Taxonomic and functional composition of the algal benthos exhibits similar successional trends in response to nutrient supply and current velocity

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Abstract
In an effort to identify the causes and patterns of temporal change in periphytic communities, we examined biomass accumulation, taxonomic and functional composition, rate of species turnover, and pairwise species correlations in response to variability in current velocity and nutrient supply in artificial stream flumes. Divergent patterns in community growth and succession were observed between nutrient treatments and, to a lesser extent, between flow treatments best described by shifts in taxonomic and functional composition. Specifically, understory low profile species, tolerant to low resource supply, became dominant under low nutrients, while overstory high profile and motile species with higher nutrient demands dominated the high nutrient treatments. Increased resource supply or current velocity did not influence the species turnover rate, measured by a time-lag analysis. Interspecific interactions, especially competition, did not appear to be driving community dynamics, as the number of positive and negative pairwise species correlations ranged between low and extremely low, respectively. The overwhelming majority of correlations were not significant, indicating that species within the biofilm matrix were not perceptibly influencing one another. Thus, temporal trends in taxonomic and functional composition were largely environmentally driven, signifying that coexistence in biofilms is defined by the same mechanism along the hierarchy from species to functional groups.

Introduction
Temporal species replacements or succession has long fascinated ecologists, who have sought to answer whether community development is strongly influenced by interspecific interactions, such as competition for limiting resources or species’ individualistic responses to local and regional environmental conditions (Odum, 1969; Drury & Nisbet, 1973; Tilman, 1985; Huston & Smith, 1987). Competitive outcomes depend on resource supply rates and over time, communities exposed to different resource conditions become more dissimilar (Inouye & Tilman, 1988, 1995). This divergence has been attributed to compositional differences (Inouye & Tilman, 1988, 1995), but little is known about whether it can also arise from the presence of unique species in each environment or presence/absence differences. As communities are commonly characterized by dominance of just a few species and lower abundances of many more rarer species, comparisons between communities based only upon species lists or presence/absence data may be misleading if there is a considerable overlap in the presence of rare species (Magurran, 1988). Additionally, if community succession proceeds according to the predictions of the ‘initial floristic composition’ hypothesis (Egler, 1954), where propagules of the entire sequence are present from the beginning, then succession is merely the development of that initial flora. As algae possess passive and generally unlimited dispersal (Finlay, 2002) and do not necessarily settle in environments matching their requirements, their succession may resemble the initial floristics model. Species that are unsuitable for the local conditions can still
remain at very low densities despite a lack of reproduction. Therefore, presence/absence data may not reflect actual differences between communities, which may be detectable only by compositional analyses.

Biofilms are complex, three-dimensional entities, composed of species differing in resource requirements and spatial position, that is, tolerant vs. sensitive forms (Passy, 2008; Passy & Larson, 2011). Tolerant species, with a short habit and an understory location, have low resource demands and can withstand nutrient limitation that is either environmental (low ambient levels) or biotic (overgrowth and subsequent resource depletion). Sensitive forms, on the other hand, need high resource levels and for that reason must obtain a beneficial spatial position in the overstory, where resource acquisition is unimpeded. This can be achieved either through late successional colonization or extended and motile habit, which explains why larger diatoms tend to be eutrophic (Pringle, 1990; Passy, 2007; Passy & Larson, 2011). Thus, nutrient enrichment in the spatially complex biofilm promotes coexistence of tolerant and sensitive species and greater species richness (Passy, 2008; Passy & Larson, 2011). Conversely, eutrophication in spatially simple communities, such as grasslands and phytoplankton, can cause competitive exclusion and diminished biodiversity (Interlandi & Kilham, 2001; Harpole & Tilman, 2007). The rate of competitive displacement, and with this the rate of succession in the latter communities, increases with resource supply (Tilman, 1988; Prach et al., 1993). As biofilm species overgrow rather than displace one another at high resource levels (Passy & Larson, 2011), it is expected that the rate of succession will remain constant across nutrient regimes. However, nutrient supply can cause biofilm succession to take divergent paths – toward tolerant low profile forms under low supply but toward the coexistence of tolerant species as well as sensitive high profile and motile forms under high supply (Passy & Larson, 2011). Consequently, functional composition changes throughout succession and displays resource-driven temporal trends, but it is not known to what extent taxonomic shifts will follow those observed at the functional level.

