic approach to understand assembly and succession in microbial communities across ecosystems.
Significance

Across ecology, and particularly within microbial ecology, there is limited understanding of the mechanisms governing the relative influences of stochastic and deterministic processes. Filling this knowledge gap is a major challenge that requires the development of novel conceptual paradigms, experiments, and ecological models. Here we (i) present a conceptual model that couples the stochastic/deterministic balance to primary and secondary ecological succession, thereby integrating previously isolated conceptual domains; (ii) evaluate this model over 105 years of ecosystem development, revealing a systematic shift in the type and strength of ecological selection; and (iii) couple empirical data with a new simulation model to elucidate underlying mechanisms and characterize their scale dependency. The insights and conceptual framework provided here represent a nexus for cross-system integration.

Introduction

A major goal in microbial community ecology is to understand the processes that underlie observed patterns in species abundances across space and time (Dumbrell et al., 2010; Stegen et al., 2013; Zhou et al., 2014). Two types of processes — deterministic and stochastic — influence the assembly of species into communities. Deterministic processes — in which abiotic and biotic factors determine the presence/absence and relative abundances of species — are associated with ecological selection [sensu Vellend (Vellend, 2010)]. Stochastic processes include probabilistic dispersal and random changes in species relative abundances (ecological drift) that are not the consequence of environmentally determined fitness (Hubbell, 2001; Chase and Myers, 2011).

Historically, microbial community assembly has been studied from a deterministic perspective (Baath, 1996; Torsvik et al., 2002), where empirical evidence shows that a variety of environmental factors — such as pH, salinity, and organic carbon — influence community establishment at different scales (Fierer and Jackson, 2006; Lozupone and Knight, 2007). However, recent studies have provided increasing support for a predominant role of stochasticity in some microbial systems (e.g. Caruso et al., 2011). As opposed to a dichotomous debate, in which one attempts to reject stochastic processes in favor of deterministic ones (or vice versa), a more comprehensive perspective should integrate both processes and work to understand how and why their relative influences vary across systems, time, and space (Chase, 2007; Chase and Myers, 2011; Chisholm and Pacala, 2011; Langenheder and Székely, 2011; Myers and Harms, 2011; Stegen et al., 2013).

The study of ecological succession provides an ideal setting for understanding mechanisms that govern community assembly processes through time and space. Although ecological succession in microbial communities has been broadly investigated (Jackson et al., 2001; Tscherko et al., 2003; Schütte et al., 2009; Fierer et al., 2010; Bengtsson et al., 2012; Ferrenberg et al., 2013), little has been done to formally link this theme with the balance in stochastic/deterministic processes. Only two studies have directly related these conceptual domains, and both have focused on secondary succession (i.e. following distur-
bance) (Ferrenberg et al., 2013; Zhou et al., 2014). These studies show that disturbance promotes a time-dependent shift in the stochastic/deterministic balance. A full understanding of linkages among community succession, disturbance, and the assembly processes, however, requires a testable conceptual framework that enables systematic evaluation of the stochastic/deterministic interplay during succession in both pristine and disturbed ecosystems.

Here we set up a framework that integrates the conceptual domains of microbial succession and the balance in stochastic/deterministic ecological processes. We first devised a conceptual model that links environmental heterogeneity to shifts in these assembly processes during microbial primary succession; for this, we purposefully followed the approach used in Ferrenberg et al. (2013) to allow a direct linkage between our model and theirs. Alternative hypotheses within the conceptual model were tested by applying an ecological null modeling approach (Stegen et al., 2013) to data from a soil chronosequence spanning 105 years of primary ecosystem succession (Olff et al., 1997). The analyses revealed scale dependency with respect to how environmental factors govern the interplay between stochastic and deterministic processes. To better understand the mechanisms underlying the observed patterns, we developed an ecological simulation model that revealed how changes in selective environments cause shifts in the processes underlying community assembly. Finally, to facilitate conceptual synthesis and to generate a priori hypotheses for future experiments, we merged our conceptual model — focused on primary succession — with an extended version of the Ferrenberg et al. (2013) secondary succession model.

**Conceptual model**

Primary succession in microbial communities has been investigated along receding glacier forelands (Sigler and Zeyer; 2002; Nicol et al., 2005; Deiglmayr et al., 2006; Nemergut et al., 2007; Zumsteg et al., 2012; Ferrenberg et al., 2013), in drinking water biofilms (Jackson et al., 2001; Jackson, 2003; Martiny et al., 2003; Revetta et al., 2013), and in the rhizosphere (Mahaffee and Klopper, 1997; Wieland et al., 2001; Marschner et al., 2002; Chaparro et al., 2014). These studies have collectively revealed a broad suite of patterns associated with shifts in community biomass, diversity, and composition, but none has directly characterized shifts in community assembly processes during succession. However, close examination of the patterns provides indirect insights. We therefore integrate these studies into a conceptual model of predicted trajectories of the stochastic/deterministic balance during microbial primary succession. The model was conceptualized as a time continuum with distinct scenarios playing out across three phases (Figure 3.1).

Our conceptual model represents a collection of alternative hypotheses focused on the dynamics of the stochastic/deterministic balance during primary succession. We do not attempt to encompass all possible scenarios but focus instead on combining straightforward hypotheses into a testable framework. The model is therefore likely to be supported in some ecosystems and rejected in others; both outcomes are equally informative.
Phase 1: Microbial Community Assembly Is Initially Governed by Stochastic Processes

It is expected that initial community establishment will be primarily dominated by stochasticity. Jackson et al. (2001, p. 564) observed that during the initial stage of drinking water biofilm formation, communities were “characterized by the colonization of different populations and lack of orderly community structure.” This phenomenon has been confirmed by additional studies (Martiny et al., 2003) and applies to a range of ecosystems. For example, during the early stages of primary succession in a glacier foreland, soil microbial communities are highly diverse and dominated by taxa capable of using many different resources (Sigler et al., 2002; Sigler and Zeyer, 2002). The authors interpreted these findings as evidence for weak competition, which implies weak selection and thus a potentially large influence of stochasticity. It has also been suggested that the sugars released by seedling roots in soil provide a resource-rich environment that reduces competitive pressures, which leads to a dominance of stochasticity during the initial establishment of rhizosphere communities (Inceoglu et al., 2011; Badri et al., 2013; Chaparro et al., 2013). More generally, when a broad range of organisms can grow successfully in a given environment, stochasticity is likely to dominate the initial phase of community assembly (Chase, 2007; Chase and Myers, 2011).

