DYNAMICS OF MICROBIAL COMMUNITY STRUCTURE AND FUNCTION IN A TALLGRASS PRAIRIE ECOSYSTEM

by

ALLISON MICHELLE VEACH

B.GS., Ball State University, 2008
M.S., Ball State University, 2011

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Due to agricultural practices and urbanization, tallgrass prairie ecosystems have become threatened as < 5% of its historical coverage exists today. The small remainder of praire that does exist is further threatened by the encroachment of woody plant species. Woody plant encroachment may not only alter prairie ecosystem function, but also prairie microbial communities responsible for these functional processes. Further, prairies are high disturbance ecosystems, especially prairie streams which are hydrologically harsh. They support communities that frequently undergo succession due to recurring flood and drought conditions, yet little is known about the response of microbial communities to these disturbances. In my dissertation, I first address the degree of woody vegetation expansion in riparian corridors (parallel to streams) in watersheds with variable fire frequency and grazing. I found that the rate of riparian woody expansion declines with higher fire intervals and is not affected by grazing, but even annual burns may not prevent woody plant expansion in riparian zones from occurring. Second, I quantified the effect of using restorations of riparian corridors, through removal of woody plants, on physical, chemical, and microbial community (bacteria and fungi) dynamics across stream to upslope soils. Removal restoration causes a decrease in NH_4^+ and soil water content, and causes streams and upslope soils to become similar in fungal community richness unlike forested landscapes. Bacterial communities were minimally impacted by removals, but were highly structured among stream to upslope soils due to multiple environmental gradients (i.e., pH, NO_3^-, soil moisture). Lastly, I examined the successional development of biofilm-associated microbial communities in a prairie stream from both a functional and structural perspective. I found that biofilm microbes exhibited strong successional trajectories, with communities developing towards net autotrophy and therefore becoming reliant upon in-stream

derived carbon. Further, bacterial communities displayed spatial differences, but much stronger temporal patterns in community composition were detected. These studies highlight how woody plant encroachment may influence stream ecosystems in addition to spatiotemporal trends in microbial community assembly.

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Preface

The contents of this dissertation are in collaboration with my two advisors, Walter Dodds and Ari Jumpponen, and others who have aided in data analysis and interpretation. Chapter 2 is published with Walter Dodds and Adam Skibbe as co-authors and formatted for the journal *PLOS ONE*. Chapter 3 is formatted for the journal *FEMS Microbiology Ecology* with both Walter Dodds and Ari Jumpponen as co-authors. Chapter 4 has been submitted to the journal *Molecular Ecology* and is formatted according to their requirements. Chapter 4 has Walter Dodds, Shawn Brown, and Ari Jumpponen as co-authors.

Chapter 1 - Introduction

Woody plant encroachment effects on prairie ecosystems

The Great Plains of North America, which once covered > 160 million hectares, has become threatened from conversion to cropland and human settlement (Sampson & Knopf 1994). Other phenomena in addition to these anthropogenic activities, such as the expansion of woody plants, may reduce the small amount of prairie land cover that still exists. Briggs et al. (2005) documented that woody vegetation has increased in cover > 70% over a 60 year period. Such a shift in plant community structure can have drastic consequences for ecosystem processes, such as carbon soil storage and respiration rates (Lett et al. 2004), and can lead to ecosystem state changes (Ratajczak et al. 2011).

Woody vegetation expansion has been well studied in terrestrial ecosystems, with previous work suggesting that increases in woody plant cover leads to declines in plant diversity (Ratajczak et al. 2012), greater carbon sequestration and annual net primary productivity (Lett et al. 2004, Van Auken 2009). While the expansion of trees and shrubs have known effects on the surrounding landscape, relatively little research has concentrated on freshwater streams that drain landscapes impacted by woody encroachment. Previous work in Kings Creek at Konza Prairie indicates that presence of woody plants in riparian corridors may increase stream respiration rates, causing a greater dependence on allochthonous carbon subsidies than grassy, open canopy prairie streams (Riley & Dodds 2011). Further, wooded riparian soils may have greater denitrification rates than grass dominated areas (Reisinger et al. 2013). In summary, stream ecosystems are as threatened by the encroachment of woody vegetation as terrestrial landscapes due to the conversion of grassy, open systems to closed canopy, forested ones.

In Chapter 2, I used aerial imagery taken at Konza Prairie Biological Station spanning 25 years and delineated riparian buffers through spatial analysis tools in ArcGIS to test if differences in fire frequency and grazing impacted rates of riparian, woody plant expansion over time. I also determined if annual water yield at 4 long-term, continuously discharge gauged watersheds increase or decline over time. This work provides one of the first spatial analyses targeting woody plant encroachment within stream-associated habitats in tallgrass prairies.

Microbial responses to woody plant encroachment and spatial effects on microbial community assembly

Biogeochemical cycling and ecosystem function is affected by the replacement of grasses by woody plants in both soil and aquatic prairie ecosystems. However, the effect on the microorganisms chiefly responsible for ecosystem function haven't been actively researched in the context of woody plant expansion (*but see* – Yannarell et al. 2014). Bacteria and fungi are important components of both soil and aquatic food-webs; they are primarily responsible for decomposition, nutrient cycling, and plant health. The conversion of prairies to forests may result in an overall reduction in total microbial enzyme activities (Brockertt et al. 2012) and can shift microbial composition such that it mimics forested soil communities (Yannarell et al. 2014). In addition, these responses to woody encroachment may be dependent on microbial distributions across landscapes.

In Chapter 3, a restoration of riparian corridors was executed by mechanically removing riparian, woody vegetation in three watersheds at Konza Prairie Biological Station. Soil cores were collected in removal and wooded areas across aquatic to upslope transects to understand the changes in both edaphic conditions and physical removal of woody plants on microbial diversity

and community structure. Both bacterial and fungal communities were assessed across these soil moisture gradients and restoration treatments.

Spatiotemporal effects on microbial structure and function

Multiple environmental gradients exist across space (e.g., pH, temperature, nutrients) that may impact bacterial community structure and functional capacity in prairie ecosystems. Environmental filtering and biotic interactions across these gradients are highly influential in microbial community assembly spatially as examined in Chapter 3. Yet, microorganisms inhabiting prairie streams which are subject to recurrent hydrological disturbances must undergo succession frequently.

Succession occurs as species abundances change over time after a disturbance event. This occurs via deterministic processes, such as selection through species interactions or environmental filtering, or via random processes, such as ecological drift (Hubbell 2001). Successional dynamics have been described for macro-organisms, but far less is known about microbial community assembly over time (Fierer et al. 2010) especially within high-disturbance stream ecosystems (but see – Jackson et al. 2001, Lyautey et al. 2005).

In Chapter 4, I placed ceramic tiles within three locations in the main reach of Kings Creek at Konza Prairie and measured microbial abundance, net biofilm productivity, and bacterial communities between 2 – 64 days after placement in the stream. This chapter tested whether microbial communities functionally and structurally follow deterministic community assembly over time.

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Chapter 2 - Fire and grazing influences on rates of woody plant expansion along grassland streams

Abstract

Grasslands are threatened globally due to the expansion of woody plants. The few remaining headwater streams within tallgrass prairies are becoming more like typical forested streams due to rapid conversion of riparian zones from grassy to wooded. Forestation can alter stream hydrology and biogeochemistry. We estimated the rate of riparian woody plant expansion within a 30 m buffer zone surrounding the stream bed across whole watersheds at Konza Prairie Biological Station over 25 years from aerial photographs. Watersheds varied with respect to experimentally-controlled fire and bison grazing. Fire frequency, presence or absence of grazing bison, and the historical presence of woody vegetation prior to the study time period (a proxy for proximity of propagule sources) were used as independent variables to predict the rate of riparian woody plant expansion between 1985 and 2010. Water yield was estimated across these years for a subset of watersheds. Riparian woody encroachment rates increased as burning became less frequent than every two years. However, a higher fire frequency (1 – 2 years) did not reverse riparian woody encroachment regardless of whether woody vegetation was present or not before burning regimes were initiated. Although riparian woody vegetation cover increased over time, annual total precipitation and average annual temperature were variable. So, water yield over 4 watersheds under differing burn frequencies was quite variable and with no statistically significant detected temporal trends. Overall, burning regimes with a frequency of every 1 – 2 years will slow the conversion of tallgrass prairie stream ecosystems to forested ones, yet over long time periods, riparian woody plant encroachment may not be prevented by fire alone, regardless of fire frequency.

Introduction

Grasslands and wooded grasslands historically covered ~ 30% of the world's total land area, are responsible for ~ 20% of global runoff, [1] and are threatened worldwide. Grasslands have become susceptible to woody plant encroachment within North America and across the globe [2-8]. Woody plant encroachment is occurring across numerous grassland ecosystems converting them into shrublands and forests. The timing required for this conversion may largely be attributed to interactions between climate, fire regime, herbivory, nitrogen deposition, and increases in CO_2 concentrations [3, 9-11]. Conversion of grasslands to shrublands and forest may lead to shifts in terrestrial ecosystem functioning [6, 12], such as heightened carbon sequestration [13] and reductions in carbon mineralization [14]. Woody plant encroachment is thus leading to widespread ecosystem changes which may not easily be reversible [15].

Several factors may interact to influence the rate of woody plant expansion, thus the primary driver of woody encroachment is not easily discernible. However, fire frequency, as well as climatic or edaphic conditions within a region, may tightly control recruitment and subsequent expansion of woody plant species across grasslands [6]. Increases in woody shrub cover within watersheds at Konza Prairie (a tallgrass prairie ecosystem) are greatest with intermediate fire intervals of every 4 years [5, 16]. Annual fires may prevent additional recruitment of upland woody plant species, but cover may still increase, albeit much less than areas with a low burn frequency [16]. Other work in savannas indicates similar trends with high fire frequency reducing tree sapling recruitment and survival [17, 18]. Hence, high fire frequencies generally prevent further woody plant expansion within grassland ecosystems.

Other factors may greatly influence success of woody plant species growth and expansion, especially when coupled with fire interval. Large, ungulate herbivores in mesic

grasslands (e.g., tallgrass prairie) may reduce the spatial extent of burning or fire intensity via grazing and removal of graminoid species [4]. Further, ungrazed watersheds with annual fires may not exhibit greater expansion of shrub cover, whereas grazed watersheds with annual fires have slight increases in expansion [19]. Alternatively, in savannas, grazing ungulates have been shown to reduce woody vegetation cover, potentially through selective grazing on woody seedlings [20, 21]. Thus, the effect of ungulate grazers on woody encroachment may depend on grazer resource preferences.

Terrestrial, grassland landscapes are globally subject to woody encroachment. However, North American tallgrass prairie streams are especially endangered because entire intact watersheds are even rarer than are remnant patches of prairie [22]. Small prairie streams have been characterized as open canopy systems with riparian zones dominated by grasses grading into riparian zones dominated by forests downstream [22]. However, riparian forests have begun to expand their native range within and outside of prairie riparian zones [2, 5]. Transitions from streams with open, grassy canopy to shaded, woody riparian areas could have consequences for stream hydrology and biogeochemistry causing potential ecosystem state changes to the streams themselves and downstream areas they drain to. Woody, riparian vegetation may reduce baseflow discharge rates and increase periods of no flow [23]. Woody plants access groundwater sources in riparian zones and can increase rates of evapotranspiration potentially causing declines in water yield [13, 24]. In addition, forested riparian zones intercept sunlight and shed leaf litter which increases terrestrial material input to streams potentially altering their trophic state [25] to an ecosystem reliant upon terrestrial carbon subsidies (i.e., strongly net heterotrophic) instead of one based on in-stream subsidies (net autotrophy). Such abrupt shifts in carbon subsidy source will likely alter resource availability for aquatic biota causing shifts in

species assemblages [26]. Investigating woody encroachment in riparian zones is pressing as this phenomenon can greatly alter stream ecosystem function and structure.

In this study, we evaluated the magnitude and direction of riparian forest expansion across tallgrass prairie watersheds exposed to variation in grazing and fire frequency treatments. Water yield was also assessed for 4 watersheds to determine if any trends in the proportion of water import to export differed with temporal changes in riparian woody plant cover. We hypothesized that (*i*) across all watersheds, riparian woody cover would increase over time, (*ii*) watersheds exposed to grazing would exhibit greater increases in woody cover relative to those that are ungrazed due to low spatial extent of burning during controlled fires, (*iii*) watersheds exposed to a high fire frequency would exhibit little woody expansion relative to those with a 4 or 20 year fire frequency. Lastly, we hypothesized that (*iv*) water yield would significantly decrease over 25 years due to the increase in cover of deeper rooting woody plant species.

Methods

Study location

Konza Prairie Biological Station (KPBS) is a 3,487 hectare tallgrass prairie preserve and is part of the Flint Hills of northeastern Kansas. KPBS is privately-owned land by both the Natural Conservancy and Kansas State University. It is located ~ 10 miles south of Manhattan, KS (KPBS Headquarters, 39°05'N, 96°35'W). KPBS granted an LTER permit (#200) for the work presented in this study. This study did not involve any protected or endangered species or involve collections of vertebrates. Any permission for research conducted at KPBS is approved through the Director of KPBS, John M. Briggs. Prescribed burning frequencies of variable intervals (every year, 2, 4, and 20 years) began in 1972. The site uses individual watersheds as experimental units under variable grazing and fire treatments. In 1987, 50 bison were introduced

to a 469 ha portion of Konza Prairie and were allowed to increase through herd reproduction and other introductions until 1992 when the bison-grazed area expanded to an additional 480 ha encompassing 10 watershed units differing in their burn frequencies [27]. Watersheds are named by fire frequency (1, 2, 4, 20 years between burns), the inclusion or exclusion of native or cattle grazers (N or C), as well as specific drainage basin (K = Kings Creek north branch, S = Shane Creek) with the final letter assigned based on replicate number (A – D). For example, N04D is the fourth replicate (D) of a native grazed watershed (N) that is burned every 4 (04) years (Fig. 2.1, additional information regarding watershed treatments found at kpbs.konza.ksu.edu).

Konza Prairie receives, on average, slightly more than 800 mm of precipitation annually, and receives 75% of its precipitation in late spring and early summer with high interannual variability [28]. The site is characterized by limestone and shale bedrock with limestone forming benches and shales forming slopes resulting in a terrace-like landscape [29]. Across watersheds, upland vegetation is dominated by C_4 grasses (e.g., *Andropogon gerardii, A. scoparius, Sorghastrum nutans*). In downstream riparian areas, oak gallery forest dominates (e.g., *Quercus macrocarpa, Q. muehlenbergii, Celtis occidenfalis, Ulmus americana*) [2]. In grazed, upland riparian zones where woody vegetation dominates, American elm (*U. americana*) and honey locust (*Gleditsia triacanthos*) are prominent, and in ungrazed headwaters woody riparian areas are dominated by bur oak (*Q. macrocarpa*), and chinkapin oak (*Q. muehlengbergii*) [30].

Spatial analysis of riparian vegetation

Aerial images of Konza Prairie were taken during years 1985, 1991, and 2010. The 1985 images were originally flown to collect ~ 1 m ground resolution data and were scanned to 200 dpi to avoid data loss. The 1985 aerial images were mosaicked and rectified using tools in ArcGIS (Version 10.1, ESRI 2012). The 1991 aerial consists of 1 m ground resolution, black and

white imagery available as part of the USGS digital orthoquad (DOQ). The 2010 aerial is part of the 2010 USDA National Agricultural Inventory Program (NAIP) and is available in color at a resolution of 1 m (metadata for 1991 and 2010 images found at http://kansasgis.org/catalog/index.cfm).

Stream networks were created using digital elevation raster data and, using the Spatial Analyst expansion, riparian zones were defined around each stream. Wooded vegetation near the stream riparian corridor was digitized manually based on visual characterization of land cover and only vegetation within a 30 m buffer (30 m perpendicular to both sides of the stream) was analyzed across the 3 years. While the 30 m width is somewhat arbitrary, it is within the range commonly assumed to have the greatest success in stream conservation [31] and wider than many U.S. states define as protective of waters [32]. While some trees occur outside these widths, they are expected to only modestly impact the stream. We viewed the 2010 color image in black and white and found no discernible difference in our characterization of land cover. The percentage of wooded vegetation (trees and shrubs) within buffers was determined and standardized by stream length within a watershed. Due to changes in fire frequency and other management treatments over time or the confounding effect of multiple wild fires and partially burned watersheds, only data for 20 out of 54 watersheds were retained for further analyses. Of these 20 watersheds, half were grazed, but only 1 was grazed by cattle so no differences between native and cattle grazed watersheds were determined in this study.

A linear regression model was performed for each watershed separately using year (1985, 1991, and 2010) as the independent variable and percentage of wooded vegetation within the buffer as the dependent variable. The regression slope estimate was then used to represent the rate of wooded vegetation increase from 1985 – 2010. All slopes were used regardless of their

statistical significance because we were interested in the direction and rate of change or lack thereof. Only using significant slopes would bias toward watersheds with large amounts of change and against watersheds where no change was evident.

Using the non-parametric, 2 dimensional Kolmogorov-Smironov test [33], we found a potentially non-linear (bi-variate) response ($p < 0.05$) of woody vegetation encroachment to burning frequency. Therefore, a multiple, linear regression model and a segmented (breakpoint) regression model were applied to determine what factors influence the rate of expansion or contraction of riparian wooded vegetation. Normal probability plots and quantile-quantile plot of slope residuals confirmed that data did not violate any assumptions regarding normality. The cumulative number of burns that had taken place between 1980 and 2010 for each watershed was collected through the Konza Prairie Biological Station LTER network burn history database [34].

The presence of grazers and whether riparian wooded vegetation was present prior to Konza Prairie were also used as predictor variables. Lastly, as a surrogate for proximity of propagule sources, a 1939 aerial image was used to visually distinguish the historic presence or absence of woody vegetation along streams within each watershed. This image was created in the same manner as the 1985 image. While the image quality was poorer than the more recent images used, the image did allow for determination of areas with high densities of large trees. Since the presence or absence of trees in the riparian zone of each watershed was a categorical variable, high precision in cover was not necessary for this analysis. This approach was taken because preliminary examination revealed some areas were largely void of woody vegetation (e.g., the southernmost watersheds), but other riparian corridors (primarily those in the northwest corner of current Konza Prairie) were already wooded before the site was established.

Water yield

If riparian wooded vegetation is increasing over time, water yield could also change temporally. Annual water yield was calculated for each of the 4 watersheds (N01B, N02B, N04D, N20B) that have long-term continuous discharge data spanning 1987 to 2010 (LTER dataset codes ASD02, ASD04, ASD05, ASD06) [35]. Discharge was measured over 5 minute periods using Druck pressure transducers at v-notch weirs. All of these watersheds impacted by bison, but have varying fire frequencies. Precipitation and air temperature were collected from the LTER Climate and Hydrology Database Projects database [36]. Discharge data were missing after 2006 for watershed N04D due to pressure transducer malfunctioning. Precipitation and mean discharge were summed per year to calculate annual water import and export, respectively, for each watershed. Water yield was then calculated as the proportion of discharge to precipitation standardized by watershed size. Summing these values on an annual basis prevented any temporal autocorrelation for each watershed as indicated by a correlogram.

A multiple, linear regression model was performed for each watershed separately to determine if water yield changed over time and with average, annual air temperature. Finally, a one-way ANOVA was performed to determine if water yield differed among watersheds. A Tukey's HSD test in conjunction with Bonferroni corrections was then used to determine watershed specific differences in water yield. All regression analyses and ANOVAs were performed in the R programming language using the *segmented*, and *stats* packages (Version 2.13.1, R Development Core Team 2013). Temporal autocorrelation for water yield estimates was tested via correlograms computed in the *stats* package as well (Version 2.13.1, R Development Core Team 2013) whereas Kolmogorov-Smironov tests were performed using Statistica (Version 10.0, Statsoft, Tula, OK).

Results

Riparian vegetation spatial analysis

Analyses of 30 m riparian buffers revealed an increase in wooded vegetation over time among all watersheds except two (Watershed 2B, $\beta = -0.06$ and White Pasture, $\beta = -0.008$). Except for these watersheds, all exhibited a positive (although not necessarily significant) rate of woody expansion, regardless of fire frequency or historical presence of woody vegetation (Fig. 2.1).

Linear regression models indicated that the cumulative number of burns between 1980 and 2010, and the historical presence of woody vegetation, significantly predicted the rate of riparian vegetation expansion ($P < 0.01$, Adj. $R^2 = 0.51$, $F_{3,16} = 7.60$; Fig. 2.2). Further, the average rate of expansion of watersheds with forest present historically was significantly greater than those without forest ($P = 0.06$, $T = -2.04$, $df = 17$; Fig. 2.2). In other words, watersheds with trees present in the 1930s exhibited more rapid expansion of woody riparian vegetation. The presence of grazers did not influence expansion rates. Since the presence or absence of bison grazers across watersheds did not affect expansion rates, we also separated out watersheds with bison introduced in 1987 and 1991 and still found that grazers did not influence rates of riparian, woody expansion regardless of timing of their introduction.