Current velocity is another environmental attribute in streams that affects community composition and physiognomy (Biggs & Hickey, 1994; Passy, 2001, 2007). Faster flows, favoring understory species and eliminating many overstory forms (e.g., without firm attachment or loosely aggregated), cause alterations in the biofilm structure similar to nutrient limitation. Intermediate flows, however, are expected to be beneficial for biofilm growth because they stimulate algal metabolism and nutrient uptake (Stevenson, 1996). Although nutrients and current velocity influence colonization, growth, and biomass accumulation in developing biofilms, thus impacting the physiognomy and successional trajectories of these communities (Sutherland, 2001; Rickard et al., 2004), experimental research examining the effects of both factors on community dynamics is limited. Several studies have examined current velocity and nutrient abundance in combination (Horner & Welch, 1981; Horner et al., 1983, 1990), but these studies focused primarily on the response of algal biomass accumulation and less on temporal trends in taxonomic composition. Here, we explored the successional trends in periphyton communities across four current-nutrient treatments and tested the following hypotheses: (1) at low to intermediate velocities, successional trajectories will diverge more strongly in response to nutrient additions than current manipulations owing to the strong nutrient dependence of sensitive forms; (2) this divergence will encompass species and functional composition much more so than species presence/absence; and (3) the rate of succession will not vary appreciably across nutrient and flow regimes because competition, implicated in driving successional rates, is expected to be weak.

**Materials and methods**

**Artificial stream flumes**

Experiments were conducted in four oval-shaped laboratory streams, each with an experimental trough measuring 80 cm in length, 12 cm in width, and 13 cm in depth. Eighty liters of modified Guillard’s WC medium (see below) was recirculated in each stream channel at uniform current velocity ($\pm 1$ cm s$^{-1}$), measured by a Marsh-Birney model 2000 flowmeter (Marsh-Birney Inc, Frederick, MD). Current velocity in each stream was maintained by adjusting a belt and multiple drive step pulleys attached to a 1.5-hp motor and a water pump. Drop-in chillers (1/5 hp; TradeWind Chillers, Escondido, CA) maintained stream water at room temperature ($\sim$ 20 °C) in the high velocity channels, while water temperature in the low-velocity channels was at room level. In each experimental trough, 4.9 × 4.9 cm unglazed porcelain tiles were placed equidistant from one another. A 250-W metal halide lamp, positioned above each experimental trough, provided light on a 14 : 10 daily light/dark ratio at levels sufficient to saturate photosynthesis of attached algae, that is, $\sim$ 200 µmol m$^{-2}$ s$^{-1}$ (Hill, 1996).

Algal succession was examined under different current and nutrient regimes. Streams were subjected to constant flows of either 10 or 30 cm s$^{-1}$, shown in natural streams to cause differences in algal immigration and emigration rates, and biomass accumulation (Stevenson, 1996). Additionally, nutrient concentration was varied across current
regimes with either high (800 μmol N-NO₃ and 50 μmol P-PO₄) or low (20 μmol N-NO₃ and 1.25 μmol P-PO₄) levels in modified Guillard’s WC media (Guillard, 1975). Other than the manipulation between treatments of nitrate and phosphate, modified WC media consisted of all constituents in their normal concentrations. Nutrient analyses of water samples, collected at the time of algal sampling, were performed with AutoAnalyzer III (SEAL Analytical Inc., Mequon, WI). In our low nutrient treatments, average concentrations (μg L⁻¹) of NO₃⁻ and PO₄³⁻ were 343–407 and 6.44–6.53, respectively, which were within the ranges shown to limit algal communities (Earl et al., 2006; Hill & Fanta, 2008). Conversely, concentrations in the high nutrient treatments, averaging 7730–9123 for NO₃⁻ and 840–1165 for PO₄³⁻, greatly exceeded these values. Hence, there were four different treatments: low nutrients at 10 and 30 cm s⁻¹, referred to as 10-low and 30-low, respectively, and high nutrients at 10 and 30 cm s⁻¹, referred to as 10-high and 30-high, respectively. The limited number of channels did not allow for replication in each flow × nutrient treatment, so the experiment itself was replicated three times in November and December 2006 and February 2007, with each experimental run lasting 35 days when sloughing occurred in some channels. For the duration of each run, 24 L of water was replaced every third day with new medium.