Phase 2: Changes in the Local Environment Progressively Increase the Importance of Deterministic Selection

Following initial microbial community establishment, deterministic selection may become progressively important as organisms affect their environment (e.g. through resource depletion): “following the disordered nature of the early communities, the bacterial assemblage may ‘simplify’ as superior competitors begin to dominate” (Jackson et al., 2001, p. 563). Thus, as the strength of selection increases, an increasingly large breadth of taxa are excluded. We extend this conceptualization with two hypothesized scenarios that relate to shifts in the level of heterogeneity in the selective environment. In our conceptual model, multiple mechanisms (e.g. abiotic habitat filtering and biotic competition) combine to generate the selective environment.

Under the homogeneous selection scenario (Figure 3.1, blue line), the selective environment is spatially homogeneous within each successional stage and does not change significantly during the relatively short time span covered by each stage. The strength of selection does, however, intensify during the longer time span covered by multiple successional stages. This occurs due to directional changes in the mean of one or more selective factors as succession proceeds, which results in specific physiological adaptations being required for positive population growth. For example, soil pH can strongly influence bacterial community composition; thus, a stringent environmental filter is imposed in high- or low-pH conditions (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). The homogeneous selection scenario could therefore emerge if (i) pH was spatially homogeneous within each successional stage and (ii) mean pH became increasingly extreme within later successional stages. The relative influence of homogeneous selection is therefore expected to increase as succession proceeds.

The selective environment may also be spatially heterogeneous, leading to variable selection [sensu Vellend (Vellend, 2010)]. In this case, taxa selected for in one place may be
**Figure 3.1.** Three-phase conceptual model composed of alternative hypotheses related to changes in the strength and type of ecological selection during primary succession. Ecological selection is weak in the center of the vertical axis and is stronger toward both extremes. In phase 1, the initial establishment of microbial communities is expected to be dominated by stochasticity such that turnover in community composition shows little deviation from the null expectation. In phase 2, changes in environmental conditions progressively increase the strength of selection leading to scenarios 1a and 1b; if the environmental factors that change through time do not impose selection, stochastic factors are expected to remain dominant such that the system will remain ecologically neutral [sensu Hubbell (Hubbell, 2001)] as in scenario 1c. When succession is associated with increasing spatial heterogeneity in selective pressures the variable selection (VS) scenario (1a) is expected; spatial environmental heterogeneity causes turnover in community composition to be greater than the null expectation. In contrast, a spatially homogeneous environment and directional changes — across successional stages — toward increasingly extreme selective conditions lead to the homogeneous selection (HS) scenario (1b); spatial environmental homogeneity causes turnover in community composition to be lower than the null expectation. In phase 3, if succession eventually leads to relatively stable environmental conditions, a relatively stable balance between stochastic/deterministic processes is expected.

selected against in a different place because of spatial variation in the selective environment. We hypothesize a variable selection scenario (Figure 3.1, red line) in which spatial environmental heterogeneity increases as succession proceeds. For example, as biofilms develop, new spatially structured ecological niches emerge (e.g. anoxic pockets), and communities are driven toward a 3D architecture where environmental conditions — and thus selective environments — vary (Jackson, 2003). In this case, increasing environmental heterogeneity is expected to cause compositional differences across local communities. The relative influence of variable selection is therefore expected to increase as succession proceeds.

*Phase 3: Emergence of Stable Environments Leads to Stable Levels of Deterministic Selection*  
In the third phase (Figure 3.1, phase 3) we hypothesize the relative influences of stochastic and deterministic ecological processes to become relatively stable under both the homoge-
neous and variable selection scenarios. This assumes that environmental factors that impose selection do not change directionally in their mean or variance. However, some ecosystems may not reach this phase before disturbance.

**Neutral Hypothesis**

As an alternative, we hypothesize that some microbial systems are consistently dominated by stochasticity. Such systems might be characterized by abundant resource supply and high levels of organismal dispersal. These characteristics are found, for example, in fluidic systems with constant resource supply (Zhou et al., 2014) such that few microbial taxa are excluded from growing. In this case, species relative abundances will likely be the result of stochastic birth/death events, instead of being environmentally determined (Figure 3.1, neutral inset).

**Results**

**Conceptual Model Evaluation**

We evaluated the conceptual model using data on soil bacterial communities and their abiotic environment across 105 years of primary succession in a salt marsh chronosequence (Olff et al., 1997), which comprised spatial and temporal community turnover within five successional stages (0, 5, 35, 65, and 105 years of soil development). We started by addressing community turnover within each successional stage. We estimated the relative influence of stochastic ecological processes and — when present — the type of deterministic ecological selection (homogeneous or variable). To do so, we combined soil bacterial community data with a previously developed null modeling strategy (Stegen et al., 2013). Second, we used linear regression and statistical model selection to evaluate which abiotic factor was most strongly associated with the relative influence of stochasticity. Below we address the details of these approaches.

First, to infer the relative influences of stochastic and deterministic processes and to differentiate between homogenous and variable selection, we studied community phylogenetic turnover; here defined as the phylogenetic distance separating bacterial operational taxonomic units (OTUs) in one community from OTUs in a second community (Graham and Fine, 2008; Stegen et al., 2012). Using phylogenetic turnover to make ecological inferences requires that phylogenetic distances among taxa approximate differences in the ecological niches they occupy. When this relation is significant, niches are said to have phylogenetic signal (Losos, 2008), and from fundamental evolutionary principles it is expected that microbial niches have phylogenetic signal despite horizontal gene transfer (Stegen et al., 2012). Testing for phylogenetic signal using Mantel correlograms (Lindemann et al., 2013; Stegen et al., 2013; Wang et al., 2013) revealed significant positive correlations between differences in OTU environmental optima and OTU phylogenetic distances ($P < 0.05$) but only across relatively short phylogenetic distances (Supplementary Figures S1 and S2). This result is consistent with previous studies on microbial communities in different environments (Stegen et al., 2012; Lindemann et al., 2013; Stegen et al., 2013; Wang et al., 2013) and suggests that within the salt marsh chronosequence, underlying ecological
processes can be inferred from analyses of phylogenetic turnover. This assertion was evaluated — and supported — via simulation (SI Material and Methods, Simulation Model).