A breakpoint was detected between burn frequency and woody expansion rate at 13 (± 9.12 S.E) burns over the 30 years or at about 2.3 years between burns (Overall model: Adj. $R^2 = 0.37$, Fig. 2.3). Only the regression model fit on the side of the breakpoint with fewer than 13 burns had a significant slope, indicating that the cumulative number of burns significantly predicts the rate of woody expansion ($P = 0.01$, $T = -4.18$; Fig. 2.3). Due to the low number of watersheds which had > 13 burns over the study period, the segmented regression did not

indicate a significant regression slope after the break. Thus, we elected to use a one-sided Student's t-test to test the hypothesis that woody vegetation increases at the greatest burn frequencies. The rate of woody expansion for watersheds with a cumulative number of burns greater than 13 were marginally significant from zero (Mean = 0.29, P = 0.03, T = 2.65, df = 7; Fig. 2.3) indicating that burning regimes implemented more frequently than every 1.6 years may not necessarily prevent woody encroachment.

Climatic variables and water yield

Annual precipitation ranged between 503 to 1115 mm across the study period and average annual temperature ranged between 11.4 to 14.8 °C. Both were highly variable over time.

Annual water yield across the four gauged watersheds never exceeded 0.72 m precipitation/mm runoff, and on average was 0.19 indicating overall only ~1/5 of precipitation was exported as stream runoff. Linear regression models indicated that none of the watersheds water yields differed over time or with temperature ($P \geq 0.28$ across all 4 models). However, N02B and N04D had greater water yield on average than N20B ($P < 0.01$; Fig. 2.4, Table 2.1).

Discussion

Factors influencing riparian, woody vegetation expansion

Nearly all watersheds within this study have experienced riparian woody vegetation expansion since the establishment of Konza Prairie (Fig 2.1). None of these studies have focused explicitly on riparian cover, but rather on total cover, and have provided similar data showing that across Konza Prairie, forested land has increased from 5 ha in 1859 to 274 ha in 2002 (72% areal increase) [5, 24]. The rate of riparian woody vegetation expansion was significantly predicted by the cumulative number of burns taken place between 1980 and 2010. High fire

frequency can reduce woody vegetation cover in some grassland ecosystems, although none of these studies have focused on riparian vegetation. For example, previous studies found that cover and density of both shrubs and trees at Kruger National Park declined by 40 years partially due to frequent, prescribed fires [37]. Moreover, woody cover declined 40-50% after 2 annual burns relative to unburned plots in the South Texas Plains [38]. Within tallgrass prairies, annual fires have prevented woody vegetation expansion, whereas watersheds subjected to intermediate fire frequencies (every 4 years) have had substantially greater tree and shrub density [4].

Our study indicates that the rate of riparian woody vegetation expansion is lessened with greater fire frequency, but even in annually burned watersheds, fires cannot prevent some encroachment of woody vegetation within riparian corridors (Fig. 2.2, 2.3). Segmented regression results suggests that a threshold may be reached for woody vegetation cover at ~ 13 burns over the 30 year study period signifying that there is a change in the way riparian woody vegetation cover responds to fire when implemented every ~ 2 years (Fig. 2.3). Shrub species can persist and still increase in cover even in frequently burned areas [5], and apparently the tree-dominated riparian zones can also persist in the face of fire. *Cornus drummondii*, a common, clonal shrub species at Konza Prairie, forms "islands" which exclude grassy species [39], but may contain other woody species, such as tree seedlings, therefore promoting the expansion of forest [40]. Further, fire may cause short-term periods of high resource availability, causing enhanced recovery and growth of *C. drummondii* [41] and similar effects may occur with respect to riparian trees.

The presence or absence of woody riparian vegetation prior to the beginning of Konza Prairie significantly predicted its rate of expansion (Fig. 2.2). Historical records suggest that tree cover was common along the nearby Kansas River (about 5 km from Konza Prairie). The earliest

written records from the Fremont expedition in 1843 taken along the Kansas River upstream of present-day Topeka note, “We halted for dinner, after a march of about thirteen miles, on the banks of one of the many little tributaries to the Kansas, which look like trenches in the prairie, and are usually well timbered.” [42]. The railroad surveys of Konza Prairie from the 1850’s indicate little tree cover on site. Hence, propagule sources have been close to Konza Prairie for at least a hundred years [43], but there was little woody vegetation on site in both the 1850’s and the 1930s. Watersheds which had forest along the riparian area in 1939 had a significantly higher rate of increase for vegetation cover relative to watersheds without forest. While presence of trees in the 1930s is a weak surrogate for proximity of propagules, this result could indicate propagule limitation for woody recruitment and expansion. However, we did not directly measure propagule production and dispersal in this study so this effect warrants further study.

Surprisingly, bison did not have any effect on riparian woody plant cover over time. This is contrary to other studies that found bison greatly increase woody vegetation cover due to their preferential grazing on graminoid plant species thereby reducing spatial extent of burning and allowing for growth of woody plant species [5, 44]. However, bison spend little of their time within or near streams. Bison spend < 6% of their time within 10 meters of streambeds at Konza Prairie and actually avoid wooded stream reaches [45]. So, although they may have an effect on woody expansion across a watershed, they likely do not within riparian zones.

Our data suggest that under current management conditions on site, woody riparian vegetation will potentially continue to expand into riparian zones, regardless of burning regimes implemented. Anthropogenic impacts on the environment, such as increased atmospheric carbon dioxide concentrations, may erase the physiological advantages that C_4 grasses have [46]. Conversely, other studies suggest that overgrazing may be more responsible for savannahs

conversion to woodlands than CO_2 effects [47]. Our data suggest that overgrazing is not strongly related to riparian, woody encroachment therefore other abiotic factors altering ecosystem states are likely to influence woody plant species increase in riparian cover.

Temporal variability of water yield

We did not detect any change in water yield over time for any watersheds (Fig. 2.4). The lowest water yield did occur in the watershed with the greatest average percentage of riparian cover. Other characteristics varied across these watersheds (number of burns, watershed area, total stream length) so we could not statistically assign the low water yield specifically to degree of riparian vegetation cover. In this study, water yield represented the proportion of stream discharge to precipitation, but one of the processes responsible for vegetation effects on water yield is evapotranspiration [48, 49], which we did not measure directly. Further, vegetation alterations can have a greater impact on the distribution of low flow periods instead of seasonal or annual water yield estimates [50]. Shrub species, such as the dominant *C. drummondii* and *Rhus glabra*, use deeper soil water sources than C_4 grass species [15, 28] therefore replacement of riparian grasses by wooded species may reduce streamflow due to deeper roots accessibility to the water table. Headwaters at Konza Prairie have become dominated by oak species and honey locust within riparian zones [30] and their large rooting systems could be withdrawing water sources and causing reductions in discharge as well, but we have not tested water source with direct isotopic methods for these species. Conversely, forestation is known to improve infiltration capacity of soils thereby potentially offsetting streamflow reductions from greater rooting depth [50]. The antagonistic effects of water table reduction and increasing soil infiltration on hydrology may have prevented any detection of change in water yield over time.

Conclusion

To our knowledge, this is one of the first long-term experimental manipulations of fire at the watershed level to assess the expansion rates of woody, riparian vegetation in a grassland ecosystem. We found that riparian woody vegetation cover is rapidly increasing at Konza Prairie, and although high fire frequency may slow this process, it does not necessarily cease it from occurring. This study indicates that grassy riparian corridors will be maintained only with a minimum of 2 years between burns in tallgrass prairies. We suspect that similar relationships will occur in other grasslands and fire frequency may control riparian, woody expansion though the exact relationships we found may not hold. Although we could not detect any influence of this landscape alteration on stream water yield, the level of variance may make the effect non-detectable. Long-term data collections are mandatory to effectively link land use modification to stream ecosystem dynamics so perhaps additional hydrologic collections will clarify the relationship of woody expansion to prairie stream hydrology. Conservation and management of grassland streams across the globe may require similar considerations in cases where the native condition is grass-lined stream channels.

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Figure 2.1 Spatial extent of woody plant species within a 30 m riparian buffer across the 4 watersheds of the Kings Creek basin monitored for stream discharge during 1985, 1991, and 2010. Woody vegetation cover within a 30 m buffer riparian zone is highlighted in gray for all three years for the 4 watersheds monitored. The 22 watersheds included in the analysis are labeled with black text whereas those not included are labeled in gray.

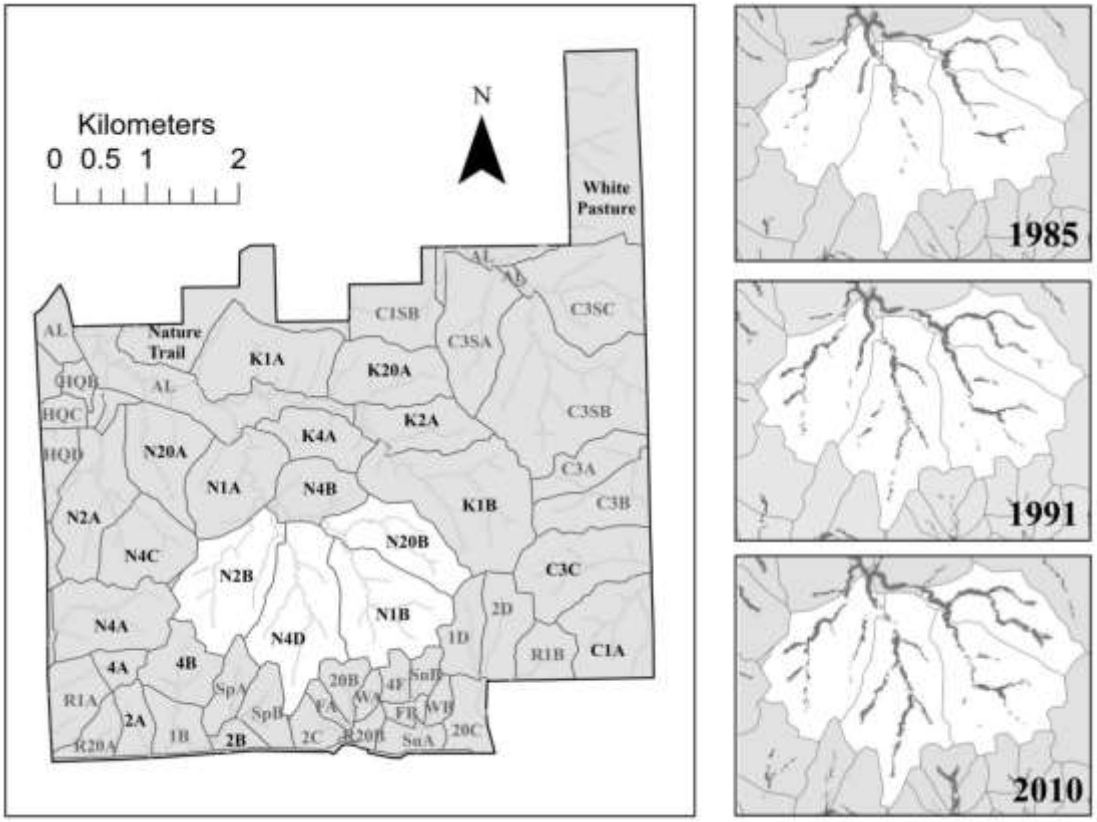

Figure 2.2 The association between the linear regression slopes calculated for each watershed's change in riparian vegetation from 1985–2010 and the cumulative number of burns since 1980 using a multiple, linear regression model. Separate regression lines are present for watersheds without riparian woody vegetation present (open circles, dotted line), and watersheds with riparian, woody vegetation present historically (closed circles, bold line). The average slope of watersheds (rate of woody riparian expansion) with riparian forest was greater than those without forest historically (upper right panel).

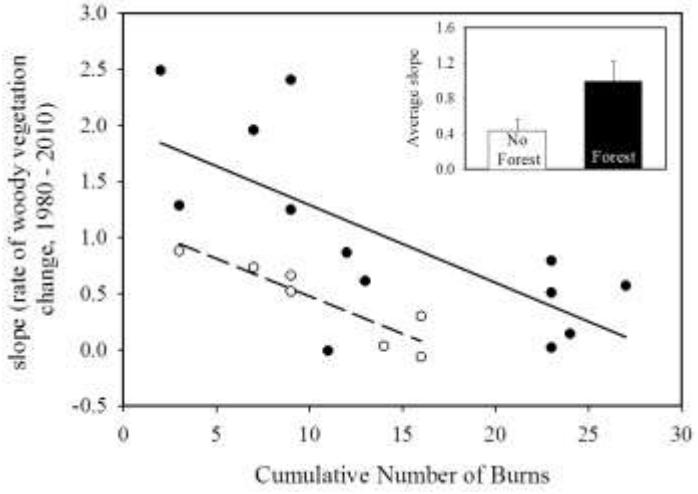

Figure 2.3 The association between the linear regression slopes calculated for each watershed's change in riparian vegetation from 1985–2010 and the cumulative number of burns since 1980 using a segmented regression model. A breakpoint was detected at ~13 burns. The bold line represents the linear regression line for the significant portion of the regression model (watersheds with burns <13 over the 25 30 year record). A dashed line represents the mean of the slope of watersheds with cumulative burns >13. Gray box represents 95% confidence bands about the mean value for watersheds burned more frequently than every 2.3 years.

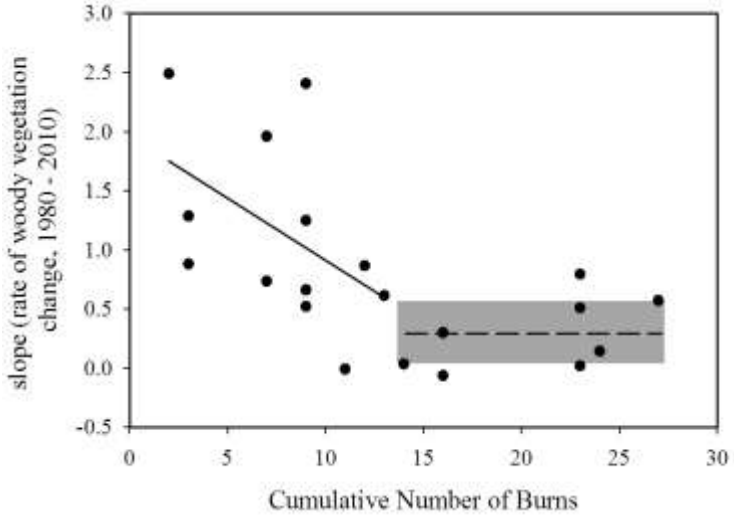

Figure 2.4 The ratio of discharge to precipitation standardized by watershed size (water yield) for a 1 year (N01B), 2 year (N02B), 4 year (N04D), and a 20 year (N20B) burned watershed. No watersheds exhibited a general trend in water yield over time, but N02B and N04D water yields were statistically higher from N20B ($P < 0.01$).

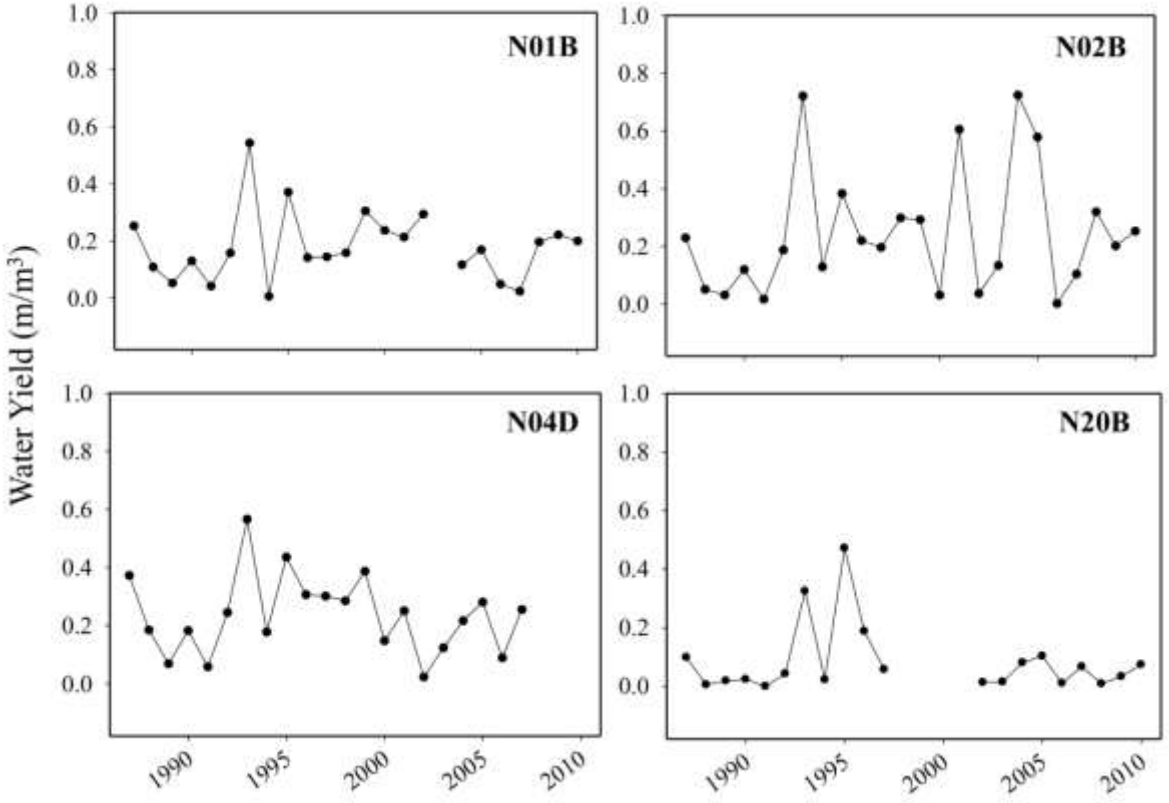

Table 2.1 Characteristics of the four study watersheds. Total burn # refers to the cumulative number of burns between 1985 and 2010. Slope refers to the linear regression slope calculated for the change in extent of riparian wooded vegetation across 1985, 1991, and 2010. The average water yield (proportion of annual precipitation in stream flow) was calculated across all years with standard error in parentheses.

Watershed	Area (ha)	Total Burn #	Slope	Total Stream Length (m)	*% woody vegetation in buffer*			Average Water Yield
					1985	1991	2010	
N01B	120.7	23	0.51	3937	40.2	56.9	57	0.18 (0.03)
N02B	77.6	12	0.87	2387	43.6	63.5	69.6	0.25 (0.05)
N04D	125.6	7	2	3886	22.3	50.9	76.3	0.24 (0.03)
N20B	84.4	4	1.3	2327	52	78.5	89.7	0.09 0.03)

Chapter 3 – Fungal and bacterial communities differ along soil moisture gradients and in response to riparian woody vegetation restorations

Abstract

Woody plant encroachment has become a global threat to grasslands and has caused declines in aboveground richness and changes in ecosystem function; yet we have a limited understanding on the effects of these phenomena on belowground microbial community structure. We carried out riparian woody plant removals at Konza Prairie Biological Station and collected soils spanning land-water interfaces in removal and woody vegetation-impacted areas. We measured soil for several edaphic variables (C and N pools, soil water content, pH) and bacterial (16S rRNA genes) and fungal (ITS2 rRNA gene repeat) communities using Illumina MiSeq metabarcoding. Bacterial richness and diversity decreased with distance from streams. Fungal richness decreased with distance from the stream in wooded areas, but was similar across landscape position in removal areas. Planctomycetes and Basidiomycota relative abundance was lower in removal areas. Cyanobacteria, Ascomycota, and Chytridiomycota relative abundance was greater in removal areas. Ordination analyses indicated that bacterial community composition shifted more across land-water interfaces than fungi and that both were marginally influenced by treatment. Woody encroachment removals cause shifts in bacterial (phyla relative abundance) and fungal communities (richness, phyla relative abundance), potentially consequential for ecosystem function across stream and terrestrial ecosystems.

Introduction

Tree and shrub encroachment into grasslands and the subsequent conversion of prairies and grasslands into woodlands and forests has shifted the fundamental character of this biome in many places (Van Auken 2000, Briggs et al. 2005, Van Auken 2009). Causes of woody encroachment, or the increase in density of woody plant cover, vary by locality, but generally are related to an increase in grazing, low fire frequency and intensity and human disturbances (Van Auken 2000, Köchy & Wilson 2001) as well as regional climatic and edaphic conditions (Archer et al. 1995, Van Auken 2009). The transition from open grasslands to forested and shrubby woodlands may alter ecosystem function which may not be easily reversible due to physiological advantages of woody plant species after recruitment (Ratajczak et al. 2011) or reduction in fire intensity at woody/grass interfaces (Engber et al. 2011, Ratajczak et al. 2011).