All artificial streams were seeded once, at the beginning of the experiment, with epilithic algae from several small streams in the Dallas-Fort Worth area encompassing diverse physicochemical conditions. Seed algae were suspended in carbon-filtered water, and 2 L of this mixture was dispensed to each stream. Not unexpectedly, taxon-omy varied between the three seed communities; however, the multivariate procedures we employed in PRIMER (see below) account for variability between replicate runs.

**Sample preparation and analysis**

After allowing an initial period of 7 days for biofilm colonization, two tiles were randomly retrieved from each stream channel. Tiles were taken from the same locations within each stream on days 7, 11, 14, 18, 21, 25, 28, and 35. Each retrieved tile was placed into an accompanying Petri dish with enough distilled water to cover the tile. Following procedures in Larson & Passy (2005), five random fields on each tile were examined with a Zeiss Axioplan 2 LSM 510 META confocal microscope (Zeiss, Jena, Germany), using an Achroplan 40 ×/0.80 numerical aperture water-immersion objective. Total algal biovolume in each field was quantified with 3-D for LSM software (Zeiss, Jena, Germany), and a mean value for the five fields was obtained and converted to total biovolume of algae per square centimeter (Larson & Passy, 2005). After bio-volume quantification with confocal microscopy, the biomass on the surface of each tile was removed with a razor blade and a toothbrush until visibly clean. The tile was then returned back into the streams but never retrieved again for the duration of the experiment. Biomass from the two tiles was consolidated, suspended in carbon-filtered water, and preserved in 4% buffered formalin solution. Samples were uniformly mixed by pulse sonification, and a subsample was placed into a Palmer-Maloney counting cell and observed under a light microscope at 400× magnification. Algal community composition was assessed by counting a minimum of 500 algal units, where an algal unit was an individual cell for unicellular organisms, a 10 μm length for filaments and a 10 × 10 μm area for colonies. Soft algae were identified in this count, and diatoms lumped into a single taxonomic category. For diatom species identification, material was acid-digested, washed with carbon-filtered water, and mounted in Naphrax® (Brunel Microscopes Ltd., Chippenham, Wiltshire, UK) mounting medium. At least 300 units (one frustule or two valves) were counted and identified for each sample at 1000× magnification. Counts were converted to density of cells per surface area of tiles (number of cells cm⁻²).

**Designation of functional guilds**

Soft algae, including cyanobacteria and green algae, and diatoms were grouped into three guilds based on growth morphology, that is, low profile, high profile, and motile species, following Passy (2007) and Passy & Larson (2011). Briefly, species in the low profile guild were short-statured, encompassing prostrate, adnate, erect, solitary centric forms, small colonies, cenobia, and slow moving species. The high profile guild comprised species of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, and stalked species, and colonial centric diatoms. The motile guild was composed of comparatively fast moving species, for example, flagellated green algae or biraphid diatoms. The low profile forms can be classified as tolerant, as they peak in oligotrophic conditions, while the high profile and motile species as sensitive because of their preference for high nutrient concentrations (Passy, 2007).

**Statistical analyses**

**Determination of successional composition**

Differences in algal community structure between treatments were analyzed with PRIMER software application (version 6.1; Plymouth Marine Labs, Plymouth, UK). Compositional and functional group similarities between
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samples were computed with the Bray–Curtis coefficient, while presence/absence similarities with the Sørensen’s coefficient. Within the PRIMER software, MDS, ANOSIM, SIMPER, and BIOENV routines were performed.