To quantitatively analyze phylogenetic turnover, we used the abundance weighted β-mean nearest taxon distance (βMNTD). This metric quantifies the phylogenetic distance between assemblages; βMNTD was used because it emphasizes phylogenetic turnover across short phylogenetic distances, where the assumption of phylogenetic signal is strongly supported (Supplementary Figure S1). To infer the ecological factor that is primarily responsible for the observed turnover between a given pair of assemblages, we use a null modeling approach that generates an expected level of βMNTD given the dominance of stochastic ecological processes. To quantify the magnitude and direction of deviation between an observed βMNTD value and the null βMNTD distribution, we used the β-nearest taxon index (βNTI). βNTI < −2 or > +2 indicates that βMNTD_{obs} deviates from the mean βMNTD_{null} by more than two standard deviations; we consider βNTI < −2 or > +2 to indicate significantly less than or greater than expected phylogenetic turnover, respectively, for a given pairwise comparison. The βMNTD_{null} distribution represents the expected level of phylogenetic turnover given the dominance of stochastic ecological processes such that we further consider a significant deviation (i.e. |βNTI| > 2) to indicate the dominance of deterministic processes and the lack of deviation (i.e. |βNTI| < 2) to indicate the dominance of stochastic processes (Hardy, 2008; Stegen et al., 2012; Stegen et al., 2013). Homogeneous and variable selection should cause less than and greater than expected community turnover; respectively; βNTI < −2 or > +2 is further interpreted as indicating the dominance of homogeneous or variable selection, respectively; this interpretation was evaluated and supported via simulation (SI Material and Methods, Simulation Model). βNTI values for all possible pairwise comparisons within, but not between, the stages of succession revealed

![Figure 3.2](attachment:figure_3.2.png)

**Figure 3.2.** Patterns of βNTI and stochasticity. (A) Box plots of βNTI distributions across successional stages showing the median (thick black line), the first quartile (lower box bound), the third quartile (upper box bound), the range of data values that deviate from the box no more than 1.5 times the height of the box (vertical dashed lines), and outliers (open circles). Horizontal dashed lines indicate upper and lower significance thresholds at βNTI = +2 and −2, respectively. (B) The estimated relative influence of stochasticity as a function of log-transformed Na concentration per gram of soil. The solid line is the linear regression model, and statistics are provided on the panel.
that the \( \beta\text{NTI} \) distribution progressively shifted with increasing successional stage, from being primarily consistent with stochastic community assembly \((-2 < \beta\text{NTI} < +2) \) to being consistent with homogeneous selection \((\beta\text{NTI} < -2) \). The means \((\mu)\) and standard deviations \((\sigma)\) were as follows: \( \mu = -0.31 \) with \( \sigma = 1.68 \), \( \mu = -1.45 \) with \( \sigma = 1.22 \), \( \mu = -3.46 \) with \( \sigma = 1.23 \), \( \mu = -4.06 \) with \( \sigma = 1.76 \), and \( \mu = -4.29 \) with \( \sigma = 1.23 \) for successional stages 0, 5, 35, 65, and 105 years, respectively (Figure 3.2A).

These results show a time-dependent shift in the relative influence of stochastic and deterministic processes along the chronosequence. As succession proceeds, the relative influence of stochasticity declined, and that of homogeneous selection increased, as in scenario 1b of our conceptual model. We further estimated the relative influence of stochasticity, within each successional stage, as the fraction of \( \beta\text{NTI} \) where \( |\beta\text{NTI}| < 2 \). These stochasticity estimates were then regressed against each measured abiotic factor or the logarithm of those factors. The best model (based on \( R^2 \)) showed a strong negative relationship between log-transformed sodium (Na) concentration and the relative influence of stochasticity \( (R^2 = 0.97, P = 0.002) \) (Figure 3.2B). This was true despite a concomitant increase \((\sim 1 \text{ to } 34 \text{ g dm}^{-3})\) in resource supply in the form of soil organic matter content (SOM) (Dini-Andreote et al., 2014), contradicting previous work (Chase, 2010) that found stochasticity to become more important with increasing resource availability.

**Community Assembly Processes Between Successional Stages**

To evaluate potential scale dependency in the mechanisms underlying community assembly, the spatiotemporal scale of analysis was expanded by including between-successional-stage comparisons. For this, \( \beta\text{NTI} \) values were determined across all pairwise community comparisons within \((\sim 15 \text{ m spatial scale and } 6 \text{ months temporal scale})\) and between \((\text{up to } 8 \text{ km spatial scale and } 105 \text{ years temporal scale})\) stages of succession. The resulting distance matrix was regressed against distance matrices associated with each measured environmental factor. This analysis showed the larger-scale patterns of \( \beta\text{NTI} \) to be most strongly related to changes in log-transformed SOM \((r = 0.73)\), and this relationship was significant via a Mantel test \((P < 0.0001)\). As the change in SOM increased, there was a continuous transition — on average — from \( \beta\text{NTI} < -2 \) at the smallest changes in SOM to \(-2 < \beta\text{NTI} < +2 \) at intermediate SOM shifts and to \( \beta\text{NTI} > +2 \) for the largest shifts in SOM (Figure 3.3A). These patterns suggested that increasing shifts in SOM lead to a transition from homogeneous selection (small to no change in SOM), to weak selection and stochasticity (moderate changes in SOM), to variable selection (large changes in SOM).