Woody encroachment significantly impacts both terrestrial and stream ecosystems, but processes governing these impacts may be substantially different between these ecosystem types. Woody encroachment has been associated with declines in plant species richness (Ratajczak et al. 2012), increases in soil and plant biomass C and N accrual (McKinley & Blair 2008), decreases in soil CO_2 flux (Lett et al. 2004), greater nutrient heterogeneity (Kleb & Wilson 1997), and increases in annual net primary productivity (ANPP) (Lett et al. 2004, Hughes et al. 2006). In riparian zones, trees and shrubs increase canopy cover, thus lowering light availability in streams and reducing algal biomass (Riley & Dodds 2012) with potential effects on stream primary productivity. Further, riparian soils with woody vegetation have greater rates of denitrification compared to grassy soils, likely due to higher soil water content and NO_3^- (Reisinger et al. 2013). The expansion of woody vegetation across prairie landscapes, and into

riparian corridors, has large consequences for both ecosystem function (C and N cycling) and structure (plant and algae) in both terrestrial and aquatic habitats.

Restoration of riparian zones is a management practice to return an area to a pre-disturbance ecological state, functionally (e.g., sediment or N retention by creating forested buffers) (e.g., Osborne & Kovacic 1993, Hill 1996) and/or structurally (e.g., removal of invasive plant species) (Richardson et al. 2007). In the context of woody encroachment into grasslands, physical removal of woody vegetation may restore riparian areas and streams to their native grass-dominated state. This approach has proven successful in conifer dominated landscapes (Provencher et al. 2000, Jones et al. 2005), but less is known about how removal restorations in grassland riparian zones may affect both riparian and stream ecosystem dynamics. Other work in a tallgrass prairie ecosystem indicates that woody vegetation riparian removals return prairie streams to their native state functionally (Riley & Dodds 2012), and therefore may serve as a means of conserving grassland ecosystems.

Woody encroachment effects carbon and nitrogen cycling (Lett et al. 2004, McKinley & Blair 2008, Reisinger et al. 2013). In general, this phenomenon, particularly shrub encroachment, is considered to cause reductions in overall ecosystem functioning (van Auken 2000, van Auken 2009). However, less is known about the impact of woody encroachment, and its subsequent removal, on grassland bacterial and fungal community dynamics (but see – Hollister et al. 2010; Yannarell et al. 2014) which are likely linked to these ecosystem changes. Woody vegetation and its removal may cause differential effects on microbial community dynamics of riparian soil (where the removal actually occurs) versus sediments associated with the nearby stream ecosystem. Our study objective was to determine how restoration of riparian areas influenced by woody encroachment affects edaphic conditions and bacterial and fungal diversity and

community composition across land-water interfaces in a managed tallgrass prairie ecosystem. Woody vegetation removals were done by mechanically removing tree and shrub species within riparian areas in three watersheds at Konza Prairie Biological Station in northeastern Kansas, U.S. We collected soils and sediments across transects spanning stream margins to upslope habitats within removal and woody vegetation impacted soils for analysis of multiple edaphic factors (inorganic N, total N and C, C:N, soil water content and pH) and microbial community composition and diversity metrics.

Methods

Study area and experimental manipulation

The study area is located at Konza Prairie Biological Station in northeastern Kansas, and sampling was executed at three watersheds draining the Kings Creek stream network (AL, N2B, N4D, Figure 3.1). Watersheds N2B and N4D are both grazed by American bison (*Bos bison*), but are burned every 2 or 4 years respectively. AL is not grazed and is burned every year. The last prescribed burn of N2B and N4D prior to our sampling occurred in April 2013, whereas AL was burned in March 2014.

At AL and N4D, woody, riparian vegetation was mechanically removed within a ~ 30 m area parallel to the stream during December 2007. Large trees were removed with chainsaws whereas shrubby vegetation was removed via brush cutting. All cut vegetation was then moved outside of the removal area (see Riley & Dodds 2012). Maintaining the removal area by removing any additional woody growth and relocating cut wood outside the removal area occurred between 2007 – 09. During February 2014, any regrowth of woody, riparian vegetation was removed again at these watersheds. AL and N4D removal reaches were 36 and 33 m in stream length (Riley & Dodds 2012). N2B had the entire western fork of the watershed cleared

of riparian, woody vegetation (4.8 km of stream length) during December 2010 similarly to AL and N4D removals. The removal areas within N2B have been maintained annually since 2010. Wooded areas are dominated by *Quercus macrocarpa* (bur oak), *Q. muehlenbergii* (chinquapin oak), *Ulmus americana* (American elm), *Gleditsia triacanthos* (honey locust), *Cercis canadensis* (Eastern redbud), *C. occidentalis* (Western redbud), and *Cornus drummondii* (Roughleaf dogwood) (Briggs et al. 2005, D. Carter, unpublished data).

Soil sampling

Soils were sampled on 6 - 7 July 2014. Within each of the three watersheds, two "treatments" were sampled: an area that had undergone riparian, woody vegetation removal (termed "removal" throughout remaining text), and an area adjacent to this with riparian, woody vegetation intact (termed "wooded"). Within each of these two treatments at each of three watersheds, soil cores were collected along four transects beginning at stream margins and ending in terrestrial, upslope areas (Figure 3.1). Specifically, for each transect, soil cores were taken at (1) the stream margin, (2) stream bank (average 0.32 m from stream margin), (3) nearby riparian soils (average 4.1 m from stream margin), and (4) more distant, upslope soil (average 11.0 m from stream margin, Figure 3.1). These categories, termed "landscape position" throughout the remaining text, were chosen as they represent a gradual continuum of habitats (e.g., water availability, soil particle size, vegetation) spanning aquatic to terrestrial environments. Stream margin samples were taken at the edge of streams in little to no flow areas and were water-saturated whereas stream banks were within the channel. Unlike stream margin samples, bank sediments were not under water, and had some vegetation present. Terrestrial riparian and upslope soils were outside of the stream channel and had much denser vegetation present (grasses in removal areas, grasses, trees, and shrubs in the wooded area), but only

differed from each other based on their distance from stream margins. At each sampling point (streams, banks, riparian, and upslope) along each of 4 replicate transects, within each treatment in a watershed, we collected 3 soil cores (top 5 cm) using a steel pipe with a 3.81 cm diameter and pooled into one for a total of 96 samples across the experiment. The samples were stored on ice until arrival to the laboratory where they were frozen at -20°C. Soil processing was completed within 2 weeks of collection. Soils were placed at 4°C until thawed (~ 48 hours), thoroughly homogenized, and sieved (2 mm mesh size).

Edaphic variables and analyses

Once sieved, the samples were analyzed for extractable NO_3^--N, NH_4^+-N, soil water content, total nitrogen (TN), total carbon (TC), C:N, and soil pH. Extractable NO_3^--N and NH_4^+-N were extracted overnight (~ 12 hrs) in a 2 M KCl solution (5:1 KCl v: soil v) and the extract filtered (Whatman Nucleopore, 0.2 um size, GE Healthcare Companies). NO_3^--N was analyzed by cadmium reduction and colorimetric reaction whereas NH_4^+-N was measured by an indophenol colorimetric reaction and both measured using a Rapid Flow Analyzer (Model RFA-300, Alpkem Corporation, Clackamas, Oregon, USA). Soil pH was measured in a 1:1 soil/deionized water solution. Additional soil was weighed and dried at 60°C for at least 48 hr to calculate soil water content and prepare soils for TN and TC analysis. TN and TC was determined by grinding dried soil into a fine powder using a ball mill and then analyzed by a Carlo Erba NA 1500 Analyzer. Due to high concentrations of calcium carbonate in Konza Prairie soils, stream and bank sediments were treated with 3% HCl to volatilize calcium carbonate prior to TN and TC analyses.

DNA Extractions, PCR, and Illumina MiSeq sequencing

Total genomic DNA was extracted from 0.25 – 0.3 g of soil from each point along each transect (32 samples per watershed, 95 total) using a MoBio PowerSoil Extraction kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). One riparian sample was potentially contaminated during thawing and therefore not prepared for microbial community analysis. DNA yield was determined using a Nanodrop ND2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) and the DNA templates adjusted to a 2 ng/μL concentration.

We analyzed DNA for both bacterial (16S) and fungal (Internal Transcribed Spacer 2, ITS2) communities using a two-step PCR approach to avoid a 3'-end amplification bias resulting from the sample-specific DNA-tags (Berry et al. 2011). For bacterial communities, we first amplified the V4 region within the 16S ribosomal RNA (rRNA) gene using 515F and 806R primers (Caporaso et al. 2012). Each sample was amplified in three independent 25 μL reactions, which consisted of 1 μM of forward and reverse primers, 10 ng of template DNA, 12.5 μL proofreading Phusion High-Fidelity Master Mix (New England Biolabs, Inc., Ipswich, MA, USA), and 5 μL of molecular grade water. For fungal communities, we first amplified the entire ITS region flanked by the 18S and 25S rRNA genes using the ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) primers. Each sample was amplified in three 25 μL reactions consisting of 1 μM of forward and reverse primers, 10 ng of template DNA, 200 μM of each deoxynucleotide phosphate, 1 μM of $MgCl_2$, 0.5 units of proofreading Phusion Green Hot Start II High-Fidelity DNA polymerase (Thermo Scientific, Wilmington, Delaware, USA), and 5 μL of 5X Green HF PCR buffer (Thermo Scientific, Wilmington, Delaware, USA). Thermal cycler parameters (Eppendorf, Hamburg, Germany) for bacterial communities consisted of an initial denaturation at 98°C for 5 min., followed by 25 cycles with denaturation at 94°C for 1 min.,

annealing for 30 sec. at 50°C, extension for 1 min. at 72°C, with final extension for 10 min. Fungal community PCRs were the same except for 30 cycles. Negative controls were included in both bacterial and fungal PCRs to detect contamination and all remained contaminant free.

The PCR amplicons were cleaned using Agencourt AmPure SPRI system (1:1 ratio of bead solution to PCR volume) to reduce carryover of primary PCR primers. The three technical replicates of the cleaned amplicons were pooled and diluted (bacteria 5:1; fungi 2:1) for secondary PCRs. The different dilutions were necessary as the initial 5:1 mixtures yielded poor amplification of fungal templates for several samples. In the secondary PCRs, 10 μL of cleaned and diluted primary PCR products were amplified as above except only using 5 cycles. In addition, the reverse primer included a 12 bp unique Multiplexing Identifier tag (MID-806R; Supplementary Table 3.1). Secondary PCRs for fungal communities were similar to those for bacteria and had 12 bp MIDs in the reverse primer (MID-ITS4; Supplementary Table 3.2), but we used a nested PCR with the fITS7 primer (Ihrmark et al. 2012) instead of ITS1F to generate optimally sized amplicons for Illumina MiSeq. This nested PCR approach also minimizes non-target plant amplicons that often result from environmental samples using this primer combination. All primary and secondary PCRs were visualized on a 1.5% agarose (w/v) gel to ensure successful amplification. Secondary PCRs were cleaned using Agencourt AmPure similarly to primary PCRs. Amplicon DNA concntration for each experimental unit was measured and pooled at equal concentrations (150 ng for bacteria, 120 ng for fungi). Both amplicon libraries were paired-end sequenced using the Illumina MiSeq. Illumina specific primers and adapters were ligated using a NEBNext® DNA MasterMix for Illumina kit (Protocol E6040, New England Biolabs Inc., Ipswich, MA, USA) and sequenced using a MiSeq Reagent Kit v2 (Illumina, San Diego, CA, USA) with 500 cycles.

Bioinformatics

Sequences (.fastq) were processed using the mothur pipeline (version 1.33.3) (Schloss et al. 2009). Both bacterial and fungal .fastq files were contiged and any sequences with any ambiguous bases, with more than 2 mismatches to the primers, any mismatches to the MID, and homopolymers longer than 8 bp were removed. Bacterial sequences were aligned against a SILVA reference, screened for chimeras with the UCHIME algorithm (Edgar et al. 2011), and non-chimeric sequences were assigned to taxa using the Naïve Bayesian Classifier (Wang et al. 2007) against the RDP training set (version 10) with 51% bootstrap threshold. Non-target sequences (mitochondria, chloroplast, Archaea) were removed. We randomly subsampled 1.5 million sequences (out of 3.03 million) from the entire dataset and calculated a pairwise distance matrix. Sequences were clustered to OTUs at a 97% similarity threshold using nearest neighbor (single linkage) joining that conservatively assigns sequences to OTUs.

After pre-processing and chimera removal as described for bacteria, the fungal sequences were assigned to taxa using the Naïve Bayesian Classifier (Wang et al. 2007) and the UNITE-curated International Nucleotide Sequence Database (INSD) reference database (Abarenkov et al. 2010). Any sequences not assigned to the Kingdom Fungi were removed and remaining sequences pairwise aligned to calculate a pairwise distance matrix. This distance matrix was used to cluster fungal sequences into OTUs at a 97% threshold using nearest neighbor joining as described for bacteria. All bacterial and fungal sequence data were accessioned into the Sequence Read Archive (SRA URL and accession numbers).

Lastly, we estimated richness and diversity metrics for both bacterial and fungal communities in mothur (Schloss et al. 2009). Observed OTU richness (S_{obs}), the complement of Simpson's diversity (1-D: $1-\sum p_i^2$), and Simpson's evenness (E_D: $1/\sum p_i^2/S$), with p_i representing

frequency of each OTU within a sample, were iteratively calculated for each sample with 8,000 sequences for bacteria and 2,000 for fungi.

Statistical Analyses

A two-way ANOVA model was used to determine the influence of landscape position (streams, banks, riparian, upslope) and treatment (removal, wooded) on edaphic conditions, microbial (bacteria and fungi) diversity and richness, as well as dominant microbial phyla (≥ 1.0 % of total sequence counts) relative abundance. We also assessed the interaction between landscape position and treatment for response variables. All edaphic variables (except soil pH and C:N) and relative abundance of Cyanobacteria, Firmicutes, Deltaproteobacteria, Chytridiomycota, and Zygomycota were $\log_{10}$ transformed prior to analyses.

Bray-Curtis distance matrices were constructed for both bacterial and fungal communities and were implemented in non-metric dimensional scaling (NMDS) ordinations to view both bacterial and fungal community composition. Both NMDS's were Wisconsin double standardized and square-root transformed. Further, Bray-Curtis distance matrices were used to calculate permutational multivariate ANOVA's (PERMANOVAs, 1000 permutations) to determine if landscape position, treatment, and their interaction influenced bacterial and fungal community composition. Lastly, we performed an indicator species analysis to determine which OTUs occurred more frequently between treatments and across landscape position. We only included the 100 most abundant OTUs in both bacterial and fungal indicator species analysis. These OTUs comprised 78% and 58% of all sequences across the experiment for bacteria and fungi, respectively. FDR corrections were used for post-hoc multiple comparisons of statistical significance for indicator species analysis.

All statistical analyses were implemented in R (version 3.1.1, R Development Core Team, 2014). ANOVAs were carried out in the *stats* package, NMDS (function *metaMDS*) and PERMANOVAs (function *adonis*) in the *vegan* package (Oksanen et al. 2014), and indicator species analysis in the *indicspecies* package (Caceres & Jansen 2014).

Results

Edaphic factors

Extractable soil NH_4^+-N was greater in wooded areas compared to removal treatments ($F_{1,90} = 10.74$, $P < 0.01$; Table 3.1), but was not influenced by landscape position ($P > 0.1$). Extractable soil NO_3^--N ($F_{4,87} = 34.02$), TN ($F_{4,83} = 225.54$), and TC ($F_{4,84} = 148.75$) differed across landscape position ($P < 0.01$) and were greater in terrestrial soils (riparian and upslope habitats) than stream and bank sediments (Tukeys HSD, $P < 0.01$; Table 3.1). C:N differed across landscape position ($F_{4,83} = 22.89$, $P < 0.01$) and was greatest in riparian soils compared to other landscape positions, whereas stream and bank sediments had the lowest C:N (Tukeys HSD, $P < 0.05$; Table 3.1). Extractable soil NO_3^--N, TN, TC, and C:N did not differ between treatments ($P > 0.1$). Soil water content differed across landscape position ($F_{4,90} = 111.44$, $P < 0.01$) and between treatments ($F_{1,93} = 5.93$, $P = 0.02$). Soil water content was greater in wooded compared to removal treatments and was greatest in stream margin sediments compared to terrestrial soils (Tukeys HSD, $P < 0.01$; Table 3.1). Soil pH differed across landscape position ($F_{4,83} = 13.62$, $P < 0.01$) and was greater in stream and bank sediments compared to terrestrial soils (Tukeys HSD, $P < 0.01$; Table 3.1), but did not differ between treatments ($P > 0.1$). There was no significant landscape position by treatment interactions for any edaphic variable.

Microbial richness and diversity

Bacterial OTU richness and diversity differed across landscape position ($F_{3,89} = 60.03$, $P < 0.01$). Both were greatest in stream and bank sediments (Tukeys HSD, $P < 0.01$), and lowest in riparian and upslope soils ($P < 0.01$, Figure 3.2, Panel A, B). Bacterial evenness weakly differed across landscape position ($F_{3,89} = 2.27$, $P = 0.09$) and between treatments ($F_{1,91} = 3.27$, $P = 0.07$). Evenness was marginally lower in upslope soils than stream sediments (Tukeys HSD, $P = 0.08$), whereas wooded treatments had marginally greater evenness than removal soils ($P = 0.07$). Multiple edaphic variables were correlated with bacterial richness, diversity, and evenness that primarily differed across landscape position (NO_3^--N, TN, C:N, soil water content, Supplementary Table 3.3).

Fungal OTU richness differed across landscape position ($F_{3,78} = 8.07$, $P < 0.01$), treatment ($F_{1,80} = 5.26$, $P = 0.02$), and had a significant landscape position by treatment interaction ($F_{3,78} = 3.71$, $P = 0.02$, Figure 3.2). Stream sediments had richer fungal communities than riparian or upslope soils (Tukeys HSD; $P < 0.01$) in wooded treatments, but removal treatments minimized these differences and resulted in similar fungal richness across landscape positions (Figure 3.2, Panel D). Fungal diversity and evenness did not differ across landscape position or treatment ($P > 0.01$). TN and pH were correlated with fungal richness (Supplementary Table 3.3).

Bacterial community compositional shifts

Twenty-nine bacterial phyla were found across the experiment, with twelve phyla (or class for Proteobacteria) dominating all samples (≥ 1 % relative abundance across all samples) collected (Supplementary Table 3.4). A small proportion of sequences (8.7%) were unclassified beyond Domain Bacteria.

Nine bacterial phyla differed in relative abundance over landscape position ($P \leq 0.01$), and two differed between treatments ($P < 0.01$; Table 3.2). Due to large differences in NO_3^--N concentrations across soils, we also included Nitrospirae in the analysis. Planctomycetes ($F_{1,92} = 8.90$) and Cyanobacteria ($F_{1,92} = 24.22$) relative abundance differed between treatments ($P < 0.01$). Planctomycetes had greater relative abundance in wooded treatments (Figure 3.3), and did not differ across landscape position, whereas Cyanobacteria abundance was greater in removal treatments ($F_{1,92} = 24.22$, $P < 0.01$). Cyanobacteria also had a significant landscape position by treatment interaction ($F_{3,90} = 4.56$, $P < 0.01$, Figure 3.3). Terrestrial soils in removal treatments had approximately 10x greater Cyanobacteria relative abundance compared to terrestrial soils in wooded treatments (Figure 3.3). Actinobacteria ($F_{3,90} = 8.48$) and Verrucomicrobia ($F_{3,90} = 21.47$) relative abundance was greater in terrestrial soils than stream-associated sediments (streams, banks; $P < 0.01$). Acidobacterial relative abundance was greater in banks, riparian, and upslope habitats compared to streams ($F_{3,90} = 10.93$), whereas Gammaproteobacteria were greatest in riparian soils ($F_{3,90} = 3.89$, $P \leq 0.01$). Chloroflexi ($F_{3,90} = 21.25$), Cyanobacteria ($F_{3,90} = 7.10$), and Nitrospirae ($F_{3,90} = 22.23$) relative abundance was greater in stream sediments ($P < 0.01$) compared to all other sediment and soil habitats. Betaproteobacteria ($F_{3,90} = 61.02$) and Deltaproteobacteria ($F_{3,90} = 23.08$) had greater relative abundance in both stream and bank sediments compared to riparian and upslope soils ($P < 0.01$). Most phyla's relative abundance was correlated with at least one edaphic variable (Supplementary Table 3.5).

Bacterial communities differed primarily across landscape position ($R^2 = 0.26$, $P < 0.01$), and weakly differed between treatments ($R^2 = 0.02$, $P = 0.09$). No variation in bacterial community composition was associated with a landscape position by treatment interaction ($P > 0.1$). A large proportion of the variation in composition remained unrelated to independent

variables (PM MANOVA: Residuals $R^2 = 0.69$). However, fitting environmental correlates with NMDS scores indicate that all edaphic variables were significantly correlated with community composition (Table 3.3, Figure 3.4).