Analysis of similarities (ANOSIM) of species abundance and presence/absence data was used to determine the differences between experimental treatments. ANOSIM calculates an R-statistic, which varies between 0 and 1. A value of 1 indicates that all replicates within a treatment are more similar to each other than to any replicate from a different treatment, while a value of 0 indicates that similarities between and within treatments are the same on average (Clarke & Warwick, 2001). Therefore, the value of the R-statistic reflects the observed differences between treatments, contrasted with differences among replicates within treatments; however, the number of replicates in the groups being compared does not bias the R-statistic (Clarke & Warwick, 2001). Ordination with nonmetric multidimensional scaling, considered a robust ordination technique for ecological analyses of successive time points, which are not independent (Clarke & Warwick, 2001), was employed to provide a graphical summary of the relationship among communities. Additionally, average abundance for each taxon was calculated across the three replicates in the different treatments for days 7, 21, and 35, representing early, mid, and late successions, respectively. For each of the 3 days, a subset of species contributing a minimum of at least 90% of the total abundance in each treatment was used in the BIOENV procedure. It calculates the Spearman correlation coefficient between the rank similarity matrices of biota (measured as Bray–Curtis similarity) and environment (measured as Euclidean distance) (Clarke & Ainsworth, 1993). The environmental data for each treatment consisted of binary variables, representing the two levels of flow and nutrient regime.

The pairwise R-statistic values for each sampling time point obtained from ANOSIM were regressed against day of colonization using the curve-fitting software TABLECURVE 2D 5.01 (SYSTAT Software Inc., Richmond, CA). Total algal biovolume accumulation and rates of succession were also regressed against day of colonization with TABLECURVE 2D. For these analyses, we adopted a parsimonious approach of selecting the simplest and biologically meaningful model with a good fit and high r².

Determination of successional rates

Successional rates for each treatment were calculated using a time-lag analytical approach shown to be an effective technique for examining rates of change (i.e., successional rates) when there are a limited number of observations in a time series (Collins et al., 2000). As the number of data points in our time series (e.g., eight time measurements) was limited, giving more weight to potentially extreme values, we chose the more robust time-lag approach than measuring distances between sequential time points. We calculated a triangular dissimilarity matrix from a species-by-time rectangular data matrix using Bray–Curtis similarity and Sørensen’s coefficient (for species composition and presence/absence, respectively). Similarity values were obtained between all pairs of each of the eight sampling periods or time lags in each treatment. For each treatment, Bray–Curtis similarity and Sørensen’s coefficient were regressed against time lag and the relationship examined for statistical significance and/or nonlinearity. A linear model best explained the relationship for each treatment, and model II regression with no constant was employed for the comparison of treatment slopes using the model:

\[ y_{ij} = b_1 x_1 + b_{1,2} x_2 + b_{1,3} x_3 + b_{1,4} x_4 + b_{0,1} + b_{0,2} + b_{0,3} + b_{0,4} + \epsilon \]

where, \( y_{ij} = \text{Bray–Curtis similarity or Sørensen’s coefficient between all pairwise sample dates } i \text{ within each treatment } j \ (j = 1–4), \) where, \( x_{1–4} = \text{lag period in 10-low (1), 30-low (2), 10-high (3), and 30-high (4)}; b_{1,1–4} = \text{slope and intercept for the respective treatments}; \) and \( \epsilon = \text{error term} \). Slope values (\( b_1 \)) for the four treatments indicated the rate of succession.

Species interactions

True measures of interactions between pairs of species in multispecies assemblages are not possible, yet a pseudo-measure of the type of interactions can be calculated using pairwise correlations (Pearson’s r) of ln-density values over the course of the experiment. Sixteen species, each comprising >1% relative abundance over at least two consecutive time periods in an experimental run, were used in these analyses. No apparent nonlinearities were observed in the pairwise species relationships when fitting with a LOWESS smoother; therefore, we proceeded with a correlation analysis. Significant correlations (\( P < 0.05 \)), either positive or negative, were taken as an indicator of whether pairwise interactions between species were ‘positive’ or ‘negative’, respectively, while nonsignificant correlations suggested ‘neutral’ interactions. Notably, the presence of a correlation between two species is ambiguous; that is, it can originate from shared responses to a common environment and/or from an interspecific interaction. However, the lack of correlation indicates that the two species do not interact perceptibly. This may
be due to the absence of direct interactions, such as facilitation or inhibition, or to community effects, like competitive networks, which weaken the strength of individual pairwise interactions (Huisman & Weissing, 1999). The three types of pairwise correlations were tabulated and converted to a percent of all interactions per experimental run. This percent was then used as a dependent variable in a two-way ANOVA, with treatment and correlation type as factors.