**Simulation-Based Evaluation of the Inferences**

In the simulation model we assembled local communities under four ecological scenarios (Figure 3.3B and Supplementary Figure S3). The four scenarios were based on conceptual interpretations of the observed \( \beta\text{NTI} \) patterns in the empirical data. That is, ecological rules governing simulated community assembly were developed from the \( \beta\text{NTI}- \) based inferences. The simulated scenarios are referred to with names similar to our conceptual model, but they are distinct from these in that succession (or dynamic changes) in the selective environment is not simulated. In the homogeneous selection scenario, local communities were assembled within a single (arbitrary) environment. The other three
scenarios invoked variable selection by assembling communities across two environments, and the strength of variable selection was manipulated by changing how different the two environments were from each other (Supplementary Figure S3).

Distributions of βNTI from the simulation model had the following means (μ) and standard deviations (σ): μ = −3.39 with σ = 1.04 from the strong homogeneous selection scenario (Supplementary Figure S3, purple panel), μ = −1.58 with σ = 1.00 from the weak variable selection scenario (Supplementary Figure S3, blue panel), μ = 1.29 with σ = 0.94 from the moderate variable selection scenario (Supplementary Figure S3, orange panel), and μ = 3.23 with σ = 0.89 from the strong variable selection scenario (Supplementary Figure S3, red panel).

The βNTI distributions arising from the simulation corresponded closely to the shifts in βNTI associated with changes in SOM (Figure 3.3 and Supplementary Figure S3) and support our inference that a continuous transition from βNTI < −2 to βNTI > +2 indicates that with progressive shifts in SOM, there is a transition from homogeneous selection, to stochasticity (i.e. weak selection), to variable selection. In addition, the simulations support our within-stage inferences of a transition from stochasticity ([βNTI] < 2 in stages 0 and 5 years) to strong homogeneous selection (βNTI < −2 in stages 35, 65, and 105 years) as succession proceeds. The simulation outcomes further indicate that robust ecological inferences can be made via βNTI-based analyses when there is phylogenetic signal across relatively short phylogenetic distances, as is the case in both the empirical and simulated regional species pools (Supplementary Figures S1 and S2).

Figure 3.3. βNTI patterns from empirical comparisons and simulated ecological scenarios. (A) βNTI for all pairwise community comparisons — within and between successional stages — as a function of the change in log-transformed soil organic matter (SOM) concentration between communities, presented as the fold change in SOM. The linear regression model is shown as the green line; statistics are provided on the panel. Horizontal dashed lines indicate the upper (+2) and lower (−2) significance thresholds. Boxes laid over the βNTI data represent our conceptual interpretation of how simulation model outputs (summarized in B) align with the empirical relationship between βNTI and changes in SOM. (B) βNTI distributions obtained from simulated ecological scenarios (see Supplementary Figure S3 for a detailed description).
Discussion

**Consistency Between the Conceptual Model and Empirical Data**

In line with our conceptual model (Figure 3.1), we observed a greater influence of stochasticity in the initial successional stages of the salt marsh chronosequence (stages 0 and 5 years). This result is consistent with previous indirect evidence from studies of microbial primary succession across diverse systems (Jackson et al., 2001; Sigler et al., 2002; Sigler and Zeyer, 2002; Martiny et al., 2003; Inceoğlu et al., 2011; Badri et al., 2013; Chaparro et al., 2013). Initial stages of the chronosequence studied here showed diverse bacterial communities (Dini-Andreote et al., 2014), in accordance with observations from glacier forelands (Sigler et al., 2002; Sigler and Zeyer, 2002) but in contrast to those from biofilms (Jackson et al., 2001; Martiny et al., 2003) and plant rhizospheres (Badri et al., 2013; Chaparro et al., 2013). These high levels of stochasticity and diversity may result from the initial physical structure of the salt marsh, which is formed through sand accumulation and sedimentation and is subjected to regular overflows by seawater (Olff et al., 1997). As a consequence, these stages are characterized by bare sand and relatively nutrient poor soils that are regularly saturated with seawater but that rapidly dry following seawater retreat (Olff et al., 1997, Dini-Andreote et al., 2014). This frequent tidal regimen may promote a dynamic environment with many opportunities for successful immigration (dispersal followed by establishment). Thus, random dispersal through both aerial and seawater vectors may be an important ecological factor at these sites. In addition, the physicochemical conditions (e.g. pH and Na concentration) are not extreme in the initial stages (Dini-Andreote et al., 2014) such that a strong environmental filter is not present. We suggest that edaphic properties during early succession lead to weak selection and high immigration rates such that random ecological drift governs spatiotemporal shifts in species abundances.

Deterministic selection was found to become increasingly strong and homogeneous toward later successional stages, which aligns with the homogeneous selection scenario in our conceptual model. Further analyses suggested that the progressive accumulation of Na was related to the decrease in stochasticity and the concomitant increase of homogeneous selection. In our system, initial stages had relatively low levels of Na (~1.8–2.4% by weight) compared with intermediate and late successional stages (~13.8–14.4%) (Dini-Andreote et al., 2014). Sodium concentrations above 13% impose a stringent filter on microbial communities, reflecting the need for physiological adaptations such as the biosynthesis of compatible solutes and the ‘salt-in’ strategy (McGenity et al., 2010; Oren, 2008).

Although our analyses encompassed 105 years of succession, we could not discern whether the relationship between stochasticity and Na was truly linear or if there was a threshold at which a small increase in Na concentration led to a large decrease in stochasticity. Future work that more finely partitions the Na concentration gradient could be used to identify such thresholds. We also note that there is covariation among physicochemical variables in our system (Dini-Andreote et al., 2014) such that caution is warranted in terms of concluding that Na concentration is the physicochemical variable that results in strong homogeneous selection. On the other hand, available literature provides evidences that
support our interpretation (Oren, 2002; Fierer and Jackson, 2006; Lozupone and Knight, 2007; Herlemann et al., 2011; Morrissey et al., 2014).