Fungal community compositional shifts

Sediments and soils were dominated by Ascomycota (48.9 % sequences) and Basidiomycota (22.6% sequences). However, Zygomycota (14.6% sequences), Chytridiomycota (3.5% sequences), and Glomeromycota (0.5 % sequences) were also present. A small proportion of sequences (9.8%) were unclassified beyond Kingdom Fungi (Supplementary Table 3.4).

Ascomycota, Chytridiomycota, and Basidiomycota differed between treatments, but not landscape position. Ascomycota ($F_{1,60} = 6.36$) and Chytridiomycota ($F_{1,60} = 8.41$) relative abundance was greater in removal ($P \leq 0.02$) whereas Basidiomycota relative abundance was greater in wooded soils ($F_{1,60} = 10.04$, $P < 0.01$). Zygomycota relative abundance differed across landscape position ($F_{3,58} = 7.25$, $P < 0.01$), but not treatment. Zygomycota were greater in riparian and upslope soils (Tukeys HSD, $P < 0.01$, Table 3.2). All fungal phyla, except Ascomycota were correlated with at least one edaphic variable (TN, NO_3^--N, or pH, Supplementary Table 3.5).

Fungal community composition significantly differed across landscape position ($R^2 = 0.09$, $P < 0.01$), treatment ($R^2 = 0.03$, $P < 0.01$), and some variation in composition was associated with a landscape position by treatment interaction ($R^2 = 0.06$, $P = 0.04$). A large proportion of variation remained unexplained (PM ANOVA: Residuals $R^2 = 0.82$). All edaphic variables were correlated with fungal community composition (Table 3.3, Figure 3.4).

Indicator Taxa

Bacteria had no indicator OTUs for removal or wooded treatment soils. For fungi, there was 1 indicator OTU for removal and wooded soils, respectively. The indicator OTU for removal soils was the mycorrhizal mushroom, OTU 72 (*Inocybe lanatodisca*, $P < 0.01$). The indicator OTU for wooded soils represented a different genus of mycorrhizal mushroom, OTU 46 (*Cortinarius sp.,* $P < 0.01$).

Bacteria had 1 indicator OTUs for sediments and soils across landscape position. OTU 92 (Bacteroidetes, $P < 0.01$) was an indicator OTU for stream and bank sediments. Fungal communities had several indicator OTUs across landscape positions. Stream sediments had 2 indicator OTUs - OTU 103 (unclassified Fungi, $P < 0.01$) and OTU 72 (*Inocybe lanatodisca*, $P < 0.01$). Further, stream and bank sediments had 5 indicator taxa, including OTU 55 (Ascomycota, $P = 0.02$), OTU 74, 56, 101, and 113 (unclassified Fungi, $P < 0.01$). There were no indicator taxa for terrestrial soils.

Discussion

Woody vegetation, and its removal, affects microbial communities

Woody encroachment in tallgrass prairie alters multiple facets of ecosystem structure and function across ecosystem compartments (Lett et al. 2004, Hughes et al. 2006, Ratajczak et al. 2011, Ratajczak et al. 2012, Riley & Dodds 2012, Reisinger et al. 2013). Our study design tested whether riparian restorations, in reference to woody encroached areas, affected microbial community diversity and composition across ecosystem types. The observational nature of the experiment prevented detection of mechanistic drivers of microbial community assembly in response to restorations. Nevertheless, this study indicates that riparian removals impact both bacterial and fungal communities, particularly within terrestrial soils, and

these effects are in part related to differences in either abiotic conditions and/or species interactions with plant communities.

Bacterial richness and diversity were similar between wooded and riparian soils, but differed across landscape positions; stream sediments harbored more bacterial OTUs and were more diverse. However, wooded stream sediments had more fungal OTUs than terrestrial soils, yet removals caused fungal richness to be similar across stream and terrestrial soils (Figure 3.2). Greater plant species richness may increase abundance of saprophytic or arbuscular mycorrhizal (AM) fungi (Chung et al. 2007) and woody encroachment causes declines in plant species richness (Ratajczak et al. 2011). Thus, removal of woody vegetation and restoration of riparian areas to their native, grassland state may increase plant species richness resulting in similar changes in fungal communities. In addition, bacterial and fungal richness and diversity may be driven by different processes. For example, resource quantity supplied by plants may be more influential in determining bacterial richness (De Deyn et al. 2010), which did not change across treatments (i.e., TC), whereas plant species richness or identity (Chung et al. 2007; De Deyn et al. 2010) may drive fungal richness. We did not measure how removals affect plant species richness - additional research is needed to address this.

The relative abundance of two bacterial phyla differed between wooded and removal soils. Planctomycetes relative abundance was lower in removal soils whereas Cyanobacteria had approximately 10x greater abundance in removal terrestrial soils compared to all wooded habitats. Planctomycetes have a wide range of metabolic capability - both a diversity of carbon and nitrogen metabolism with some lineages that are anaerobic ammonium oxidizers (Strous & Jetten 2004, Glöckner et al. 2003). Planctomycetes were positively correlated with NH_4^+ (Supplementary Table 3.5), so wooded soils may provide more available nutritive sources for

this bacterial group as wooded areas had greater NH_4^+ than removals. Interestingly, Cyanobacteria abundance was substantially greater and more variable in removal terrestrial soils (Figure 3.3). All cyanobacteria are photosynthetic; many are known to be desiccation-resistant (Potts 1994, Singh et al. 2002, Lüttge 2011) so could proliferate in drier soils with higher light conditions. They were negatively correlated with NH_4^+ and NO_3^- (Supplementary Table 3.5) suggesting they may be affected not only by the abiotic environment, but potentially by species interactions (e.g., out-competed for NH_4^+).

Ascomycota and Chytridiomycota relative abundance was greater in removal treatments, whereas Basidiomycota was greater in wooded. Basidiomycota are known lignin decomposers so their reduced prevalence in removal soils may be due to a lower amount of lignocellulose (Kirk & Farrell 1987, Paláez et al. 1995), but some ascomycetes are also lignin degraders (Kirk & Farrell 1987, Rodríguez et al. 1997). Since the ecological inference related to a fungal phylum is limited (e.g., Ascomycota encompasses fungi that have varying ecologies – saprobes, pathogens, mycorrhizae for example), and no finer taxonomic groupings of fungi, besides 2 indicator OTUs which are both mycorrhizal basidiomycetes, were different across the experiment, the specific reasons for these differing frequencies of fungal phyla between treatments is unknown.

Microbial community composition was marginally impacted by treatment, especially for bacteria (PM ANOVA, $R^2 = 0.02$, $P = 0.09$). The composition of bacterial communities may be more impacted by abiotic conditions (for example, pH, Fierer & Jackson 2006, see Table 3.3), more so than by differing plant communities. In fact, composition was correlated with all edaphic variables measured with TN, TC, and water content being most influential (Table 3.3). Fungal richness substantially differed between treatments (Figure 3.2), yet composition was marginally different between removal and wooded treatments ($R^2 = 0.03$, $P < 0.01$). Similar to

bacteria, composition was correlated with all edaphic variables and TN, TC, and water content was the most influential (Table 3.3). These data suggest that although differences present in removals versus wooded sites impact richness of fungi and relative abundance of bacterial and fungal phyla, there is relatively no effect on microbes at a fine taxonomic resolution. Instead, bacterial and fungal community composition is influenced by edaphic conditions.

Microbial communities shift across stream and terrestrial habitats

In this ecosystem, multiple environmental gradients exist across stream and terrestrial soil habitats and are highly influential in structuring microbial communities. Bacterial and fungal richness differed across these environmental gradients, with stream sediments (greater water and pH, lower C and N concentrations) holding the most species (at least for wooded soils, Figure 3.2). In general, soils are typically considered to be the most microbially rich and diverse habitat (Torsvik et al. 2002). Several reasons may explain why we found contrasting results in this study. First, during precipitation events, microbes associated with adjacent soils are flushed into stream networks and transported down-stream and eventually deposited when baseflow resumes. So, stream sediments would have both stream-exclusive microbes as well as those primarily found in soil. Second, stream sediments may have a richer consortium of microbial life due to multiple chemical and physical gradients that exist vertically (Lozupone & Knight 2007). This latter explanation may be appropriate for bacteria, but bacteria *and* fungi were richer in streams and most fungal taxa are not aquatic (excluding Ingoldian fungi). One of the indicator taxa for fungi in stream sediments was *Inocybe lanatodisca,* a mycorrhizal mushroom, which would only have higher frequencies in streams if its spores are being deposited there. Further, we exclusively used DNA-based methods, which capture the entire community (active and inactive). This suggests that sediments in low to no flow areas (e.g., stream margins, pools) may act as

depositional habitats for microbes in this ecosystem, particularly fungi, and may serve as a seed bank (Lennon & Jones 2011) reservoir with proportionally greater numbers of fungal, and perhaps bacterial, cells that lie dormant.

Although streams had higher microbial richness which may be due to a large proportion of inactive cells, many bacterial phyla had differential relative abundance across land-water interfaces (Table 3.2). Some phyla were more abundant in stream sediments, such as Chloroflexi, known to be found in water-saturated habitats (Costello & Schmidt 2006), Betaproteobacteria and Nitrospirae. Others were more abundant in terrestrial soils, such as Actinobacteria, Acidobacteria, and Verrucomicrobia. As expected, phyla more abundant in streams positively correlated with soil water content, whereas those more abundant in soils were positively correlated with nutrients (Supplementary Table 3.5). These differences in abundance of dominant phyla and their correlation with edaphic variables across landscape position indicate that bacteria, even when evaluating low taxonomic resolutions such as phylum, may undergo environmental filtering (Fierer et al. 2007). Many other variables not measured in this study, such as sediment particle size (Jackson & Weeks 2008), or location of sampling near vegetation, for example, may heavily impact these conclusions so teasing apart specific processes contributing to these results is limited. Regardless, bacteria assembled differentially across landscape position more so than fungi, whereas fungal richness, and relative abundance of phyla, was more impacted by treatment implying different processes controlling their assembly are likely at work.

These data serve as a first step towards understanding (1) if woody encroachment, and its restoration, affects bacteria and fungi across ecosystem types, and (2) what processes may affect microbial community assembly in streams and soils. Although this study focused on edaphic

conditions, with emphasis on concentrations of N and C, and its relationship to microbial communities in the context of woody encroachment restorations, we did not measure ecosystem process rates. However, the removal effects (lower soil extractable NH_4^+ and soil moisture, substantially greater Cyanobacteria abundance, and higher fungal richness) found here imply that restorations do affect both bacteria and fungi taxonomic groups which may have implications for multiple ecosystem processes. In addition, patterns of microbial diversity and community composition may be more driven by species-sorting (Crump et al. 2010) mechanisms such as species interactions (e.g., plant – fungi interactions) or by physiochemical controls (e.g., bacterial community composition shift across ecosystem types due to abiotic conditions, Leibold et al. 2004). Further research is needed to link the effect of woody encroachment on ecosystem processes and microbial community dynamics within stream and terrestrial habitats.

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Figure 3.1 Location of the three watersheds (A) located at Konza Prairie Biological Station near Manhattan, KS. At each watershed, ~ 30 m area had woody vegetation removed parallel to the stream channel (AL is shown as an example in Panel B), except at N2B with removal in entire western fork (reach upstream of sampling point shown). In both removal and wooded treatments, samples were collected from stream margin sediments to upslope soils (see Panel C for stream channel cross section schematic). In Panel C, dashed line denotes stream water surface and ranges of distance from stream (S) for each position (B = bank, R = riparian, U = upslope) are given.

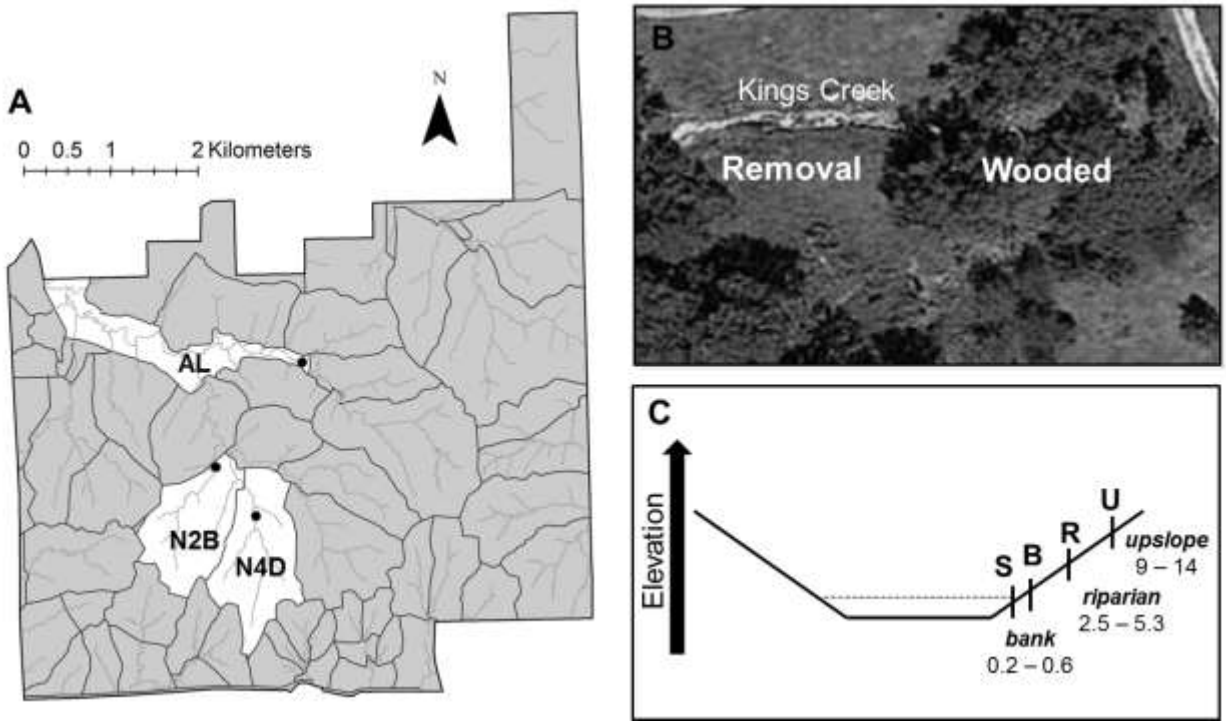

Figure 3.2 The distribution of bacterial (A, B, C) and fungal (D, E, F) richness, diversity, and evenness across landscape positions within removal and wooded treatments. Letters denote Tukeys HSD post-hoc comparisons. Gray boxes = wooded treatment, white boxes = removal treatment.

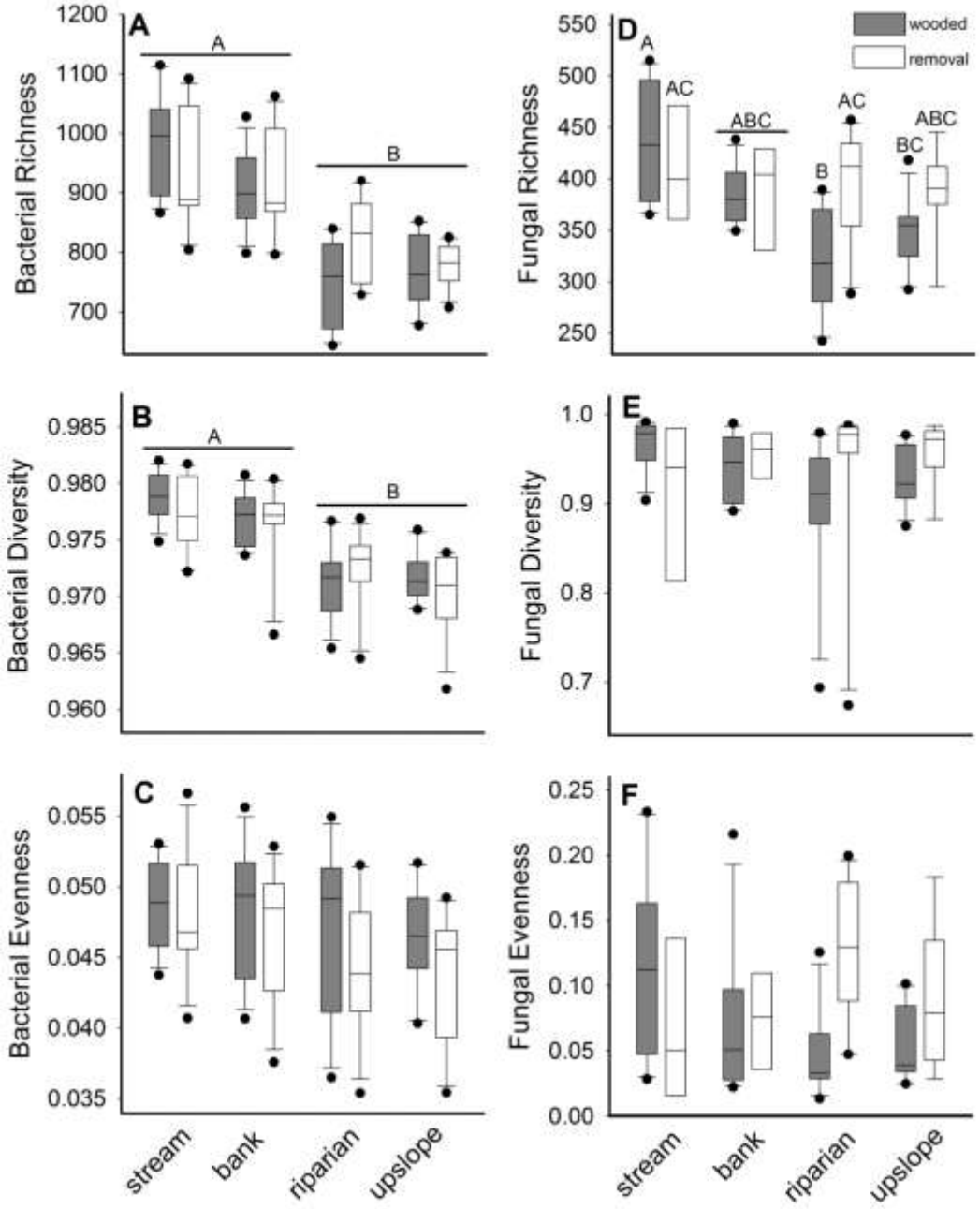

Figure 3.3 Relative abundance of Planctomycetes (A) and Cyanobacteria (B) across landscape position in removal (gray boxes) and wooded (white boxes) treatments. Letters denote Tukeys HSD post-hoc comparisons.

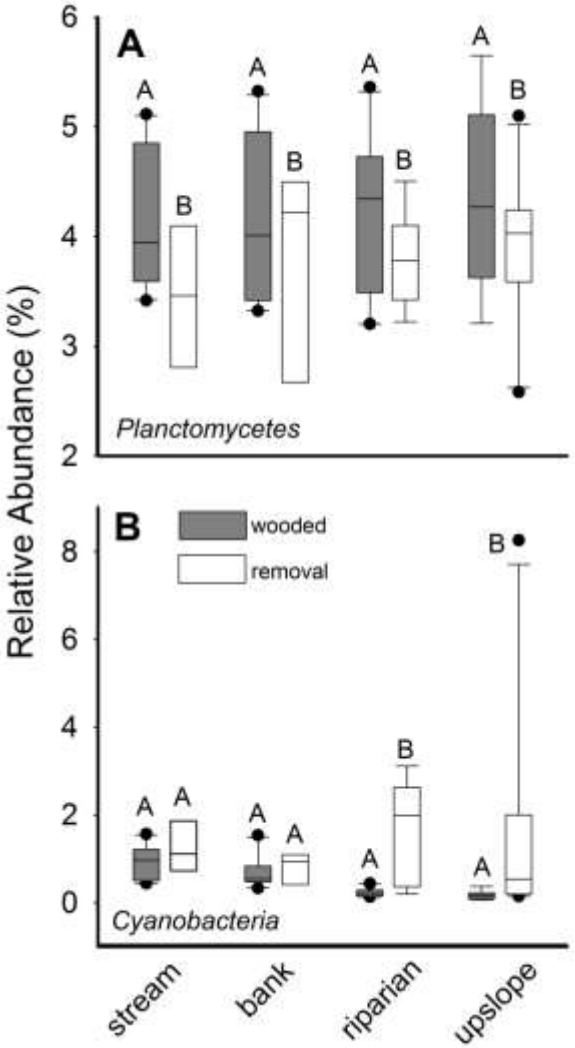

Figure 3.4 NMDS plots for bacterial (A) and fungal (B) communities. Differences in colors represent landscape position: white = streams, light gray = banks, dark gray = riparian, black = upslope soils. Removal soils are represented by circles; wooded soils are represented by triangles. Edaphic associations with community composition are in bold with arrows. Gray axis tick labels correspond to edaphic variable vectors.