**Results**

**Total community biovolume**

Total algal biovolume increased initially and subsequently leveled off in all treatments, captured by the model \( y = a + b/x^2 \) (Fig. 1). The highest total biovolume (as indicated by the ‘ceiling’ parameter \( a \) in this model) was observed in the 30-high treatment and the lowest total biovolume in the 30-low treatment. The temporal response of total biovolume in the high nutrient treatments was similar between current treatments, with higher biovolume in the 30-high treatment late in succession as evidenced by a higher ‘ceiling’ parameter (95% CI: 20.41–20.59 for 30-high vs. 20.00–20.28 for 10-high). In the low nutrient treatments, total biovolume was higher in the 10-low treatment compared to the 30-low treatment (95% CI: 18.98–19.49 for 10-low vs. 18.26–18.95 for 30-low).

**Community composition, presence/absence, and functional groups**

Examination of an MDS of mean species densities revealed early separation between nutrient treatments, which remained distinct throughout succession (Fig. 2a). Within nutrient treatments, differences between flow regimes were more subtle, with dissimilarity arising early in succession at low nutrients but later at high nutrients. Specifically, early in succession, the flora across experiments was largely composed of *Achnanthidium minutissimum*, *Gloeocystis ampla*, and *Gomphonema parvulum*; however, the most discriminative species were the dominant *A. minutissimum* and the subdominant *Nitzschia palea* (Supporting Information, Table S1). Mid-successional communities were dominated by *Lyngbya vandenberghenii*, *N. palea*, *Scenedesmus bernardii*, and *G. ampla* in the high nutrient treatments, while in the low nutrient treatments, *Ankistrodesmus braunii* was abundant in 10-low and *A. minutissimum* in 30-low. With the exception of *A. braunii*, all of the above species were good discriminative species on day 21. In the later stages, the low nutrient treatments became dominated by *A. braunii* in 10-low and *A. minutissimum* in 30-low, with the cyanobacterium *Chamaesiphon fuscus* abundant in both flow treatments. All these species were tolerant to low nutrient conditions (Sládeček, 1973; Van Dam et al., 1994; Rott et al., 2006). Late successional communities in the high nutrient treatments were dominated by several species of *Scenedesmus*, several motile diatoms (e.g., *N. palea* in both current treatments, *Luticola mutica* in 30-high, and *Fistulifera saprophila* in 10-high), and several filamentous cyanobacteria, for example *Phormidium inundatum* in 30-high and *L. vandenberghenii* in 10-high, all of which were characteristically eutrophic and pollution-tolerant (Palmer, 1969; Pringle, 1990; Vis et al., 1998). On day 35, *S. bernardii*, *A. braunii*, *C. fuscus*, *L. mutica*, and *F. saprophila* were the species responsible for discriminating among treatments.

An MDS of mean presence/absence data revealed some separation between nutrient and current treatments, albeit not as pronounced as that seen for densities (Fig. 2b). Furthermore, points were more scattered and the stress for this MDS was fairly high (0.18), making the interpretation of the plot difficult. Many species in these experiments were observed in all treatments (even if at very low densities), further contributing to the lack of considerable separation between treatments. Of the species that were abundant, *Scenedesmus bijugatus* and *Ankistrodesmus fusi- formis* were unique to the high nutrient treatments. The same successional patterns as seen for densities were detected using only three functional groups, indicating high congruence between compositional and functional
response to nutrients and flow (Fig. 2c). In both low nutrient treatments, low profile species were dominant, while all three guilds were abundant in both high nutrient treatments.

R-statistic values using densities and presence/absence were plotted against time, and the trends were similar to those seen in the MDS plots. Values based on abundance increased for all pairwise treatment combinations, revealing an increasing dissimilarity through time (Fig. 3a–f); however, divergence was the strongest between nutrient treatments irrespective of the current treatment (Fig. 3c–f vs. Fig. 3a and b). In the MDS plots, divergence between current treatments was greater and arose sooner in low than in high nutrient treatments. Examination of values obtained with presence/absence data showed that only one comparison was statistically significant, that is, 10-high vs. 10-low treatments with differences starting to emerge at mid-succession (Fig. 3c).