Contrary to macroecological theory in which the relative influence of stochasticity is thought to increase with resource supply (Chase, 2003; Chase, 2010), we observed a negative resource supply–stochasticity relationship. This points to an interaction between resource supply and other physicochemical conditions that impose selection. More specifically, we suggest that increased resource supply can increase stochasticity under physicochemical conditions that do not impose strong selection; when physicochemical conditions are extreme and require specific physiological adaptations, changes in resource supply should have little influence over levels of stochasticity. From a macroecological perspective, high levels of diversity may therefore be maintained in regions characterized by high resource supply and relatively benign abiotic conditions, consistent with Chase’s (Chase, 2010) assertion that high productivity enhances tropical biodiversity through elevated levels of stochasticity.

Evidence for Scale Dependency in the Mechanisms Underlying Ecological Selection
Our analyses revealed that selection was primarily imposed by Na at a relatively small scale (i.e. within successional stages; spatial scale up to 15 m, temporal scale up to 6 months) but that it was imposed by SOM at a larger scale (i.e. among successional stages; spatial scale up to 8 km, temporal scale up to 105 years). We hypothesize that this scale dependence arises because (i) there are locations within the larger-scale domain where Na concentrations are relatively low and thus do not impose ecological selection and (ii) changes in SOM across the larger-scale domain result in different selective environments across successional stages, but the strength of selection imposed by SOM does not vary substantially across successional stages. From this perspective, the strength of selection imposed by Na or SOM is hypothesized to be dependent (Na) or independent (SOM) of their respective concentrations. Application of the simulation model provided an initial evaluation of this hypothesis and showed that progressive shifts in an environmental variable that imposes a consistent strength of selection are expected to cause βNTI patterns that closely align with our among-stage βNTI observations (Figure 3.3).

Although these simulation results are not definitive, they corroborate the above conceptual inferences and provide a point of departure for future experimental research. Although the larger-scale analyses point to an important influence of SOM, we cannot distinguish among potential mechanisms that may be mediated by shifts in SOM concentration and/or composition. We note, however, that underlying physiological mechanisms are likely related to the composition of SOM, which is a heterogeneous mixture of organic compounds derived from marine and terrestrial sources and that are found at different states of degradation/lability (Bianchi, 2011). Spatiotemporal shifts in SOM composition — that covary with SOM concentration — may therefore cause shifts in the selective environment and, in turn, promote turnover in soil bacterial community composition among successional stages (Jorgensen et al., 2012). Manipulative experiments are required to characterize the degree to which selection is imposed by SOM concentration versus composition.
Conclusions and the Path Forward: Integrating Primary and Secondary Succession with Ecological Processes that Govern Microbial Community Assembly

In this study we aimed to synthesize and evaluate concepts related to shifts in the strength and type of ecological processes governing microbial community assembly during primary succession. In this effort, we set up a framework that allows the balance between stochastic/deterministic processes and the mechanisms mediating their relative influences to be systematically quantified and understood. Empirical and simulation analyses revealed that the relative influences of underlying mechanisms are scale-dependent. This is

Figure 3.4: Hypothesized conceptual model linking primary and secondary succession to the stochastic/deterministic balance. Phase 3 is the final phase of primary succession and is consistent with phase 3 in Figure 3.1; vertical axis is as in Figure 3.1. Following a disturbance event the relative influence of stochastic factors can shift in ways that are dependent on both the outcome of primary succession and the type of disturbance. In scenario 2a (red line), primary succession has resulted in strong variable selection. A significant shift away from strong variable selection — following disturbance — is expected if the environment is homogenized; the system may become dominated by strong homogeneous selection (not displayed) or become neutral if the resulting environmental condition does or does not, respectively, impose strong selection. In scenario 2b (purple line), a strong influence of stochastic factors has been maintained throughout primary succession, and the disturbance itself imposes a strong and spatially homogeneous selective pressure similar to patterns observed in soil microbial communities following fire (Ferrenberg et al., 2013). In scenarios 2c and 2d (blue lines), primary succession has resulted in strong homogeneous selection as in our field system. In scenario 2c, disturbance removes the strong selective pressure that developed over the course of primary succession; in our field system this could occur if shifts in topography lead to an increased frequency of tidal inundation at an older part of the chronosequence, thereby causing a decline in Na concentration. In scenario 2d, disturbance does not impact the primary selective pressure such that strong homogeneous selection is expected to be maintained; in our field system this could occur if SOM was artificially added to the later successional stages — high Na concentrations in late primary successional stages impose a dominant selective pressure that would not be alleviated by the addition of SOM.
a critical result that resonates with previous work (Martiny et al., 2011) and that will strongly influence the conceptual foundation of studies linking ecological processes to community assembly. We showed that microbial communities do not follow macroecological assumptions because the mechanisms driving stochasticity at a small scale were not related to resource supply.

We further hypothesize that the position of a community along the stochastic/deterministic continuum during primary succession will influence how community assembly processes operate during secondary succession (i.e. following disturbance). We used this hypothesis to couple our primary succession conceptual model with an extended version of the secondary succession model provided by Ferrenberg et al. (2013) (Figure 3.4). As summarized in Figure 3.4, this broader conceptual model provides an experimentally testable framework composed of a priori hypotheses that represent a theoretical basis for future experiments aimed at linking microbial community assembly with successional dynamics across pristine and disturbed systems. A systematic approach to understanding community assembly and succession in microbiomes across ecosystems is thereby enabled. Relevant new studies designed to test the broader conceptual model will significantly advance fundamental knowledge of ecological systems, within and beyond microbial ecology.

**Material and Methods**

Details for all methods are provided in SI Material and Methods. Briefly, soil samples were collected in triplicated plots at five stages of soil development in a salt marsh chronosequence, estimated as 0, 5, 35, 65, and 105 years of soil development. Total soil DNA was extracted using a MoBio PowerSoil DNA isolation kit (MoBio Laboratories), and communities were profiled targeting the V4–V6 region of the bacterial 16S rRNA using a Roche GS-FLX 454 automated pyrosequencer running the Titanium chemistry. Sequence data were analyzed in QIIME (Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2010b). Samples were individually subjected to measurements of soil physical structure (clay:silt:sand %) and chemical content of total organic matter (OM), nitrate (N-NO₃⁻), ammonium (N-NH₄⁺), sulfate (S-SO₄²⁻), sodium (Na), and pH.