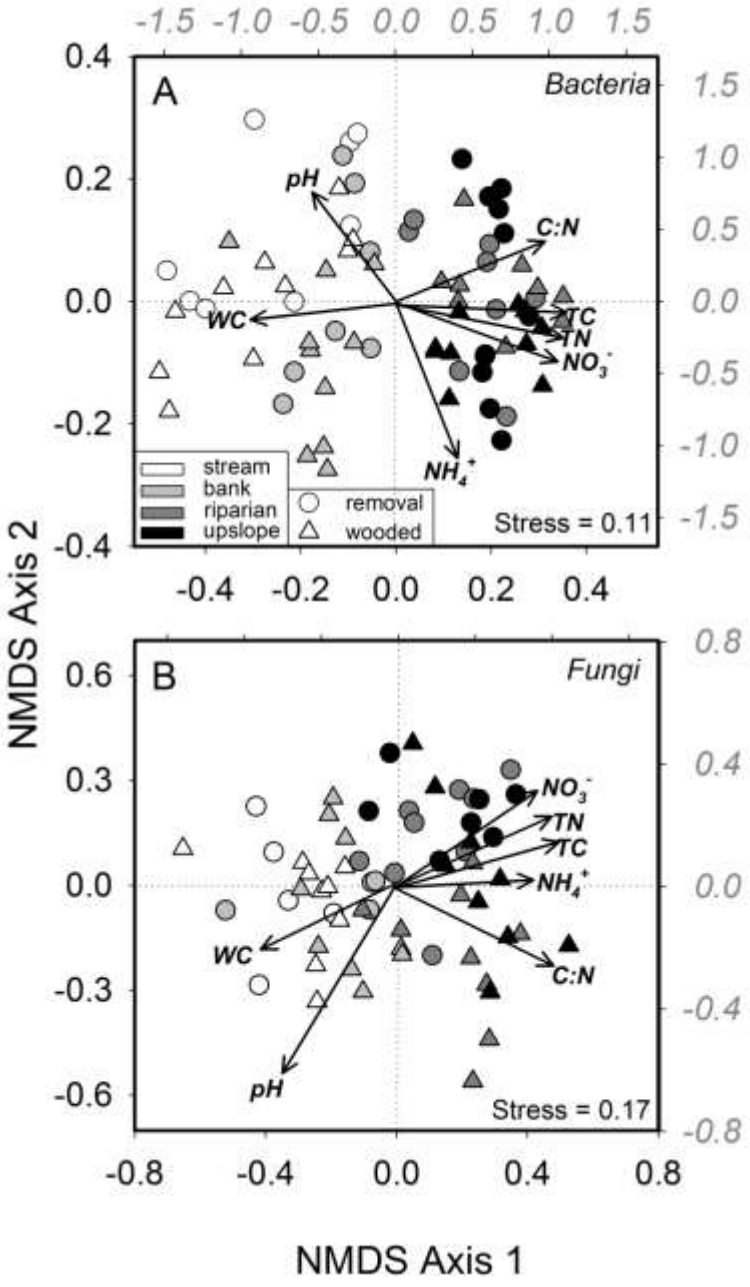

Table 3.1 Summary statistics for edaphic variables measured across landscape position (streams, banks, riparian, upslope habitats) and treatments (W = wooded, R = removal areas). Means (standard deviation) are reported. Letters denote Tukey's HSD post-hoc comparisons for edaphic variables across landscape position only. † denotes edaphic variables that differed across treatment.

Landscape Position	Treatment	NH_4^+-N ($\mu g\ g^{-1}$ soil)†	NO_3^--N ($\mu g\ g^{-1}$ soil)	TN ($mg\ g^{-1}$ soil)	TC ($mg\ g^{-1}$ soil)	C:N	Soil Water Content (%)†	Soil pH
Stream	W	7.60 (3.94)	0.31 (0.15)^A	1.57 (0.66)^A	17.74 (11.99)^A	10.44 (2.45)^A	0.51 (0.11)^A	7.82 (0.09)^A
	R	5.97 (2.39)	0.32 (0.15)	1.60 (0.40)	16.71 (6.36)	10.13 (1.63)	0.53 (0.16)	7.80 (0.13)
Bank	W	8.82 (5.38)	0.48 (0.52)^A	1.39 (0.39)^A	13.74 (6.14)^A	9.33 (2.76)^A	0.27 (0.06)^B	7.85 (0.23)^A
	R	6.13 (3.05)	0.29 (0.30)	1.27 (0.24)	11.17 (4.72)	9.19 (1.57)	0.26 (0.04)	7.87 (0.11)
Riparian	W	8.82 (3.63)	3.0 (1.55)^B	9.41 (2.06)^B	150.11 (36.58)^B	16.30 (3.54)^B	0.14 (0.04)^C	7.55 (0.32)^B
	R	6.42 (2.69)	2.21 (2.15)	8.19 (1.84)	123.22 (23.98)	15.29 (2.80)	0.12 (0.03)	7.51 (0.42)
Upslope	W	12.05 (3.42)	3.70 (2.65)^B	11.64 (3.74)^B	154.60 (55.66)^B	13.18 (1.06)^C	0.18 (0.03)^C	7.33 (0.26)^B
	R	7.73 (4.73)	3.19 (2.43)	9.15 (3.85)	121.44 (40.68)	13.95 (3.16)	0.13 (0.04)	7.48 (0.43)

Table 3.2 Bacterial and fungal phyla relative abundance across landscape position and treatment. Means (SD) are reported. Letters after means denote pairwise differences between landscape position based on Tukey HSD post-hoc tests. Bolded phyla labels denote a phylum which differed in relative abundance between treatments.

		Landscape Position			***Treatment***	
Phylum	**Stream**	**Bank**	**Riparian**	**Upslope**	**Removal**	**Wooded**
Bacteria						
Alphaproteobacteria	5.6 (0.8)	5.6 (0.9)	5.7 (0.8)	5.9 (0.8)	5.7 (0.8)	5.7 (0.8)
Betaproteobacteria	9.2 (1.3)^{A}	8.7 (1.1)^{A}	5.6 (0.8)^{B}	5.4 (1.1)^{B}	7.1 (2.0)	7.4 (2.1)
Deltaproteobacteria	5.6 (1.3)^{A}	4.9 (1.0)^{A}	3.9 (0.5)^{B}	3.9 (0.5)^{B}	4.5 (1.1)	4.7 (1.3)
Gammaproteobacteria	3.8 (0.8)^{A}	4.5 (1.2)^{B}	3.9 (1.1)^{A}	3.6 (0.7)^{A}	3.9 (1.2)	4.0 (0.8)
Acidobacteria	12.5 (1.8)^{A}	14.6 (2.1)^{B}	14.9 (2.1)^{B}	15.5 (1.6)^{B}	14.4 (2.2)	14.3 (2.2)
Actinobacteria	10.9 (3.7)^{A}	11.5 (4.0)^{A}	14.7 (2.1)^{B}	14.9 (2.7)^{B}	13.2 (3.4)	12.7 (3.9)
Bacteroidetes	13.8 (2.8)	13.3 (3.1)	15.7 (2.6)	14.7 (2.0)	14.1 (2.6)	14.6 (2.9)
Chloroflexi	10.6 (3.4)^{A}	9.4 (2.3)^{A}	6.7 (2.3)^{B}	5.3 (1.8)^{B}	7.9 (2.9)	8.2 (3.6)
Cyanobacteria	1.2 (0.7)^{A}	1.0 (1.2)^{AB}	0.8 (1.0)^{B}	0.8 (1.8)^{C}	1.4 (1.5)	0.5 (0.5)
Firmicutes	3.1 (1.0)	2.7 (1.3)	3.1 (1.6)	3.5 (1.9)	3.3 (1.6)	2.9 (1.4)
Nitrospirae	0.7 (0.2)^{A}	0.5 (0.2)^{B}	0.4 (0.1)^{BC}	0.3 (0.1)^{C}	0.5 (0.2)	0.5 (0.2)
Planctomycetes	3.9 (0.7)	4.0 (0.8)	4.0 (0.6)	4.2 (0.8)	3.8 (0.7)	4.2 (0.7)
Verrucomicrobia	6.0 (1.0)^{A}	5.5 (0.7)^{A}	8.5 (1.9)^{B}	9.3 (2.7)^{B}	7.6 (2.6)	7.0 (2.0)
Fungi						
Ascomycota	40.5 (15.5)	39.8 (11.5)	41.5 (13.0)	42.2 (16.3)	46.5 (13.3)	37.3 (13.2)
Basidiomycota	27.2 (18.2)	29.9 (15.5)	39.2 (19.9)	38.2 (20.3)	26.6 (15.5)	39.5 (19.7)
Chytridiomycota	1.4 (0.8)	1.2 (1.3)	1.9 (1.8)	1.5 (1.0)	2.1 (1.7)	1.2 (0.8)
Zygomycota	3.1 (2.2)^{A}	4.9 (3.1)^{A}	7.0 (4.0)^{B}	6.5 (2.8)^{B}	6.0 (3.9)	5.2 (3.1)

Table 3.3 Correlation statistics for environmental vectors fitted with Bray-Curtis dissimilarity matrices calculated for both bacterial and fungal communities.

Edaphic variable	R^2	P-value
Bacteria		
NH_4^+	0.28	< 0.01
NO_3^-	0.54	< 0.01
TN	0.74	< 0.01
TC	0.67	< 0.01
C:N	0.29	< 0.01
pH	0.43	< 0.01
Water Content	0.64	< 0.01
Fungi		
NH_4^+	0.19	< 0.01
NO_3^-	0.39	< 0.01
TN	0.69	< 0.01
TC	0.64	< 0.01
C:N	0.29	< 0.01
pH	0.6	< 0.01
Water Content	0.49	< 0.01

Chapter 4 - Freshwater bacterial communities display rapid compositional and functional successional trajectories

Abstract

Biofilms represent a metabolically active and structurally complex component of freshwater ecosystems. Ephemeral prairie streams are hydrologically harsh and prone to frequent perturbation. Elucidating both functional and structural community changes over time within prairie streams provides a holistic understanding of microbial responses to environmental disturbance. We examined microbial succession of biofilm communities at three sites in a third-order stream at Konza Prairie over a 2 – 64 day period. Microbial abundance (bacteria abundance, chlorophyll *a* concentrations) increased and never plateaued during the experiment. Net ecosystem productivity of the developing biofilms was not statistically different from zero (net balance of oxygen consumption and production) until 64 days which suggests a balance of the use of autochthonous and allochthonous energy sources until late succession. In contrast to the gradual development of NEP rates, the bacterial communities (queried via MiSeq sequencing of the V4 region of 16S rRNA complex) established quickly and both richness and diversity were high after 2 days and remained stable thereafter. Sequences and operational taxonomic units (OTUs) across the study period were dominated by Bacteroidetes, Betaproteobacteria, and Verrucomicrobia. However, other dominant phyla, such as Alphaproteobacteria, Cyanobacteria, Gemmatimonadetes, and Planctomycetes, increased in relative abundance over time. Bacterial community composition differed across space and successional time, but strong temporal patterns in composition were detected suggesting distinct successional trajectories exist for bacteria-associated biofilm communities in this ecosystem.

Introduction

Most bacteria exist attached to surfaces and survive in complex, multi-species communities. Biofilms form by initial adhesion of bacteria to substrata and subsequently grow into an interdependent, matrix-enclosed system (Davey & O'Toole, 2000). In streams, the proportion of respiring bacteria is typically higher in streambed biofilms relative to flowing waters (Araya et al., 2003), thus bacteria-associated biofilm communities represent a highly metabolically active component of freshwater ecosystems and provide an ecologically relevant system to study microbial succession.

Biogeochemical cycling in freshwater is strongly influenced by biofilm communities and their developmental stage (Battin et al., 2003). Further, biogeochemical cycling controlled by stream biofilms can consequently influence nutrient transport (Mulholland et al., 2008) and be a significant source of nitrous oxide globally (Beaulieu et al., 2011). Grassland and wooded grassland streams drain approximately $1/4^{th}$ of the world's land area and $1/5^{th}$ of total continental runoff originates from them (Dodds, 1997). Therefore, understanding the compositional and functional dynamics of microbial communities in general, and grassland streams more specifically, is necessary to predict responses of nutrient cycles to global change (Wrona et al., 2006).

Successional ecology aims to characterize community assembly over time either after an initial colonization or following a disturbance and has provided mechanistic insights into community development across taxa, especially for plants and animals (e.g., Cowles, 1899; Clements, 1916; Gleason, 1926; Keever, 1950; Connell & Slatyer, 1977; Sousa, 1979). Succession occurs as species abundances change over time via deterministic (i.e., niche-based) processes, such as selection through species interactions or environmental filtering, or stochastic

(i.e., random) processes, such as ecological drift (Hubbell, 2001). Although successional ecology has been well characterized for macro-organisms, far less is known about temporal dynamics of bacterial community assembly (Fierer et al., 2010) especially in flowing waters (*but see* – Jackson et al., 2001; Lyautey et al., 2005). Other work has found that bacterial communities exhibit successional dynamics highly influenced by deterministic processes such as physical conditions (Lyautey et al., 2005, Nermergut et al., 2006), or biotic interactions (algal-bacterial associations; Besemer et al., 2007). Alternatively, bacterial communities may be controlled by stochasticity (e.g., random speciation or extinction events) during certain stages of succession (Zhou et al., 2014). The impact of deterministic and stochastic processes during succession depends on the degree and timing of community development.

In this study, we characterized primary successional dynamics of biofilm-associated microbial communities in a native, tallgrass prairie stream by measuring both compositional and functional aspects of microbial development. First, we hypothesized that microbial abundance (algae, bacteria) would increase over time, but peak ca. 30 d (indicative of late stage succession) as seen in previous metabolic studies in prairie streams (Dodds et al., 1996). Second, biofilm net ecosystem productivity (NEP) would initially be net heterotrophic (greater O_2 consumption than production) because of low abundances and biomass of autotrophs relative to heterotrophs, but would shift to net autotrophy as a result of increasing autotrophic biomass. Third, we hypothesized that early stage bacterial communities would be composed of a few pioneer species capable of adhering to substrata, and these communities would be amended with additional species resulting in a gradual increase in species richness and diversity over time. Lastly, as other studies have found that specific bacterial groups may be responsible for biofilm formation and succession (e.g., Proteobacteria; Dang & Lovell, 2000, Hall-Stoodley et al., 2004, Lyautey et al.,

2005), we hypothesize that although spatial differences will be apparent, biofilm bacterial communities will more strongly display deterministic successional trajectories as determined through community compositional changes over time.

Materials and Methods

Study location

We used the main reach of Kings Creek at Konza Prairie Biological Station (KPBS) located in the Flint Hills of northeastern Kansas in this study. Unglazed ceramic tiles (N = 200 per site to allow for random sampling; 4.8 x 2.8 cm) were autoclaved and then adhered to large bricks (40.6 x 40.6 cm) using aquarium silicon and submerged in three separate pools (Figure 4.1; see Supplementary Table 4.1 for abiotic characteristics of sites over time). Tiles were placed within each of three stream locations on April 5th, 2013 and removed 2, 4, 8, 16, 35, and 64 days later. These pools were disconnected from one another at the beginning of the experiment (zero surface flow), but potentially became connected after a 38.4 mm rainfall event at 13 d post tile placement (Supplementary Figure 4.1).

Microbial abundance and biofilm metabolism

We randomly sampled tiles to estimate algal biomass, bacterial cell abundance, and biofilm metabolism (NEP). From each of the three pools and at each time (2, 4, 8,16, 35, 64 d post placement); i) three tiles were collected for algal biomass, placed in Whirl-Pak bags (Nasco International, Fort Atkinson, WI, USA), and stored at -20°C; ii) three tiles were collected for bacterial cell abundance, placed in a nuclease-free 50 mL centrifuge tube, preserved in a 3% formalin solution, and stored at 4°C and; iii) two tiles were collected into a 50 mL centrifuge tube containing stream water from each respective sampling pool, and kept upright (to prevent

biofilm disturbance) to measure NEP upon arrival in the laboratory. Day 2 samples did not have adequate biomass to obtain NEP measurements.

Algal biomass tiles analyzed for chlorophyll *a* were placed in a 95% ethanol:H_2O solution, heated at 78°C for 5 minutes, and kept at 4°C for ~ 12 hours (Sartory & Grobbelaar, 1984). Extract solution was analyzed using a spectrophotometer (Hitachi High Technologies America, Inc., Schaumburg, IL, USA) according to standard methods (APHA, 1995) and corrected for tile surface area.

Bacterial cell abundance tiles were scraped with a sterile razor to remove biofilm biomass and preserved in 3% formalin. A subsample of 1 mL from the total volume of preserved biofilm was incubated with 4',6-diamidino-2-phenylindole (DAPI) nucleic acid stain (5 mg/mL) for 5 min., and filtered onto a black, polycarbonate membrane (Whatman Nucleopore, 0.2 um size, GE Healthcare Companies). The number of bacteria on the membranes was estimated by counting 10-15 optical fields under an epifluorescent miscrocope (Nikon Labophot-2, Nikon Corporation, Tokyo, Japan). Bacterial cell abundance was then determined per sample by averaging the number of cells counted across replicate optical fields, accounting for total sample volume, optical field size, dilution, and filter area. Replicates for both bacterial abundance and chlorophyll *a* were averaged to obtain one value for each site across time points for statistical analyses.

We estimated NEP by placing 2 tiles into a closed, circulating chamber containing stream water from the location where the tiles were collected. The chamber was constructed using clear, acrylic plastic (US Plastics, Lima, OH) which allows for ~ 92% transmittance of photosynthetically available radiation. A logging membrane oxygen probe (YSI 600-XLM, Yellow Springs, OH) was placed horizontally in the chamber. A fluorescent, full-spectrum light

(20 watt mini-compact bulb, Central Aquatics, Frankin, WI) was placed over the chamber to mimic daylight conditions (~140 µmol quanta m^{-2} s^{-1}). Both temperature and dissolved oxygen (DO) were measured every 5 minutes for 20-25 minutes to obtain gross primary productivity (GPP) rates. Subsequently, the chamber was placed in the dark to measure community respiration (CR) rates as above. The slope of DO concentration change over time in light and dark incubations was used to calculate metabolic rates (GPP, CR) whereas NEP is the balance between GPP and CR rates, as in Bott (1996).

DNA extractions and Illumina MiSeq analysis

Total genomic DNA for three tiles from each site at each sampling time was extracted using a Qiagen DNeasy Plant Maxi kit (Qiagen, Venlo, Netherlands) using the manufacturer's protocol with the following modifications: tiles were sonicated (FS20, Fisher Scientific, Pittsburgh, PA, USA) in the cell lysis solutions until biomass was removed from the tiles. Two tiles did not yield enough DNA extract (Day 16 at two sites) and were omitted (N=52). Extracted DNA was then quantified using a Nanodrop 1000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA). Template DNA was aliquoted into a 96-well plate at a concentration of 2 ng/uL.

We used a two-step PCR approach (*see* Berry et al., 2011) to avoid a 3'-end amplification bias generated with DNA-tags. In the first PCR step, the 16S rRNA gene V4 region was amplified using the 505F and 806R primers (Caporaso et al., 2012). Each sample was amplified in three independent 50 µL PCRs. Each reaction consisted of 2 µM of forward and reverse primers, 10 ng of template DNA, 25 µL Phusion High-Fidelity Master Mix (New England Biolabs, Inc., Ipswich, MA, USA), and 10 µL of molecular grade water. Thermal cycler parameters (Eppendorf, Hamburg, Germany) included: 5 min. denaturation at 94°C, followed by

25 cycles with denaturation at 94°C for 1 min., annealing for 30 sec. at 50°C, extension for 1 min. at 72°C, with final extension for 10 min. Negative controls were included in PCRs to detect contamination and all controls remained contaminant free. For the secondary PCR, 10 µL of primary PCR products were amplified as above with the exception of using only 5 cycles and the inclusion of a reverse primer joined with 12 bp unique Molecular Identifier tags (MID-806R; Caparoso et al., 2012; Supplementary Table 2). All technical replicates for both primary and secondary PCRs were visualized on a 1.5% agarose (w/v) gel to check for amplification. After secondary PCR visualization, the remaining PCR volume was pooled per experimental unit and cleaned with the Agencourt AmPure (Beckman Coulter Inc., Pasadena, CA) as per manufacturer's instructions except that we used a 1:1 ratio of AmPure bead solution to PCR volume to further discriminate against short PCR fragments. Each experimental unit was quantified for DNA yield, and pooled at equal molarity (115 ng per sample). Amplicons were submitted to the Integrated Genomics Facility at Kansas State University (Manhattan, KS, USA). Amplicons then had Ilumina specific primers and adapters ligated using a NEBNext® DNA MasterMix for Illumina kit (Protocol E6040, New England Biolabs Inc., Ipswich, MA, USA) and using a MiSeq Reagent Kit v2 (Illumina, San Diego, CA, USA) with 500 cycles.