**Rate of succession**

Increasing dissimilarity in periphyton communities with time was measured with both Bray–Curtis similarity and Sørensen’s coefficient (Fig. 4). Linear models best described the trends, meaning that the rate of change was consistent through time; however, the rate was greater using Bray–Curtis similarities, as evidenced by the steeper slopes (Fig. 4a). The rates of change in species composition and presence/absence were not significantly different across treatments as indicated by the considerable overlap in the 95% confidence intervals of the slopes. However, a lack of observed differences in successional rates between treatments should not be construed as a lack of treatment effects, as communities in each of the treatments, most notably in the nutrient manipulations, were progressing toward different end points, that is, tolerant low profile forms at low nutrients and sensitive high profile and motile forms at high nutrients.

**Species interactions**

In each treatment, the overwhelming majority of pairwise comparisons was nonsignificant, suggestive of predominance of ‘neutral’ interactions (Fig. 5). The comparatively few significant correlations were generally positive and only occasionally negative, implying that ‘positive’ but especially ‘negative’ interactions were uncommon, the latter comprising no more than 3% in each treatment. The three interaction types had significantly different percentages across all treatments, that is ‘neutral’ (mean of
Fig. 3. Change in ANOSIM R-statistic with day of colonization for species abundance and presence/absence data for comparison between flow and nutrient supply treatments. The fits were generated by the following models with all parameters significant at $P < 0.05$. (a) 10-high vs. 30-high, abundance: stepwise regression, presence/absence: no model significant; (b) 10-low vs. 30-low, abundance: $y = -0.79 + 0.12x - 0.0023x^2$, $r^2 = 0.72$, $P = 0.042$, presence/absence: no model significant; (c) 10-high vs. 10-low, abundance: $y = -0.32 + 0.09x - 0.0017x^2$, $r^2 = 0.92$, $P = 0.0019$, presence/absence: $y = -0.085 + 0.0018x^{1.5}$, $r^2 = 0.81$, $P = 0.0009$; (d) 30-low vs. 30-high, abundance: $y = 0.15 + 0.07x - 0.0013x^2$, $r^2 = 0.76$, $P = 0.027$, presence/absence: no model significant; (e) 10-high vs. 30-low, abundance: $y = 1.02 - 25.13/x^2$, $r^2 = 0.98$, $P = 0.000001$, presence/absence: no model significant; (f) 30-high vs. 10-low, abundance: $y = 1.01 - 43.09/x^2$, $r^2 = 0.94$, $P = 0.00002$, presence/absence: no model significant. Nonsignificant trends shown as dashed lines.

Fig. 4. Rates of temporal change for the different flow and nutrient supply treatments. Sampling days were 7, 11, 14, 18, 21, 24, 28, and 35 with lag period representing the number of days between sampling days. Linear models significant for all treatments and model II regression were performed to estimate 95% confidence intervals for slopes using (a) Bray–Curtis similarities (slope: 10-low: $-3.98$ to $-2.24$, 10-high: $-3.27$ to $-1.53$, 30-low: $-3.75$ to $-2.00$, 30-high: $-3.59$ to $-1.86$) and (b) Sørensen's coefficient (slope: 10-low: $-0.56$ to $-0.21$, 10-high: $-0.58$ to $-0.23$, 30-low: $-0.77$ to $-0.42$, 30-high: $-0.63$ to $-0.29$).
that is, tolerant species at low nutrient supply but sensitive forms in the high nutrient supply. Early in these experiments, there was considerable similarity in species composition between treatments, dominated by small tolerant forms, for example, *A. minutissimum*, which is not unexpected, given treatments were seeded the same. Later in succession, both nutrient treatments sustained their low profile tolerant flora, but high nutrient treatments also accumulated high densities of various high profile sensitive forms, as predicted by the recently proposed benthic model of coexistence (Passy, 2008). Our results support the premise that succession in algal biofilms is largely influenced by environmental or abiotic factors. It proceeds toward dominance of the functional form, both trophic and morphological, that is selected for by the particular environment. These results are in agreement with a growing body of research in both natural and experimental settings, which reveals a predictable response of the present algal ecological guilds to a broad array of environmental impacts, including eutrophication, light availability, flow disturbance, organic pollution, and pesticide exposure (Passy, 2007; Berthon et al., 2011; Lange et al., 2011; Passy & Larson, 2011; Rimet & Bouchez, 2011).