To test for phylogenetic signal (in both empirical and simulation data analyses), we used phylogenetic Mantel correlograms, as described elsewhere (Lindemann et al., 2013; Stegen et al., 2013; Wang et al., 2013). To characterize the turnover in phylogenetic community composition, we quantified the β-mean nearest taxon distance (βMNTD), calculated as follows:

\[
βMNTD = 0.5 \left[ \sum_{i=1}^{n_k} f_{ik} \min(Δ_{ik}/m) + \sum_{i=1}^{n_m} f_{im} \min(Δ_{im}/k) \right],
\]

where \(f_{ik}\) is the relative abundance of OTU \(i\) in community \(k\), \(n_k\) is the number of OTUs in \(k\), and \(\min(Δ_{ik}/m)\) is the minimum phylogenetic distance between OTU \(i\) in community \(k\) and all OTUs \(j\) in community \(m\) \(\min(Δ_{ik}/j)\). βMNTD was calculated using the R function ‘comdistnt’ (abundance.weighted = TRUE; package ‘picante’), see SI Material and Methods.
To quantify the magnitude and direction of deviation between an observed $\beta$MNTD value and the null $\beta$MNTD distribution, we used the $\beta$-Nearest Taxon Index ($\beta$NTI), calculated as follows:

$$\beta\text{NTI} = \frac{(\beta\text{MNTD}_{\text{obs}} - \overline{\beta\text{MNTD}_{\text{null}}})}{sd(\beta\text{MNTD}_{\text{null}})},$$

where $\beta\text{MNTD}_{\text{obs}}$ is observed $\beta$MNTD, $\beta\text{MNTD}_{\text{null}}$ are null values of $\beta$MNTD, and $sd$ indicates the standard deviation of the $\beta\text{MNTD}_{\text{null}}$ distribution. We quantified $\beta$NTI for all pairwise comparisons, using a separate null model for each comparison.

Detailed information on the development of the simulation model, including the regional species pool evolution and local community assembly, is provided in SI Material and Methods.

Conflict of Interest
The authors declare no conflict of interest.

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References


Chaparro JM, Badri DV, Bakker MG, Sugiyama A, Manter DK et al. (2013). Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. PLoS One, 8: e55731.


Chase JM, Myers JA. (2011). Disentangling the importance of ecological niches from stochastic processes across scales. Philosophical Transactions of the Royal Society B: Biological Sciences, 366: 2351–2363.


Langenheder S, Székely AJ. (2011). Species sorting and neutral processes are both important during the initial assembly of bacterial communities. The ISME Journal, 5: 1086–1094.


Supporting information

Material and Methods

Sampling Location and Data Collection
Soil samples were collected at five different stages of soil development in a salt marsh chronosequence spanning over a century of ecosystem development, in the island of Schiermonnikoog (N53°30’ E6°10’), The Netherlands (for a detailed description of sample collection, see ref. 1). Successional stages were identified and estimated as 0, 5, 35, 65, and 105 years of soil development (referred to as “years”) in 2012. For detailed information on chronosequence establishment and calibration (see Olff et al., 1997; Dini-Andreote et al., 2014). Briefly, plots were established at the same base of elevation [position at the initial elevation gradient on the bare sand flats with a base elevation of 1.16 m ± 2.2 cm (mean ± SE) above Dutch Ordinance Level]. Sampling was performed in all sites in four time points: May, July, September, and November 2012. Total soil DNA was extracted using a MoBio PowerSoil DNA isolation kit (MoBio Laboratories), and communities were profiled targeting the V4–V6 region of the bacterial 16S rRNA using a Roche GS-FLX 454 automated pyrosequencer running the Titanium chemistry. Sequences were analyzed in QIIME (Caporaso et al., 2010b), using PyNast (Caporaso et al., 2010a) and the Greengenes core set (DeSantis et al., 2006) for alignment and FastTree (Prince et al., 2009) to generate a phylogeny containing all OTUs observed across all samples. OTUs were defined at 97% of nucleotide identity. OTU table was rarified to a depth of 2,600 sequences per sample (the fewest in a single sample), to minimize effects of sampling effort upon analysis. Each individual sample was subjected to measurements of soil physical structure (clay:silt:sand %) and chemical content of total organic matter (OM), nitrate (N-NO₃⁻), ammonium (N-NH₄⁺), sulfate (S-SO₄²⁻), sodium (Na), and pH (for methods, see Dini-Andreote et al., 2014).

Phylogenetic Signal
To test for phylogenetic signal, we used a phylogenetic Mantel correlogram (Lindemann et al., 2013; Stegen et al., 2013; Wang et al., 2013). The inputs to this analysis are two distance matrices with OTUs as both rows and columns. One matrix contains between-OTU differences in environmental optima, and the other matrix contains between-OTU phylogenetic distances. Differences in environmental optima among OTUs were quantified using all measured physicochemical variables (Stegen et al., 2013; Wang et al., 2013). For each OTU we calculated its relative-abundance-weighted mean value for each physicochemical variable; the resulting values estimate the magnitude of each physicochemical variable at which a given OTU is most abundant, which we interpret as a proxy for the level of each physicochemical variable at which a given OTU has its highest fitness (i.e. the OTU’s optimal environmental condition with respect to a given physicochemical variable) (Anderson et al., 2010; Pei et al., 2011; Stegen et al., 2012; Lindemann et al., 2013; Stegen et al., 2013; Wang et al., 2013). After estimating environmental optima for all OTUs with respect to all physicochemical variables, we generated a matrix containing these estimates, with OTUs as rows and physicochemical variables as columns; estimates for each physicochemical variable were normalized as standard normal deviates. Among-OTU differences
in environmental optima were then quantified as Euclidean distances simultaneously using all (normalized) physicochemical axes.