Bioinformatics

Sequences (.fastq) were processed using the mothur pipeline (version 1.32.1, Schloss et al., 2009). Paired-end .fastq files were contiged with a minimum of 50 bp overlap. Sequences with ambiguous bases, with greater than 2 mismatches to the primers, 1 mismatch to the MID, and homopolymeric regions greater than 8 were removed. Remaining sequences were aligned against a mothur implemented SILVA reference. Likely sequence generated errors were screened using a pseudo-single linkage algorithm (pre.cluster with diff=2; see Huse et al., 2010).

Remaining sequences were screened for chimeric properties with the mothur implemented UCHIME algorithm (Edgar, 2010). Sequences were assigned to taxonomic affinities using the Naïve Bayesian Classifier (Wang et al., 2007) with a bootstrap threshold of 80% against the RDP training set, version 9. Sequences not assigned to Domain Bacteria (including Archaea, mitochondria, and chloroplasts) were omitted. A pairwise sequence distance matrix was calculated (extended gaps not penalized) and sequences were clustered to Operational Taxonomic Units (OTUs) at a 97% similarity threshold using an average neighbor joining method. Rare OTUs (abundance < 10 across all experimental units) that may have limited metabolic function in the system were removed. Taxonomic affinities were assigned to clustered OTUs. All sequence data was accessioned into the Sequence Read Archive (SRA URL and accession numbers).

Statistical Analyses

Multiple regression models were used to determine if microbial abundance and biofilm metabolism (NEP, GPP, CR) differed over time and across sites. Biofilm metabolism regression models had the y-intercept at zero as it is logical to assume metabolism rates equal zero at the onset of the experiment. Most microbial abundance and biofilm metabolism data visually exhibited curvature in the data over time so we performed two multiple regression models: one which included time and site as predictor variables and another that also included a quadratic term for time ($time^2$). Comparison of model residual sum of square errors indicated models that included time, $time^2$, and site obtained the best fit based on reduced error and statistically significant $time^2$ terms; therefore, we only report results for the multiple, quadratic regression models. Chlorophyll *a* and bacteria abundance data were highly skewed and were log10

transformed prior to the analyses. An outlier was detected (high Cook's D value; Cook, 1979), for a 4 day measurement taken for GPP and was removed.

For bacterial communities, Good's coverage (the complement of the ratio of local OTU singletons to total number of sequences) was calculated to determine how well each sample represented the resident bacterial communities. Observed OTU richness (S_{obs}), the complement of Simpson's diversity (1-D: $1-\sum p_i^2$), and Simpson's evenness (E: $1/\sum p_i^2/S$), with p_i representing frequency of each OTU within a sample, were also calculated for each site across each sampling time after randomly subsampling at a depth of 12,000 sequences per experimental unit (Gihring et al., 2012). Richness was log10 transformed whereas both diversity and evenness were arcsine square root transformed. A multiple linear regression model was used to determine if observed OTU richness, diversity, and evenness differed over time and across sites.

Multiple regression models were also used to determine if the relative abundance of dominant bacterial phyla (represented $\geq 1\%$ of all sequences at any time point) differed over time and across sites. Proteobacteria were partitioned into respective classes unless unclassified at the class level. Alphaproteobacteria, Cyanobacteria, and Gemmatinoadetes exhibited curvature in the data so time^2 was added to regression models for these groups as it reduced residual sum of square errors. In addition, individual linear regression models were used to test whether the 100 most abundant OTUs changed in frequency over successional time. The 100 most abundant OTUs represented approximately 60% of sequences (after subsampling and removal of rare OTUs) and are likely contributing the most functionally. All regression models, except for microbial abundance, were deemed significantly meaningful after Bonferroni-corrections for multiple comparisons.

Compositional differences among the communities across time and sites were determined by computing Bray-Curtis dissimilarity distances and visualized using non-metric multidimensional scaling (NMDS). The number of dimensions was defined by Monte Carlo tests of significance for each level of dimensionality by comparisons with 250 runs of empirical versus randomized data. A multiple linear regression model was then used to test whether NMDS axis scores differed over time and across sites. Further, a permutational MANOVA was used, with 1000 permutations, to determine sources of variation in community composition across time and site after converting sequence abundance data to a Bray-Curtis distance matrix.

Community composition may vary spatially because of random or non-random processes resulting from environmental or biotic filtering (Legendre et al., 2005). Beta diversity, the variation in community composition between samples, reflects two different biological processes (species replacement and nestedness) that in turn are influenced by abiotic or biotic interactions. Species replacement describes replacement of taxa in a local community by other taxa whereas nestedness refers to local communities containing subsets of species found in other locations with richer communities (Ulrich & Gotelli, 2007; Baselga, 2010). We estimated beta diversity over time (comparisons of beta diversity across time points, not across sites) and determined beta diversity into its additive components of species replacement and nestedness (as in Baselga, 2010). Temporal beta diversity was calculated using Sørensen-based multiple-site dissimilarity (β_{SOR}). Subsequently, Simpson-based multiple-site dissimilarity ($\beta_{SIM;}$ estimate of species replacement, Simpson, 1943), and nestedness-resultant dissimilarity ($\beta_{NES;}$ estimate of nestedness, Lennon et al., 2001) were calculated to partition beta diversity into species replacement and nestedness components (*see* Baselga, 2010).

All richness and diversity estimates were calculated in mothur (version 1.32.1, Schloss et al., 2009). All regression and posthoc analyses were performed using the *stats* package, β diversity calculations were executed using the *betapart* package (Baselga & Orme, 2012), and permutative ANOVA was executed using the adonis function in the *vegan* package (Oksanen et al., 2011) in the R programming language (version 2.13.1, R Development Core Team, 2011). NMDS was performed in PC-ORD (version 5; McCune & Mefford, 2006).

Results

Microbial abundance and biofilm metabolism

Chlorophyll *a* and bacterial abundance increased over time (Table 4.1, Figure 4.2). Early stages of succession (2 days of incubation) were characterized by low microbial abundance (*chlorophyll*: 0.25 μg cm^{-2}; *bacteria*: $7.5 \cdot 10^5$ cells cm^{-2}), whereas later during succession (64 d), abundance was more than an order of magnitude greater (*chlorophyll*: 4.77 μg cm^{-2}; *bacteria*: $2.71 \cdot 10^6$ cells cm^{-2}). Chlorophyll *a* concentrations and bacteria neither differed among sites nor had any site by time interactions ($P > 0.25$, Table 4.1).

All biofilm metabolism rates increased over time (Table 4.1, Figure 4.3). CR rates were greatest at 35 d (average: $-1.92 \cdot 10^{-4}$ mg O_2 cm^{-2} min^{-1}) then decreased by 64 d, whereas GPP rates increased with later successional stages (64 d, average: $3.71 \cdot 10^{-4}$ mg O_2 cm^{-2} min^{-1}) with rates more than twice those of early stages (4 d, average: $1.6 \cdot 10^{-4}$ mg O_2 cm^{-2} min^{-1}). A one-sample t-test for each time point indicated that NEP did not differ from zero until 64 days ($T = 7.5$, $df = 2$, $P = 0.017$). These data indicate that during primary succession, relative rates of O_2 production and consumption are similar until the late stage when communities become net autotrophic. Lastly, sites differed in GPP and CR rates and had a significant site by time

interaction (Table 4.1) indicating that biofilm-associated microbial communities display functional differences in metabolic rates spatiotemporally.

Bacterial community richness, diversity, and composition

Bacterial communities established rapidly on submerged tiles. Observed OTU richness, diversity, and evenness did not change over time; all metrics plateaued only after 2 days of incubation (Average over time: S_{obs} = 1,497, 1-D = 0.99, E = 0.06, P > 0.1). No richness or diversity metric differed among sites.

Across all sampling times, dominant phyla (or classes of Proteobacteria) (≥ 1% of total abundance) belonged to Bacteroidetes (25.2% sequences, 25.5% OTUs), Betaproteobacteria (23.6% sequences, 17.9% OTUs), Verrucomicrobia (20.7% sequences, 15.1% OTUs), Alphaproteobacteria (8.3% sequences, 8.4% OTUs), Gammaproteobacteria (6.9% sequences, 7.2% OTUs), Deltaproteobacteria (3.2% sequences, 7.4% OTUs), Proteobacteria unclassified at class level (3.2% sequences, 4.8% OTUs), Acidobacteria (1.1% sequences, 2.9% OTUs), and Gemmatimonadetes (3.6% sequences) (Figure 4.4). While these phyla comprised the majority of the community, many abundant OTU's represented other taxa, such as Chloroflexi (3.6%), Planctomycetes (2.2%), Actinobacteria (2.2%), Cyanobacteria (1.3%), and OD1 (1.1%) (Figure 4). Alphaproteobacteria, Gemmatimonadetes, Cyanobacteria, and Planctomycetes increased in relative abundance over time with Alphaproteobacteria, Gemmatimonadetes, and Planctomycetes having over twice as high relative abundance by the latest successional stage (2 d average: 5.1%, 1.9%, 0.4% and 64 d average: 10.4%, 5.0%, 1.0% respectively). Cyanobacteria increased over an order of magnitude over time (2 d average: 0.2% and 64 d average: 2.6%) (Table 2, Figure 5). Further, Alphaproteobacteria and Gemmatimonadetes displayed differences in relative

abundances across sites and exhibited site by time interactions (Table 4.2). Proteobacteria unclassified at the class level differed across sites, but not over time (Table 4.2).

A total of 14 of the 100 most abundant OTUs changed in frequency over time (Table 4.3). Four OTUs declined (affinities to Comamonadaceae, *Flavobacterium sp.*, and *Luteolibacter sp*.) and ten (affinities to Proteobacteria, *Gemmatimonas sp.*, *Haliscomenobacter sp.*, Bacteroidetes, *Roseomonas sp.*, *Runella sp.*, and *Luteolibacter sp.*) increased in frequency during the experiment (Table 4.3).

In addition to the observed shifts in frequencies of individual OTUs, bacterial communities overall changed compositionally over time and sites (NMDS, 2D Stress = 9.58; Figure 4.6). Temporal trends in composition were most apparent visually across NMDS Axis 2 (56.6% variance represented, $P < 0.01$). Based on a linear regression model, NMDS Axis 2 scores decreased over time ($T = 9.55$, $F_{2,15} = 46.01$, $P < 0.01$, Adj. $R^2 = 0.84$; Figure 4.6). In addition, NMDS Axis 1 (29.7% variance represented, $P < 0.01$) scores significantly differed across sites ($T = -2.67$, $F_{3,14} = 4.76$, $P = 0.02$, Adj. $R^2 = 0.40$), but showed no differences over time. Permutational MANOVA of bacterial community data indicated that community composition differed over both time ($R^2 = 0.34$, $P < 0.01$) and sites ($R^2 = 0.21$, $P < 0.01$), with a significant time by site interaction ($R^2 = 0.20$, $P < 0.01$).

Analyses of compositional changes by partitioning β_{SOR} into respective β_{SIM} and β_{NES} constituents indicates that compositional changes were primarily due to species replacement over time and to a lesser extent due to species additions (Average: $\beta_{SIM} = 0.47$, $\beta_{SIM} = 0.44$, $\beta_{NES} = 0.03$).

Discussion

Development of biofilm microbial abundance and metabolic rates

Contrary to our hypothesis, chlorophyll *a*, and bacterial cell abundance increased over time but did not appear to plateau (Figure 4.2). Our results contrast similar studies that evaluate the return time of benthic algae in prairie streams after a hydrologic disturbance (Fisher et al., 1984; Dodds et al., 1996). Those studies concluded that algal communities reach pre-disturbance levels within two weeks (Dodds et al., 1996). These studies examined secondary successional dynamics after a flooding event instead of a primary succession sequence therefore habitat differences (e.g., geochemistry, discharge) may partially explain discrepancies. However, primary succession and colonization of biofilm microorganisms may be delayed or require conditioning (i.e., polymeric substance present from the overlying water) before significant biofilm construction occurs. In addition, nutrient availability, environmental temperature, hydrophobocity of substrata, or ionic interactions with bacteria and substrata may affect the timing and rate of biofilm development (Brading et al., 1995; Melo & Bott, 1997; Siboni et al., 2007). Although temperature was likely not low enough to slow biofilm growth (Supplementary Table 4.1), substrata may have required conditioning of organic materials (Siboni et al., 2007) before significant growth of biofilm communities occurred. Further, grazing scars were noticeable on biofilms across sampling times and sites (personal observation, A. Veach), therefore continual removal of biomass by consumers may have also slowed recruitment of microbial biomass. Bacterial OTU richness was very high within 2 days, so although unlikely that colonization was delayed (at least for the heterotrophic component of biofilms), the growth and establishment of microbial biomass within biofilms may be stunted because of unfavorable habitat conditions or grazing.

In agreement with our hypothesis, net ecosystem productivity gradually increased over time, and was closely related to changes in GPP (Figure 4.3). CR rates were high within 4 days indicating that an active heterotrophic microbial community capable of using stream water derived dissolved organic carbon established very quickly. Biofilms exhibited no relative change in O_2 consumption versus production until late stage succession (64 d), which is likely due to high algal biomass during this period. Thus, generalizations about the importance of allochthonous or autochthonous carbon sources during early biofilm succession may not be discernible. Further, the relative abundance of Cyanobacteria sequences increased ten-fold from early to late stage communities, which could have contributed to increased primary production at 64 days. Pohlon et al., (2009) found that pioneer biofilm communities (7 d of growth) had low ratios of β-xylosidase: β-glucosidase enzymes suggesting biofilms relied upon carbon sources derived from algae (as would be expected for net autotrophic communities). They found that late stages of succession (5 mo.) resulted in biofilms using carbon sources likely from allochthonous compounds. Contrary to this, we found biofilms became net autotrophic after 2 months of growth and exhibited a trajectory towards net autotrophy instead of one directed to net heterotrophy. Allowing longer timeframes of primary succession to occur in this study system (e.g., several months) may eventually lead to communities dependent upon autochthonous sources, as seen in other studies examining ecosystem metabolism in prairie streams (Riley & Dodds, 2011).

Successional development of biofilm bacterial communities

Succession has been described as a random, stochastic arrival and assembly of taxa that, over time, converge into similar community types due to local, deterministic effects (Del Moral, 2009). In this study, bacterial communities changed substantially both over time and space (Figure 4.5, 4.6). The relative abundance of Alphaproteobacteria, Gemmatimonadetes, and

Proteobacteria differed across sites (Table 4.2) and, overall community composition differed across sites (NMDS Axis 1 scores) as well. Nevertheless, bacterial communities displayed stronger temporal trends as communities across sites exhibited similar successional trajectories (Perm MANOVA: Time R^2 = 0. 34, Site R^2 = 0.21; Figure 4.6).

Previous work on primary succession of bacteria associated with apple flowers (Shade et al., 2013), leaf surfaces (Redford & Fierer, 2009) and deglaciated soils (Nemergut et al., 2006) have also observed stronger temporal patterns than spatial suggesting bacterial communities develop predictably over time, but with some degree of variability across space. Biofilms in particular may exhibit strong temporal trends compositionally due to colonization of specific pioneer species that possess gene factors required for initial attachment and exopolymeric secretion (Whitchurch et al., 2002; Hall-Stoodley et al., 2004). Biofilm differentiation may also be highly influenced by cell signaling indicating a degree of genetic regulation (Davies et al., 1998) that controls bacterial biofilm community assembly via both physiological and biochemical mechanisms. Conversely, these communities may merely represent subsets derived from the regional species pool (propagules in the water column) or differentiate as a result of environmental conditions. Although substrata were all placed in pools, with little to no flow, other abiotic characteristics were quite variable spatiotemporally (Supplementary Table 4.1). Thus, temporal patterns in bacterial community structure may be more influenced by local processes, such as environmental filtering or species interactions, although regional processes (i.e., immigration and emigration) likely contribute to variability in community composition spatiotemporally. The relative contributions of local and regional processes in structuring community development remain indiscernible in this study, but the presence of strong

successional trajectories give evidence that local processes are probably more influential for bacterial community assembly.

Bacterial biofilm communities clearly changed compositionally over time and this was primarily driven by species replacement more so than species additions (i.e., nestedness). Shade et al., (2013) found similar successional trends in bacteria associated with apple flowers and attributed community variability to turnover of transient (or OTUs with low abundance) species. As many dominant OTUs and bacterial phyla remained in high abundance over time, our data indicates similar trends for temporal beta diversity of biofilm bacterial communities. These patterns in beta diversity may be driven by ecological strategies of microbes. Such small organisms may have high temporal turnover of taxa due to high rates of dispersal, especially during certain stages of biofilm growth (Hall-Stoodley et al., 2004) or their expedient life cycles relative to the sampling duration (Brown et al., 2004; Korhonen et al., 2010). Likewise, these life history strategies may explain why bacteria rapidly established hyper-diverse communities within the first 2 days of the experiment.

The majority of OTUs and sequences represented Bacteriodetes, Betaproteobacteria, and Verrucomicrobia (Figure 4.4). Other studies (Boucher et al., 2006, Pohlon et al., 2009, Besemer et al., 2007, Besemer et al., 2012) have found that Proteobacteria (especially Betaproteobacteria and Alphaproteobacteria) and Bacteroidetes dominate bacterial biofilms in freshwater but relatively few have documented dominance of Verrucomicrobia members in freshwater biofilms (but see Boucher et al., 2006). Fierer et al., (2013) found that Verrucomicrobia are very abundant in tallgrass prairie soils but remained underrepresented in previous studies because of sequencing or primer biases (Bergmann et al., 2011). Suspended, freshwater bacterial communities can be compositionally very similar to soil inoculum (Crump et al., 2012). If true, it is unsurprising that

the regional species pool in tallgrass prairie streams (i.e., bacteria in the water column) are dominated by species of Verrucomicrobia as well as Proteobacteria and Bacteroidetes that form, and continue to dominate, freshwater biofilms during succession. Although Bacteroidetes, Betaproteobacteria, and Verrucomicrobia were most dominant in bacterial communities, their frequency in abundance remained high and stable unlike other phyla (Figure 4.4, 4.5). Alphaproteobacteria, Gemmatimonadetes, and Planctomycetes increased two-fold by late stage succession whereas Cyanobacteria increased by over an order of magnitude over time (Figure 4.5). In general, bacterial succession may be characterized not only by taxa in Bacteroidetes and Betaproteobacteria, which have shown to be strong competitors in algal dominated biofilms (Besemer et al., 2009), but also by eventual increases in Gemmatimonadetes, Planctomycetes, and Cyanobacteria, with especially heightened abundances of Alphaproteobacteria.

In summary, our study suggests that microbial communities develop quickly and predictably over time, regaining ecosystem rates of productivity with biofilms becoming reliant upon autochthonous resources. Further, biofilm-associated bacteria converge to similar communities that may be influenced by stochastic processes, but are likely driven by determinism. Although we examined several microbial components over time (algal and bacterial abundance, ecosystem process rates, bacteria assemblages), additional research is needed to mechanistically link bacterial function and structure throughout ecological succession.

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Figure 4.1 Locations of the three pools sampled (sites 1 -3) during the study period. Sites are located at Konza Prairie Biological Station within a third-order reach of the Kings Creek basin.