The separation in species composition between current regimes within nutrient treatments was not as striking. Current can have both positive and negative effects on periphyton communities (Stevenson, 1996), but limited influence on nutrient uptake when benthic algae are nutrient replete (Borchardt, 1994). In our experiments, dissimilarity between current treatments at high nutrients only emerged toward the end of our experiments and was largely because of the differences in the relative abundance of a few species at each treatment. Similarly, total biovolume accumulation in current treatments at high nutrients differed only at the end of succession. Under low nutrient conditions, differences between current treatments were apparent earlier as *A. minutissimum* became very abundant at 30-low, while *A. braunii*, with no mode of attachment, at 10-low. In the low nutrient treatment, accumulation of total algal biovolume was reduced in the high current treatment; perhaps reflecting the difficulty some species had dealing with current stress. These results indicate that the flow gradient exerted stronger control over succession in the nutrient-deplete communities than in the nutrient-sufficient communities.

Admittedly, divergence between treatments may have been partially facilitated by a limited supply of propagules emanating only from the developing communities within each stream, as high immigration rates can lead to greater homogenization in species composition (Leibold et al., 2004). However, in microbial communities, the importance of local factors such as resource availability may be
more important than immigration after initial coloniza-
tion. In the periphyton, colonization ability influences
early dynamics on bare substrates (Stevenson, 1983; Ste-
verson & Peterson, 1991), while specific differences in
growth rates become important once surfaces are colo-
nized (Peterson, 1996a, b). Despite continual immigration
in natural streams, periphyton communities are highly
responsive to environmental conditions, especially water
chemistry, which has led to their frequent use as bioindi-
ators of stream conditions (Hill et al., 2000). In our
experiments, many of the late dominant species were ini-
tially nearly undetectable, either in the seed algae or early
in community development, but became very abundant
later in succession, for example, *Scenedesmus* and *C. fuscus*
in high and low nutrients, respectively. This is more
consistent with the predictions of the ‘initial floristics’
than the ‘relay-floristics’ model of succession (Egler,
1954), which is to be expected in a community marked
by species overgrowth rather than by displacement.

Using a time-lag analytical approach to examine the
differences in species composition between samples at
increasing time intervals, we observed significant linear
trends, which confirmed that our assemblages were
undergoing strong directional changes. Temporal trends
were best explained by linear models, indicating the rate
of succession was uniform for the duration of our experi-
ments. This was consistent with our initial predictions
but contrary to other studies which have shown a
decreasing species turnover through time (Prach et al.,
1993; Myster & Pickett, 1994; Anderson, 2007). Transient
dynamics or competitive displacement of successive spe-
cies has been suggested as a mechanism for higher rates
of succession in fertilized systems (Tilman, 1988). This
does not seem to be the case in our experiments, where
most interactions in the benthos appeared to be ‘neutral’.
The very low number of ‘negative’ interactions suggests
that strong competition was not a major driving force in
our periphyton communities. A diminished role of ‘negat-
ive’ interactions is expected under the benthic model
(Passy, 2008) and in agreement with field observations of
‘three-dimensional’ communities, shown to be driven by
tolerance to overgrowth rather than competitive exclusion
(McCormick & Stevenson, 1991; Airoldi, 2000; Passy,
2008). Furthermore, the high spatial complexity of bio-
films translates into greater internal resource gradients,
providing more opportunities for coexistence and easing
negative interspecific interactions (Passy & Legendre,
2006; Passy, 2008). ‘Neutral coexistence’ of functional
groups, neither facilitating nor suppressing one another,
was proposed as a mechanism of succession in the bio-
film at a functional level (Passy & Larson, 2011). Here,
we see that at a species level, coexistence throughout bio-
film succession was largely neutral as well. This suggests
that contrary to many terrestrial systems, in periphytic
biofilms, interspecific interactions may play a marginal
role in temporal community organization, while the envi-
ronment and particularly nutrient supply control both
the taxonomic and functional composition of this com-
unity. These findings also emphasize the effectiveness of
algal functional groups in capturing as general a commu-
nity pattern as primary succession.

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Authors’ contribution
C.A.L. and S.I.P. conceived and designed the experiments.
C.A.L. performed the experiments. C.A.L. and S.I.P. ana-
alyzed the data and wrote the manuscript.

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Divergent succession in benthic algal communities


**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Species abundance (density cm$^{-2}$) averaged across replicates for each treatment on days 7, 21, and 35.

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