Using a phylogenetic Mantel correlogram (R function mantel.correlog in package vegan), correlation coefficients between differences in environmental optima and phylogenetic distances were quantified for 50 phylogenetic distance bins, and significance of those correlations was evaluated using 1,000 permutations and a progressive Bonferroni correction (Legendre and Legendre, 1998) (Supplementary Figures S1 and S2). A positive correlation coefficient indicates that more closely related species are more similar ecologically (and conversely that more distantly related species are more ecologically dissimilar). For each phylogenetic distance bin — phylogenetic distances were normalized to vary between 0 and 1 before analysis — we consider there to be significant phylogenetic signal if the correlation coefficient is positive and significantly larger than the coefficient expected following permutation (Lindemann et al., 2013; Stegen et al., 2013; Wang et al., 2013).

**Turnover in Phylogenetic Community Composition**

To characterize the turnover in phylogenetic community composition, we quantified the \( \beta \)-mean nearest taxon distance (\( \beta \)MNTD). This metric was calculated as follows:

\[
\beta \text{MNTD} = 0.5 \left[ \sum_{i=1}^{n_k} f_{ik} \min(\Delta_{ik}/m) + \sum_{m=1}^{n_m} f_{im} \min(\Delta_{im}/j) \right],
\]

where \( f_{ik} \) is the relative abundance of OTU \( i \) in community \( k \), \( n_k \) is the number of OTUs in \( k \), and \( \min(\Delta_{ik}/m) \) is the minimum phylogenetic distance between OTU \( i \) in community \( k \) and all OTUs \( j \) in community \( m \min(\Delta_{ik}/j) \). \( \beta \)MNTD was calculated using the R function ‘comdistnt’ (abundance.weighted = TRUE; package ‘picante’). The value of \( \beta \)MNTD provides a raw estimate of phylogenetic turnover between assemblages; by itself the level of \( \beta \)MNTD does not indicate whether turnover between a pair of communities is the result of stochastic or deterministic processes. To infer the ecological process primarily responsible for observed turnover between a given pair of assemblages, we used a null modeling approach that generates an expected level of \( \beta \)MNTD given a dominance of stochastic ecological factors. Each iteration of the null model randomly shuffles OTUs across the tips of the phylogeny, and \( \beta \)MNTD is thereafter recalculated; this provides one null value for \( \beta \)MNTD (Stegen et al., 2012). Going through 999 iterations of the null model provides a null distribution of \( \beta \)MNTD values. To quantify the magnitude and direction of deviation between an observed \( \beta \)MNTD value and the null \( \beta \)MNTD distribution, we used the \( \beta \)-nearest taxon index (\( \beta \)NTI), calculated as follows:

\[
\beta \text{NTI} = (\beta \text{MNTD}_{\text{obs}} - \bar{\beta \text{MNTD}}_{\text{null}}) / sd(\beta \text{MNTD}_{\text{null}}),
\]

where \( \beta \text{MNTD}_{\text{obs}} \) is observed \( \beta \)MNTD, \( \bar{\beta \text{MNTD}}_{\text{null}} \) are null values of \( \beta \)MNTD, and \( sd \) indicates the standard deviation of the \( \beta \text{MNTD}_{\text{null}} \) distribution. We quantified \( \beta \)NTI for all pairwise comparisons, using a separate null model for each comparison. Detailed interpretation of \( \beta \)NTI values are provided in the Results.
Simulation Model

Regional species pool evolution
We aim to develop a model containing the minimal set of elements necessary to evolve a regional species pool that includes information on species evolutionary relationships and species environmental optima. The purpose is to provide a regional species pool that can be used to assemble local communities under defined ecological scenarios that relate to our conceptual interpretation of the βNTI patterns observed in the empirical data. The use of βNTI to make ecological inferences requires significant phylogenetic signal in species’ environmental optima; phylogenetic signal occurs when more closely related species are more ecologically similar (Losos, 2008). The evaluation of phylogenetic signal is discussed below, after the description of the regional species pool simulation model.

To simulate regional species pool evolution we begin with one ancestor species that is defined by the environmental conditions that lead to its maximal growth. We use an arbitrary environmental gradient that takes on values from 0 to 1. In the model, new species arise asexually through mutations according to the environmental optimum of the ancestor such that new species (like the ancestor) are defined simply by their environmental optimum. For our purposes, there is no reason for fitness to vary across the environmental gradient such that we set fitness to be constant, leading effectively to Brownian evolutionary dynamics in species environmental optima. To facilitate comparisons between simulated and empirical patterns we constrained the number of simulated species to be similar to the number retained following rarefaction of the empirical data. To control the number of species in the regional pool, we imposed the following constraints, which are similar to those in Hurlbert and Stegen (2014) that ensure equilibrium species richness: (i) We constrained the total number of individuals summed across all species’ populations to be 2 million such that population sizes decline as species richness increases; this is the zero-sum assumption (Hurlbert and Stegen, 2014). (ii) We assume that the probability of a species going (stochastically) extinct increases as its population size decreases; this assumption was implemented such that extinction probability decreased following a negative exponential function [population extinction probability ∝ \exp(-0.001 * \text{population size})].

As a consequence of these constraints, as species richness increases, population sizes decline, which causes extinction rates to increase. The increase in extinction rates with increasing species richness ultimately leads to a balance between speciation and extinction and thus an equilibrium number of species. Parameters were adjusted so that equilibrium species richness arising in the simulation model (4,748) was very close to the empirically observed species richness (4,746).

In the model, mutation occurs probabilistically and increases with population size. When a mutation occurs, a descendant is produced, and the descendant’s environmental optimum deviates from its ancestor’s by a quantity randomly sampled from a Gaussian distribution (\(\mu = 0, \sigma = 0.15\)). After a new species arises, all population sizes are adjusted so that all population sizes are equal and so that the total number of individuals is 2 million. Population sizes are equal because there are no fitness differences across the environmental axis. Mutation and extinction occur (or not), and population sizes are adjusted
within each time step. The simulation model was run for 250 time steps, which was sufficient to reach equilibrium.

To evaluate phylogenetic signal within the simulated regional species pool, we used a phylogenetic Mantel correlogram as described above. The large number of species within the regional species pool made it computationally unfeasible to evaluate statistical significance within the Mantel correlogram. For this reason we randomly selected 2,500 species from the regional pool for evaluation of statistical significance.