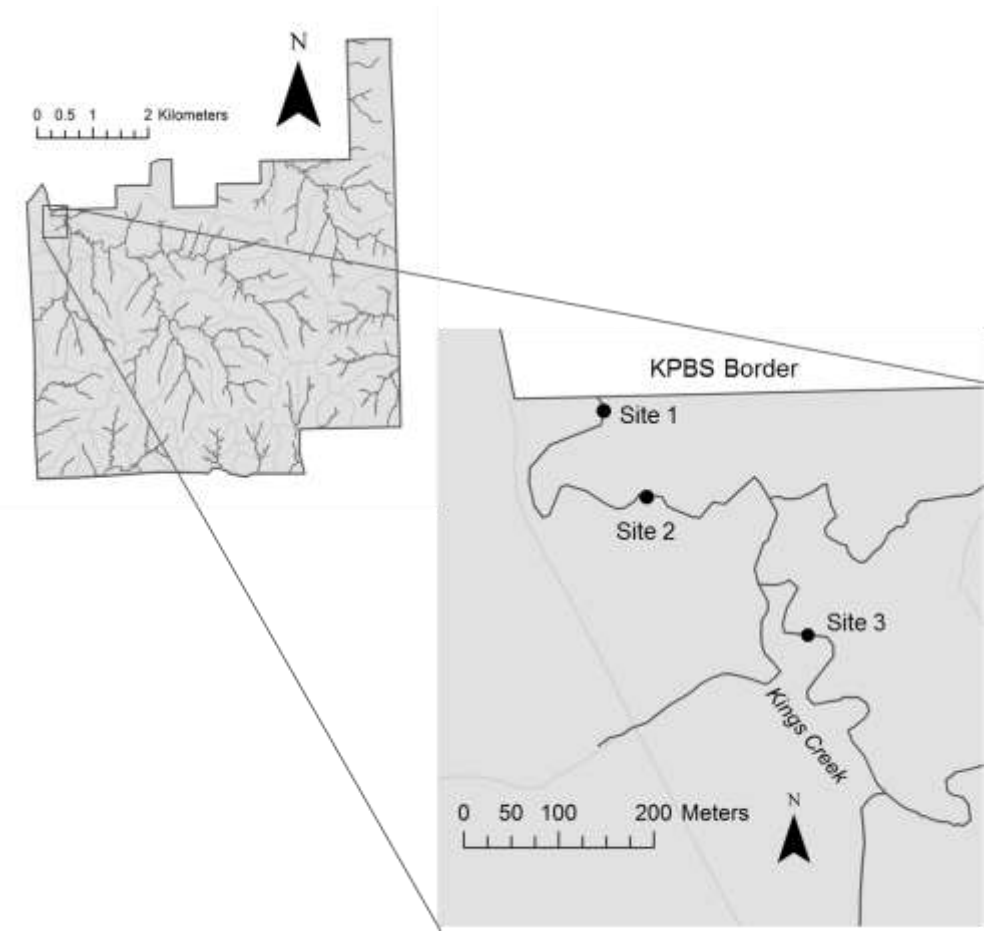

Figure 4.2 Algal biomass (chlorophyll *a;* Panel A) and bacterial cell abundance (Panel B) on tiles incubated in Kings Creek at Konza Prairie across time (2 – 64 days). Both measures of microbial abundance increased over time since placed in Kings Creek ($P < 0.01$, Adj. $R^2 \geq 0.67$). Both panels are displaying raw data. Regression statistics using transformed data are listed in Table 1.

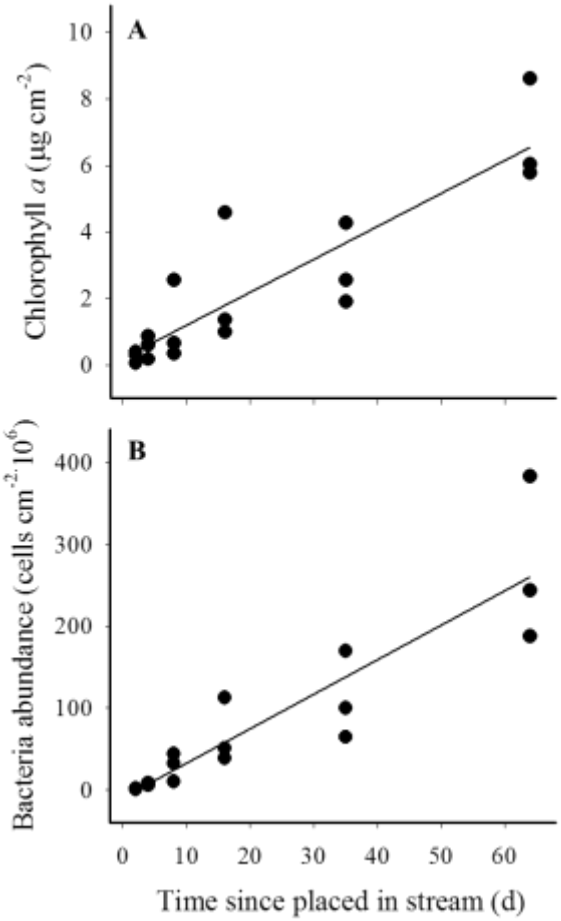

Figure 4.3 Gross primary productivity, community respiration (Panel A), and net ecosystem productivity (Panel B) measured on tiles incubated in Kings Creek across time (4 – 64 days). CR, GPP, and NEP increased over time ($P < 0.01$, Adj. $R^2 > 0.80$). Note that respiration signifies oxygen consumption therefore all values are negative. Different shapes represent the 3 sites samples (circles = site 1, triangle = site 2, square = site3). Regression statistics are listed in Table 1.

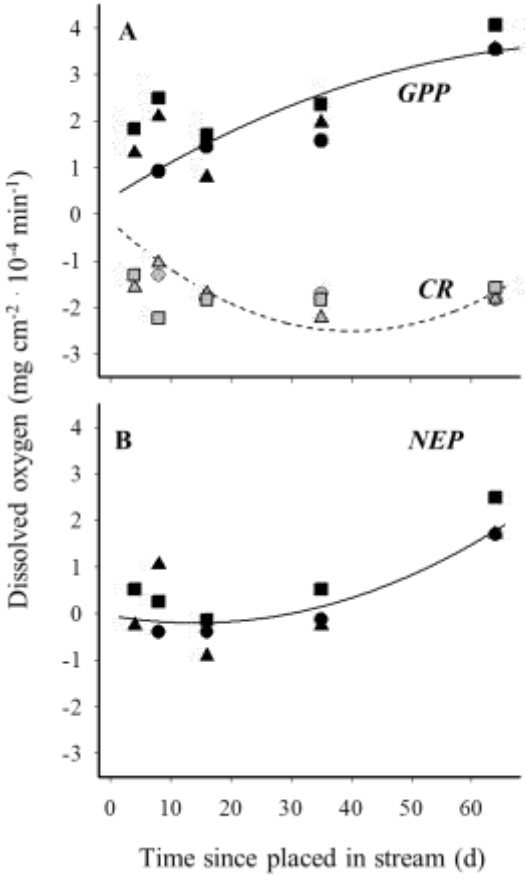

Figure 4.4 The proportion of sequences (A) and number of OTUs (B) designated to bacterial phyla across the experiment. Only phyla that represented ≥ 1% of all sequences or OTU's are included.

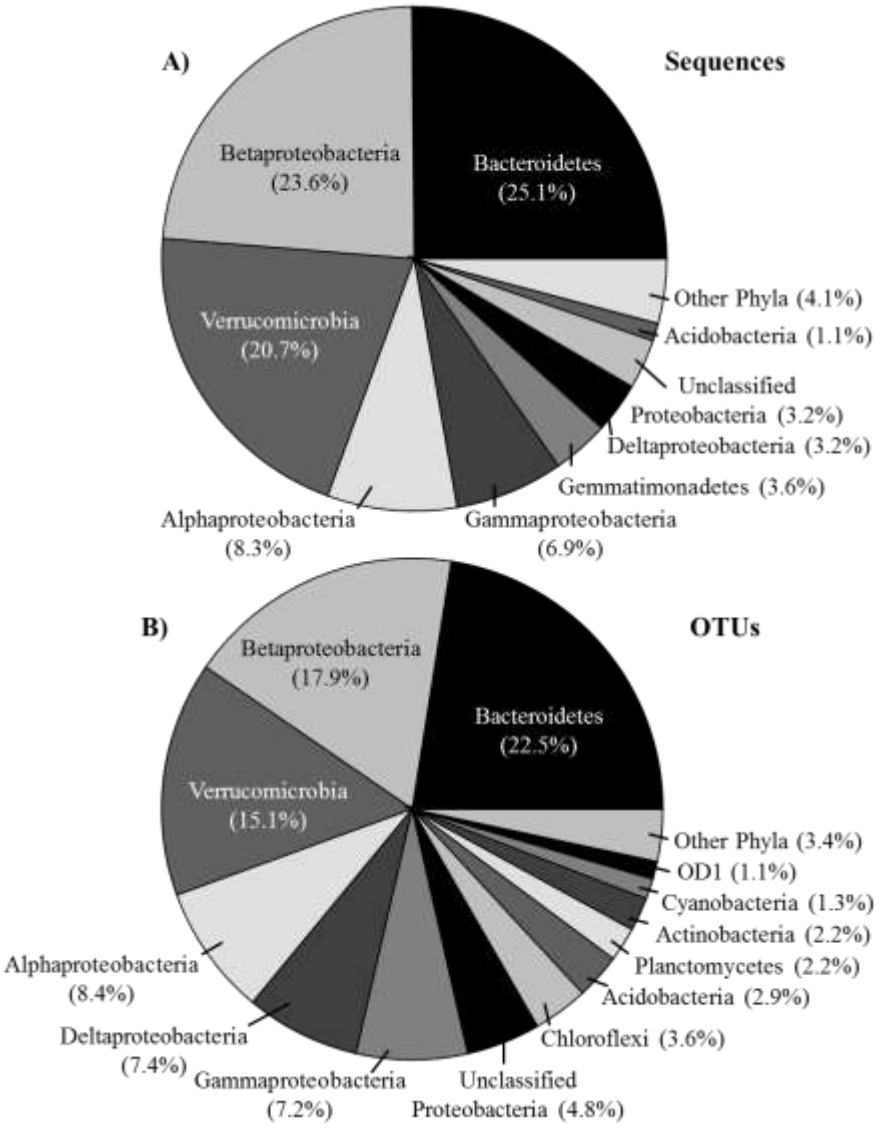

Figure 4.5 Relative abundance of dominant (≥ 1% of sequence abundance at any time point) bacterial phyla over time. Only phyla which exhibited statistically significant (after Bonferroni-correction) increases or decreases over time are displayed. Different shapes represent the 3 sites samples (circles = site 1, triangle = site 2, square = site3). Regression statistics are listed in Table 2.

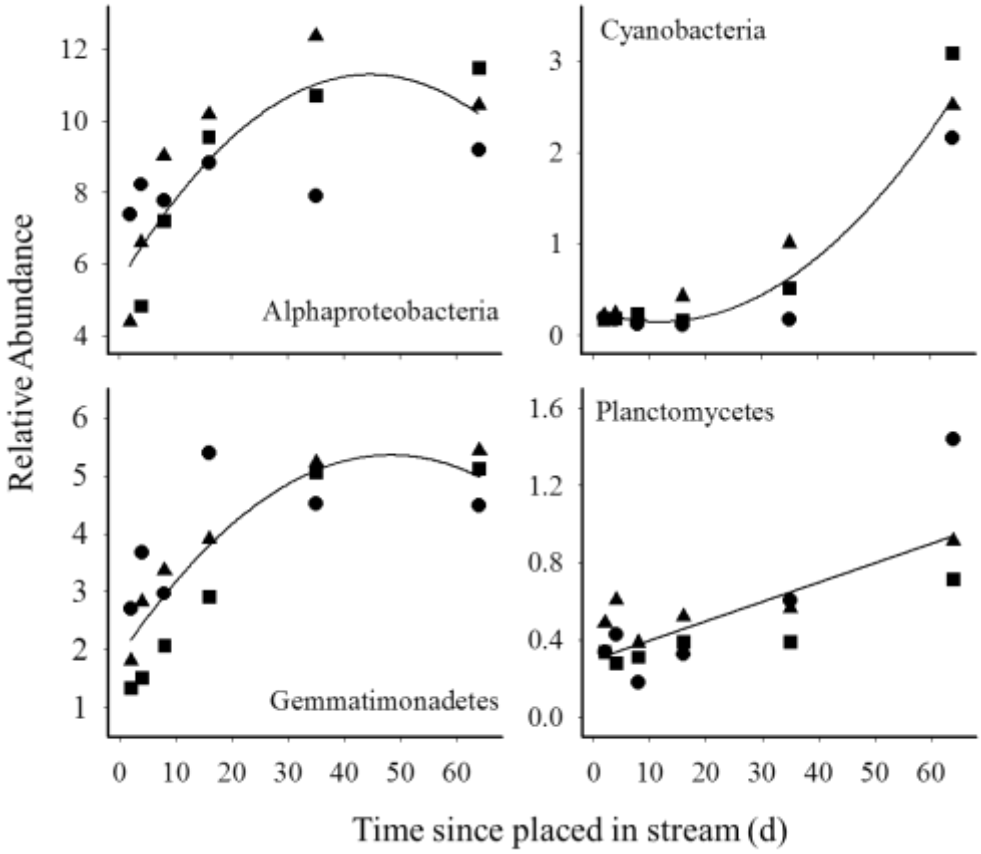

Figure 4.6 Non-metric dimensional scaling (NMDS) ordination of bacterial communities sampled across time (2D Stress = 9.58, cumulative variance represented = 86.3%; Panel A). Communities sampled at each time point are denoted by a gradient of black/gray color scheme (2 day communities are black, 64 day communities are white). Different shapes represent the 3 sites sampled (circles = site 1, triange = site 2, square = site 3). Bacterial community composition significantly changed over time based on NMDS 2 axis scores (P < 0.01, Adj. R^2 = 0.84; Panel B). Linear regression equation included in Panel B.

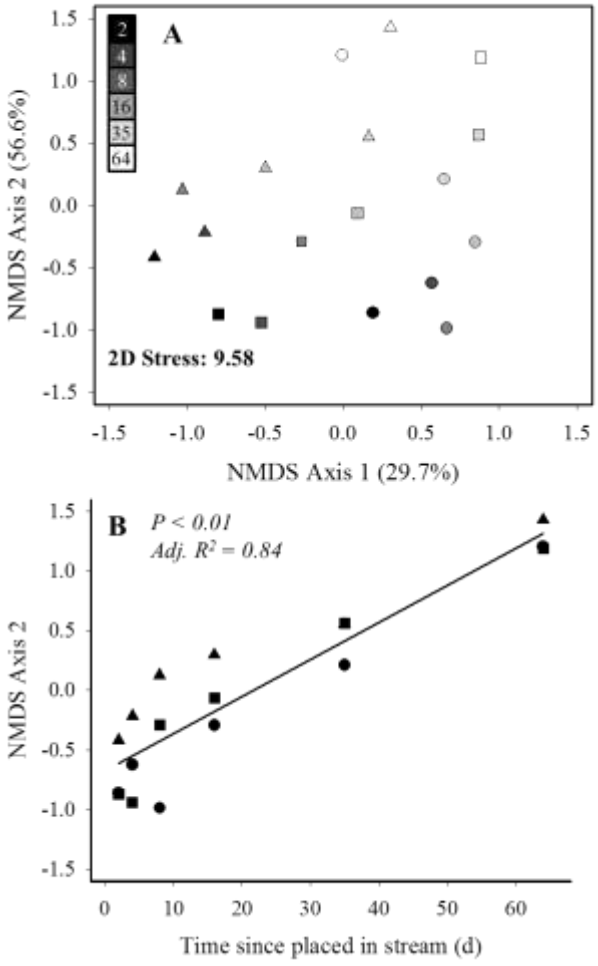

Table 4.1 Multiple regression model statistics for microbial abundance and biofilm metabolism data. Model results indicate all response variables change over successional time. Note that chlorophyll *a* and bacterial abundance data were log10 transformed.

				Full model statistics			
	Estimate	T-value	P-value	F-value	DF	Adj. R^2	P-value
Chlorophyll a				9.815	13	0.67	< 0.01
Intercept	-0.92	-2.93	0.01				
Time	0.05	2.98	0.01				
$Time^2$	$-4 \cdot 10^{-4}$	-1.97	0.07				
Site	0.16	1.2	0.25				
Site*Time	-0.001	-0.26	0.8				
Bacteria				12.29	13	0.73	< 0.01
Intercept	5.84	13.33	< 0.01				
Time	0.1	4.12	< 0.01				
$Time^2$	-0.001	-3.12	< 0.01				
Site	0.2	1.06	0.31				
Site*Time	-0.003	-0.45	0.66				
GPP				107.4	10	0.97	< 0.01
Time	$1 \cdot 10^{-6}$	-0.58	0.58				
$Time^2$	$6 \cdot 10^{-8}$	2.39	0.04				
Site	$7 \cdot 10^{-5}$	6.77	<0.01				
Site*Time	$-8 \cdot 10^{-7}$	-1.92	0.08				
CR				88.86	10	0.96	<0.01
Time	$-7 \cdot 10^{-6}$	-5.28	<0.01				
$Time^2$	$7 \cdot 10^{-8}$	2.82	0.02				
Site	$-5 \cdot 10^{-5}$	-5.86	<0.01				
Site*Time	$1 \cdot 10^{-6}$	$3 \cdot 10^{-7}$	0.01				
NEP				15.1	10	0.80	< 0.01
Time	$-6 \cdot 10^{-6}$	-3.36	<0.01				
$Time^2$	$1 \cdot 10^{-7}$	4.21	< 0.01				
Site	$2 \cdot 10^{-5}$	1.77	0.11				
Site*Time	$2 \cdot 10^{-7}$	0.55	0.59				

Table 4.2 Multiple regression model statistics for bacterial groups which differed in relative sequence abundance over successional time after Bonferroni-correction. Regression statistics for the same analysis for all other bacterial groups are found in Supplementary Table 3.

				Full model statistics			
	Estimate	T-value	P-value	F-value	DF	Adj. R^2	P-value
Alphaproteobacteria				11.11	13	0.7	< 0.003
Intercept	7.82	6.44	<0.01				
Time	0.17	2.46	0.03				
$Time^2$	-0.003	-3.37	<0.01				
Site	-1.19	-2.23	0.04				
Site*Time	0.05	2.74	0.02				
Cyanobacteria				111.5	13	0.96	< 0.003
Intercept	0.36	2.17	0.05				
Time	-0.04	-3.94	<0.01				
$Time^2$	$9 \cdot 10^{-4}$	7.64	< 0.01				
Site	-0.04	-0.55	0.6				
Site*Time	0.007	3.14	< 0.01				
Gemmatimonadetes				29	13	0.87	< 0.003
Intercept	3.77	9.09	<0.01				
Time	0.1	3.89	<0.01				
$Time^2$	-0.002	-4.5	<0.01				
Site	-0.94	-4.63	<0.01				
Site*Time	0.02	3.25	<0.01				
Planctomycetes				17.5	13	0.74	<0.003
Intercept	0.2	1.53	0.15				
Time	0.02	5.18	<0.01				
Site	0.05	0.85	0.41				
Site*Time	-0.006	-3	0.01				
Proteobacteria				18.31	13	0.75	<0.003
Intercept	3.24	10.48	<0.01				
Time	-0.002	-0.24	0.82				
Site	-0.32	-2.21	0.04				
Site*Time	0.01	3.02	<0.01				

Table 4.3 The 100 most abundant OTUs (represent ~ 60% of all sequences) that differ in frequency over time using linear regression models with Bonferroni-correction. The direction of change (increase or decrease in sequence frequency across time) is denoted by regression slope estimates (positive = increase, negative = decrease). Taxonomic affinities of the OTUs were determined by the Naive Bayesian Classifier with 100% bootstrap support. 1 denotes taxonomic affiliation to Family, 2 denotes affiliation to Genus. Four of the 13 OTU's only had high bootstrap support only at the Phylum level. * denotes an OTU within Proteobacteria which has the Class noted instead of only the Phylum.

OTU	P-value	T statistic	Adj. R^2	Slope	Taxonomic Affiliation	Phylum
3	$2.6 \cdot 10^{-4}$	-4.67	0.55	-0.08	Comamonadaceae[1]	Betaproteobacteria*
5	$4.3 \cdot 10^{-4}$	-4.14	0.52	-0.07	Comamonadaceae[1]	Betaproteobacteria*
10	$4.3 \cdot 10^{-5}$	-5.56	0.64	-0.17	*Flavobacterium*[2]	Bacteroidetes
12	$1.8 \cdot 10^{-5}$	6.02	0.67	0.28	Proteobacteria	Proteobacteria
16	$5.0 \cdot 10^{-4}$	4.35	0.51	0.18	*Gemmatimonas*[2]	Gemmatimonadetes
44	$5.2 \cdot 10^{-7}$	8.04	0.79	0.3	*Haliscomenobacter*[2]	Bacteroidetes
50	$1.1 \cdot 10^{-8}$	10.67	0.87	0.27	Bacteroidetes	Bacteroidetes
55	$3.2 \cdot 10^{-5}$	5.72	0.65	0.21	Bacteroidetes	Bacteroidetes
62	$4.7 \cdot 10^{-8}$	9.62	0.84	0.55	Bacteroidetes	Bacteroidetes
78	$6.0 \cdot 10^{-6}$	6.61	0.72	0.74	*Roseomonas*[2]	Alphaproteobacteria*
84	$2.7 \cdot 10^{-4}$	-4.64	0.55	-0.87	*Luteolibacter*[2]	Verrucomicrobia
85	$3.0 \cdot 10^{-6}$	7.0	0.74	0.64	*Runella*[2]	Bacteroidetes
91	$7.7 \cdot 10^{-8}$	9.28	0.83	0.62	*Luteolibacter*[2]	Verrucomicrobia

Chapter 5 – Conclusions

Prairie streams are affected by woody encroachment

Grasslands, and especially tallgrass prairies, are heavily impacted by the increase in woody plant cover. Although grasslands are traditionally described as having few shrubs and trees, and dominated by herbaceous plants and grasses, this may not be the case for future scenarios especially along stream channels. In Chapter 2, I used aerial imagery to define riparian corridors along stream networks at Konza Prairie and analyzed the spatial extent of woody plant cover within corridors in years 1985, 1991, and 2010. These analyses were done across 22 watersheds with differing fire frequencies and grazing regimes. In addition, annual water yield was calculated at four of these watersheds from1987-2010 to determine if water yield changed over time in response to increases in woody cover. We found that indeed high fire frequencies reduced the rate of riparian woody, vegetation expansion, but it did not cease it. Grazing had no detectable impact on the rate of riparian, woody expansion. Water yield had no detectable temporal trends. This work primarily highlights the importance of fire frequency on proliferation of woody plant species in riparian corridors of prairie ecosystems and suggests that it is essential for managers to consider frequent burns to maintain a native prairie state.