We were specifically concerned with the presence/absence of significant phylogenetic signal across relatively short phylogenetic distances because in natural microbial systems, phylogenetic signal is consistently observed but only across relatively short phylogenetic distances (Stegen et al., 2012; Lindemann et al., 2013; Stegen et al., 2013; Wang et al., 2013). Significant phylogenetic signal across short phylogenetic distances is also the pattern observed within our study system (Supplementary Figure S1). In the simulated regional species pool we likewise find significant phylogenetic signal across relatively short phylogenetic distances (Supplementary Figure S2).

Consistency between simulated and empirically observed phylogenetic signal and species richness in the regional pool suggests that local community assembly from the simulated regional pool will have utility in terms of facilitating conceptual understanding of phylogenetic turnover patterns observed in the natural system. That is, we use local community assembly (next section) from the simulated regional species pool primarily as a device to facilitate understanding; the models are coarse-grain abstractions of the system and are not meant to reflect highly resolved system details.

**Local community assembly**

We assembled local communities from the regional species pool to evaluate whether our conceptual interpretations of the relationship between βNTI and changes in organic matter concentrations represent reasonable approximations of how ecological selection operates in the natural system. Simulations captured four ecological scenarios that include strong homogeneous selection, weak variable selection, moderate variable selection, and strong variable selection.

For the strong homogeneous selection scenario we assembled 12 communities, which is consistent with the number of communities sampled within each successional stage; homogeneous selection should be strongest within successional stages because organic carbon varies relatively little within (as opposed to between) successional stages. For the other three scenarios we assembled 12 communities within each of two environments meant to represent two successional stages.

The βNTI–organic matter relationship is well approximated by a linear model, and this leads to the hypothesis that the strength of the selective pressure imposed by organic carbon (potentially through both composition and concentration of organic matter) is relatively consistent across organic matter concentrations; nonlinearity in the βNTI–organic matter relationship should arise if the strength of selective pressure imposed by organic matter depends on the absolute value of organic matter concentration. For this reason we set the strength of ecological selection to be consistent across environments.

All communities had 817 species, which is the median OTU richness across samples in
the empirical data. To assemble each local community, 10,000 individuals were drawn probabilistically from the regional species pool using ecological rules summarized below. A relatively large number of individuals were used to assemble local communities to generate robust estimates of species’ relative abundances; using fewer or more individuals would have generated more or less uncertainty, respectively, in the estimates of species’ relative abundances but would not influence species richness.

To assemble each local community in a given environment, 817 species were drawn without replacement from the regional species pool with probabilities proportional to their fitness in that environment; fitness was quantified with a Gaussian function centered on the prevailing environmental condition and with variance of 0.1. To simulate relative abundances, individuals were probabilistically drawn into the selected species until reaching 10,000 individuals; the probability of drawing an individual from a given species into the local community (that is within a given environment) was proportional to the fitness of that species within the environment, as defined by the Gaussian fitness function. For the scenario of strong homogeneous selection, local communities were assembled within one environment, which took on a value of 0.1; recall that the environmental axis is arbitrary and takes on values from 0 to 1. For the scenarios of weak, moderate, and strong variable selection, local communities were assembled into two environments taking on values of 0.1 and 0.3, 0.1 and 0.5, and 0.1 and 0.6, respectively.

Following community assembly the distributions of βNTI were quantified. For the scenario of strong homogeneous selection, all 12 communities were compared with each other, and the βNTI distribution was generated from the resulting 66 unique pairwise comparisons. For all other scenarios, only between-environment comparisons were used to generate the βNTI distribution, resulting in 144 pairwise comparisons within each βNTI distribution. The distributions were subsequently estimated and visualized using a kernel density function within R (function density within the stats package, using default parameters).
**Supplementary figures**

**Figure S1.** Phylogenetic Mantel correlogram evaluating phylogenetic signal in the soil microbial communities sampled in this study. The plot relates Pearson correlation coefficients to phylogenetic distances classes. Significant correlations (P < 0.05; solid symbols) indicate significant phylogenetic signal in OTU ecological niches but only within the associated phylogenetic distance class. The analysis reveals significant phylogenetic signal but only across relatively short phylogenetic distances.

**Figure S2.** Phylogenetic Mantel correlogram evaluating phylogenetic signal in the simulated regional species pool. The plot relates Pearson correlation coefficients to phylogenetic distances classes. Significant correlations (P < 0.05; solid symbols) indicate significant phylogenetic signal in species ecological niches but only within the associated phylogenetic distance class. The analysis reveals significant phylogenetic signal but primarily across relatively short phylogenetic distances. There is significant phylogenetic signal across somewhat larger phylogenetic distances than the results obtained for the empirical data (Supplementary Figure S1), but this difference is not expected to substantially alter inferences drawn from the simulation model.
Figure S3. βNTI patterns from empirical comparisons and simulated ecological scenarios, and linkages to underlying simulation model structure. A and B are as in Figure 3.3. (B) The βNTI distribution from simulations of strong HS (purple) aligns with the cluster of empirical βNTI values (A) that are centered below –2 and associated with very small changes in SOM, βNTI from weak VS simulations (blue) align with the cluster of empirical βNTI values that are centered near –2 and associated with SOM changes that approach threefold, βNTI from moderate VS simulations (orange) align with the cluster of empirical βNTI values that are centered near +2 and associated with roughly 10- to 20-fold changes in SOM, and βNTI from strong VS simulations (red) align with the cluster of empirical βNTI values centered above +2 and associated with the largest changes in SOM. (Top and Bottom) Displayed Gaussian functions underlie the ecological simulation model and show the relative performance of an OTU that has a given environmental optimum. In the strong HS scenario (purple), for example, an OTU with an optimal environment near 0.1 will have much higher performance (i.e. higher fitness that translates into higher abundance) relative to an OTU with an environmental optimum near 1.0. Strong HS was achieved with one Gaussian curve such that communities were assembled under a consistent selective pressure. Increasingly strong VS (blue to orange to red) was achieved by increasing the separation between environmental conditions; the increasingly deep valley between performance curves results in increasingly distinct selective environments that select for increasingly distinct ecological communities.