Riparian, woody plant removals affect microbes

By executing mechanical removals of riparian, woody vegetation, I found that removals cause changes to edaphic conditions, primarily by decreasing NH_4^+ and soil organic matter (Chapter 3). Removal treatments also caused fungal communities across stream and terrestrial soils to exhibit similar community richness patterns, unlike intact woody areas. Removals also had higher abundance of Pleosporales (Ascomycota) and lower abundance of Agaricomycetes

(Basidiomycota) suggesting that removing woody plant species may also be removing ectomycorrhizal fungal symbionts.

Spatiotemporal variability in prairie microbial communities

Microbial diversity (e.g., bacteria, fungi) and its fundamental drivers have been understudied and underestimated due to technological limitations until recent years. Further, freshwater ecosystems, especially streams, have not received as much attention for ecological study regarding microbes. In this dissertation, I assessed both spatial (Chapter 3) and temporal (Chapter 4) patterns of microbial community assembly in prairie streams.

I found that bacteria and fungi are structured by environmental filtering, but the specific mechanisms controlling this process are likely dependent on taxon specific (bacteria vs. fungi) ecological strategies. Bacterial richness and diversity were strongly influenced by the abiotic environment whereas fungal taxa were more sensitive to biotic interactions, responding more so to the removal of woody plant species (Chapter 3). Further, other research has suggested that terrestrial ecosystems hold the highest prokaryotic diversity compared to freshwater environments, but Chapter 3 results suggest that freshwater ecosystems may be as, if not more, rich and diverse as soils. This study is informative but warrants further research regarding the causal mechanisms acting upon bacterial and fungal community assembly over space.

I also found that microbial communities not only have strong associations with environmental conditions spatially, but also exhibit deterministic community development over time. By placing clean tiles within stream reaches at Konza Prairie and estimating microbial abundance, biofilm productivity, and bacterial community composition over time, I found that microbial communities exhibit strong successional trajectories, both compositionally and functionally (Chapter 4). Freshwater biofilms in prairie streams become net autotrophic over

successional time. Bacterial communities' exhibit spatial differences across sites, but stronger temporal trends in composition were detected. This work indicates that local processes, such as environmental filtering or biotic interactions are likely structuring microbial communities more so than stochastic processes.

Appendix A - Chapter 3 Supplementary Files

This section contains supplementary tables and figures for Chapter 3.

Supplementary Table 3.1 Twelve bp unique Multiplexing Identifiers (MIDs) used for each sample with DNA amplified for fungal communities. "Replicate" refers to transect number (1-4), "Position" refers to landscape position (Str = stream, Rip = riparian, Up = upslope).

Watershed	Replicate	Position	Treatment	MID
K2A	1	Str	Woody	TCCCTTGTCTCC
K2A	2	Str	Woody	ACGAGACTGATT
K2A	3	Str	Woody	GCTGTACGGATT
K2A	4	Str	Woody	ATCACCAGGTGT
K2A	1	Bank	Woody	TGGTCAACGATA
K2A	2	Bank	Woody	ATCGCACAGTAA
K2A	3	Bank	Woody	GTCGTGTAGCCT
K2A	4	Bank	Woody	AGCGGAGGTTAG
K2A	1	Rip	Woody	ATCCTTTGGTTC
K2A	2	Rip	Woody	TACAGCGCATAC
K2A	3	Rip	Woody	ACCGGTATGTAC
K2A	4	Rip	Woody	AATTGTGTCGGA
K2A	1	Up	Woody	TGCATACACTGG
K2A	2	Up	Woody	AGTCGAACGAGG
K2A	3	Up	Woody	ACCAGTGACTCA
K2A	4	Up	Woody	GAATACCAAGTC
K2A	1	Str	Removal	GTAGATCGTGTA
K2A	2	Str	Removal	TAACGTGTGTGC
K2A	3	Str	Removal	CATTATGGCGTG
K2A	4	Str	Removal	CCAATACGCCTG
K2A	1	Bank	Removal	GATCTGCGATCC
K2A	2	Bank	Removal	CAGCTCATCAGC
K2A	3	Bank	Removal	CAAACAACAGCT
K2A	4	Bank	Removal	GCAACACCATCC
K2A	1	Rip	Removal	GCGATATATCGC
K2A	2	Rip	Removal	CGAGCAATCCTA
K2A	3	Rip	Removal	AGTCGTGCACAT
K2A	4	Rip	Removal	GTATCTGCGCGT
K2A	1	Up	Removal	CGAGGGAAAGTC
K2A	2	Up	Removal	CAAATTCGGGAT
K2A	3	Up	Removal	AGATTGACCAAC
K2A	4	Up	Removal	AGTTACGAGCTA
N2B	1	Str	Woody	GCATATGCACTG

N2B	2	Str	Woody	CAACTCCCGTGA
N2B	3	Str	Woody	TTGCGTTAGCAG
N2B	4	Str	Woody	TACGAGCCCTAA
N2B	1	Bank	Woody	CACTACGCTAGA
N2B	2	Bank	Woody	TGCAGTCCTCGA
N2B	3	Bank	Woody	ACCATAGCTCCG
N2B	4	Bank	Woody	TCGACATCTCTT
N2B	1	Rip	Woody	GAACACTTTGGA
N2B	2	Rip	Woody	GAGCCATCTGTA
N2B	3	Rip	Woody	TTGGGTACACGT
N2B	4	Rip	Woody	AAGGCGCTCCTT
N2B	1	Up	Woody	TAATACGGATCG
N2B	2	Up	Woody	TCGGAATTAGAC
N2B	3	Up	Woody	TGTGAATTCGGA
N2B	4	Up	Woody	CATTCGTGGCGT
N2B	1	Str	Removal	TACTACGTGGCC
N2B	2	Str	Removal	GGCCAGTTCCTA
N2B	3	Str	Removal	GATGTTCGCTAG
N2B	4	Str	Removal	CTATCTCCTGTC
N2B	1	Bank	Removal	ACTCACAGGAAT
N2B	2	Bank	Removal	ATGATGAGCCTC
N2B	3	Bank	Removal	GTCGACAGAGGA
N2B	4	Bank	Removal	TGTCGCAAATAG
N2B	2	Rip	Removal	CATCCCTCTACT
N2B	3	Rip	Removal	TATACCGCTGCG
N2B	4	Rip	Removal	AGTTGAGGCATT
N2B	1	Up	Removal	ACAATAGACACC
N2B	2	Up	Removal	CGGTCAATTGAC
N2B	3	Up	Removal	GTGGAGTCTCAT
N2B	4	Up	Removal	GCTCGAAGATTC
N4D	1	Str	Woody	AGGCTTACGTGT
N4D	2	Str	Woody	TCTCTACCACTC
N4D	3	Str	Woody	ACTTCCAACTTC
N4D	4	Str	Woody	CTCACCTAGGAA
N4D	1	Bank	Woody	GTGTTGTCGTGC
N4D	2	Bank	Woody	CCACAGATCGAT
N4D	3	Bank	Woody	TATCGACACAAG
N4D	4	Bank	Woody	GATTCCGGCTCA
N4D	1	Rip	Woody	CGTAATTGCCGC
N4D	2	Rip	Woody	GGTGACTAGTTC
N4D	3	Rip	Woody	ATGGGTTCCGTC
N4D	4	Rip	Woody	TAGGCATGCTTG
N4D	1	Up	Woody	AACTAGTTCAGG

N4D	2	Up	Woody	ATTCTGCCGAAG
N4D	3	Up	Woody	AGCATGTCCCGT
N4D	4	Up	Woody	GTACGATATGAC
N4D	1	Str	Removal	GTGGTGGTTTCC
N4D	2	Str	Removal	TAGTATGCGCAA
N4D	3	Str	Removal	TGCGCTGAATGT
N4D	4	Str	Removal	ATGGCTGTCAGT
N4D	1	Bank	Removal	GTTCTCTTCTCG
N4D	2	Bank	Removal	CGTAAGATGCCT
N4D	3	Bank	Removal	GCGTTCTAGCTG
N4D	4	Bank	Removal	GTTGTTCTGGGA
N4D	1	Rip	Removal	GGACTTCCAGCT
N4D	2	Rip	Removal	CTCACAACCGTG
N4D	3	Rip	Removal	CTGCTATTCCTC
N4D	4	Rip	Removal	ATGTCACCGCTG
N4D	1	Up	Removal	TGTAACGCCGAT
N4D	2	Up	Removal	AGCAGAACATCT
N4D	3	Up	Removal	TGGAGTAGGTGG
N4D	4	Up	Removal	TTGGCTCTATTC

Supplementary Table 3.2 Twelve bp unique Multiplexing Identifiers (MIDs) used for each sample with DNA amplified for fungal communities. "Replicate" refers to transect number (1-4), "Position" refers to landscape position (Str = stream, Rip = riparian, Up = upslope).

Watershed	Replicate	Position	Treatment	MID
K2A	1	Str	Woody	TCCCTTGTCTCC
K2A	2	Str	Woody	ACGAGACTGATT
K2A	3	Str	Woody	TACCGCTTCTTC
K2A	4	Str	Woody	ATCACCAGGTGT
K2A	1	Bank	Woody	TGGTCAACGATA
K2A	2	Bank	Woody	ATCGCACAGTAA
K2A	3	Bank	Woody	GTCGTGTAGCCT
K2A	4	Bank	Woody	GATTATCGACGA
K2A	1	Rip	Woody	ATCCTTTGGTTC
K2A	2	Rip	Woody	GCCTAGCCCAAT
K2A	3	Rip	Woody	ACCGGTATGTAC
K2A	4	Rip	Woody	GATGTATGTGGT
K2A	1	Up	Woody	TGCATACACTGG
K2A	2	Up	Woody	AGTCGAACGAGG
K2A	3	Up	Woody	ACCAGTGACTCA
K2A	4	Up	Woody	GAATACCAAGTC
K2A	1	Str	Removal	GTAGATCGTGTA
K2A	2	Str	Removal	TAACGTGTGTGC
K2A	3	Str	Removal	ACTCCTTGTGTT
K2A	4	Str	Removal	CCAATACGCCTG
K2A	1	Bank	Removal	ACTTGGTGTAAG
K2A	2	Bank	Removal	TCACCTCCTTGT
K2A	3	Bank	Removal	CAAACAACAGCT
K2A	4	Bank	Removal	GCAACACCATCC
K2A	1	Rip	Removal	GCACACCTGATA
K2A	2	Rip	Removal	CGAGCAATCCTA
K2A	3	Rip	Removal	AGTCGTGCACAT
K2A	4	Rip	Removal	GCGACAATTACA
K2A	1	Up	Removal	CGAGGGAAAGTC
K2A	2	Up	Removal	TCATGCTCCATT
K2A	3	Up	Removal	AGATTGACCAAC
K2A	4	Up	Removal	AGTTACGAGCTA
N2B	1	Str	Woody	GCATATGCACTG
N2B	2	Str	Woody	CAACTCCCGTGA
N2B	3	Str	Woody	GAGAGCAACAGA
N2B	4	Str	Woody	TACGAGCCCTAA
N2B	1	Bank	Woody	CACTACGCTAGA

N2B	2	Bank	Woody	TGCAGTCCTCGA
N2B	3	Bank	Woody	ACCATAGCTCCG
N2B	4	Bank	Woody	TCGACATCTCTT
N2B	1	Rip	Woody	GAACACTTTGGA
N2B	2	Rip	Woody	GAGCCATCTGTA
N2B	3	Rip	Woody	TTGGGTACACGT
N2B	4	Rip	Woody	CGTGCTTAGGCT
N2B	1	Up	Woody	CACTCATCATTC
N2B	2	Up	Woody	TATCTATCCTGC
N2B	3	Up	Woody	TTGCCAAGAGTC
N2B	4	Up	Woody	CATACCGTGAGT
N2B	1	Str	Removal	TACTACGTGGCC
N2B	2	Str	Removal	GGCCAGTTCCTA
N2B	3	Str	Removal	GATGTTCGCTAG
N2B	4	Str	Removal	CTATCTCCTGTC
N2B	1	Bank	Removal	ACTCACAGGAAT
N2B	2	Bank	Removal	ATGATGAGCCTC
N2B	3	Bank	Removal	GTCGACAGAGGA
N2B	4	Bank	Removal	TGTCGCAAATAG
N2B	2	Rip	Removal	CATCCCTCTACT
N2B	3	Rip	Removal	ATGTGTGTAGAC
N2B	4	Rip	Removal	TTCTCTCGACAT
N2B	1	Up	Removal	ACAATAGACACC
N2B	2	Up	Removal	CGGTCAATTGAC
N2B	3	Up	Removal	GCTCTCCGTAGA
N2B	4	Up	Removal	GCTCGAAGATTC
N4D	1	Str	Woody	AGGCTTACGTGT
N4D	2	Str	Woody	TCTCTACCACTC
N4D	3	Str	Woody	ACTTCCAACTTC
N4D	4	Str	Woody	CTCACCTAGGAA
N4D	1	Bank	Woody	GTGTTGTCGTGC
N4D	2	Bank	Woody	CCACAGATCGAT
N4D	3	Bank	Woody	TATCGACACAAG
N4D	4	Bank	Woody	GATTCCGGCTCA
N4D	1	Rip	Woody	CGTAATTGCCGC
N4D	2	Rip	Woody	GGTGACTAGTTC
N4D	3	Rip	Woody	ATGGGTTCCGTC
N4D	4	Rip	Woody	TAGGCATGCTTG
N4D	1	Up	Woody	AACTAGTTCAGG
N4D	2	Up	Woody	ATTCTGCCGAAG
N4D	3	Up	Woody	AGCATGTCCCGT
N4D	4	Up	Woody	GTACGATATGAC
N4D	1	Str	Removal	GTGGTGGTTTCC

N4D	2	Str	Removal	ATGCCATGCCGT
N4D	3	Str	Removal	GACATTGTCACG
N4D	4	Str	Removal	ATGGCTGTCAGT
N4D	1	Bank	Removal	GTTCTCTTCTCG
N4D	2	Bank	Removal	CGTAAGATGCCT
N4D	3	Bank	Removal	GCGTTCTAGCTG
N4D	4	Bank	Removal	GTTGTTCTGGGA
N4D	1	Rip	Removal	GGACTTCCAGCT
N4D	2	Rip	Removal	CTCACAACCGTG
N4D	3	Rip	Removal	CTGCTATTCCTC
N4D	4	Rip	Removal	ATGTCACCGCTG
N4D	1	Up	Removal	TGTAACGCCGAT
N4D	2	Up	Removal	AGCAGAACATCT
N4D	3	Up	Removal	GCCAACAACCAT
N4D	4	Up	Removal	TTGGCTCTATTC

Supplementary Table 3.3 Linear regression statistics for edaphic variables and microbial richness, diversity, and evenness. Only edaphic variables selected after a stepwise selection via AIC minimization procedure are given. Fungal diversity and evenness was not correlated with any edaphic variable.

Model variables	Coef.	T value	P value
Bacteria			
Richness			
Intercept	616.31	2.83	< 0.01
NO_3^--N	-149.12	-4.15	< 0.01
Water	197.15	5.55	< 0.01
pH	54.66	1.98	0.05
Diversity			
Intercept	0.94	121.02	< 0.01
Water	0.01	6.78	< 0.01
C:N	< 0.01	2.36	0.02
pH	< 0.01	5.79	< 0.01
Evenness			
Intercept	0.01	0.95	0.34
NO_3^--N	< 0.01	2.04	0.05
Water	< 0.01	3.53	< 0.01
C:N	< 0.01	2.75	< 0.01
pH	< 0.01	2.45	0.02
Fungi			
Richness			
Intercept	747.3	4.17	< 0.01
TN	-71.56	-4.0	< 0.01
pH	-42.19	-1.86	0.07

Supplementary Table 3.4 The proportion of sequences and OTUs for both bacterial and fungal phyla across the experiment.

Phylum	Sequences (%)	OTUs (%)
Bacteria		
Bacteroidetes	19.25	9.09
Actinobacteria	14.89	3.98
Acidobacteria	14.19	4.30
Verrucomicrobia	9.27	3.95
Betaproteobacteria	5.14	1.28
Alphaproteobacteroa	4.70	4.17
Chloroflexi	4.15	11.30
Firmicutes	3.98	2.54
Deltaproteobacteria	3.77	6.81
Gammaproteobacteria	3.57	4.14
Planctomycetes	3.21	9.95
Unclassified Proteobacteria	3.04	3.11
Gemmatimonadetes	0.63	1.22
WPS2	0.39	0.48
Nitrospirae	0.35	0.13
Armatimonadetes	0.19	1.44
Latescibacteria	0.15	0.16
Chlamydiae	0.13	0.61
Cyanobacteria	0.11	2.22
Parcubacteria	0.06	1.64
Ignavibacteriae	0.01	0.13
BRC1	0.06	0.29
WPS1	0.01	0.45
Spirochaetes	0.01	0.26
Microgenomates	< 0.01	0.29
Epsilonproteobacteria	< 0.01	0.10
Hydrogenedentes	< 0.01	0.13
Saccharibacteria	< 0.01	0.22
SR1	< 0.01	0.10
Unclassified	8.73	25.52
Fungi		
Ascomycota	48.92	34.38
Basidiomycota	22.63	25.61
Zygomycota	14.64	1.25
Chytridiomycota	3.51	2.94
Glomeromycota	0.50	1.32
Unclassified	9.81	40.00

Supplementary Table 3.5 Linear regression statistics for edaphic variables and relative abundance of bacterial and fungal phyla. Only edaphic variables which were selected after a stepwise selection via AIC minimization procedure, and then deemed significantly correlated with relative abundance are given. Firmicutes and Ascomycota relative abundance was not correlated with any variable.

Phylum	Coef.	T value	P value
Bacteria			
Aphaproteobacteria			
Intercept	6.45	5.05	< 0.01
NH_4^+-N	1.31	2.98	< 0.01
Betaproteobacteria			
Intercept	4.79	0.99	0.32
Water	4.19	5.24	< 0.01
TC	-6.94	-2.22	0.03
C:N	0.26	2.04	0.05
pH	1.33	2.53	0.01
Deltaproteobacteria			
Intercept	0.85	14.94	< 0.01
NH_4^+-N	-0.11	-2.45	0.02
Water	0.27	4.72	< 0.01
C:N	< 0.01	2.67	< 0.01
Gammaproteobacteria			
Intercept	-7.16	-1.85	0.07
NH_4^+-N	1.96	3.89	< 0.01
pH	1.51	3.52	< 0.01
Acidobacteria			
Intercept	2.30	0.67	0.51
NH_4^+-N	2.86	2.49	0.02
Water	-8.61	-5.77	< 0.01
TC	11.74	2.27	0.03
TN	-13.4	-2.45	0.02
C:N	-0.62	-3.04	< 0.01
Actinobacteria			
Intercept	12.37	1.01	0.32
TC	-27.51	-3.46	< 0.01
TN	33.14	3.96	< 0.01
C:N	0.78	2.39	0.02
Bacteroidetes			
Intercept	9.52	7.98	< 0.01
NH_4^+-N	3.49	2.72	< 0.01

TC	1.11	2.0	0.05
Chloroflexi			
Intercept	8.66	2.78	< 0.01
NH_4^+-N	-4.21	-2.59	0.01
Water	4.41	2.17	0.03
TC	8.03	2.88	< 0.01
TN	-12.66	-3.57	< 0.01
Cyanobacteria			
Intercept	0.11	0.50	0.62
NH_4^+-N	-0.78	-4.42	< 0.01
NO_3^--N	-0.49	-5.52	< 0.01
Gemmatimonadetes			
Intercept	1.08	13.88	< 0.01
Water	-0.27	-2.18	0.03
TC	-0.15	-1.99	0.05
C:N	-0.02	-1.97	0.05
Nitrospirae			
Intercept	1.11	12.53	< 0.01
NH_4^+-N	-0.35	-4.46	< 0.01
Water	0.53	7.30	< 0.01
Planctomycetes			
Intercept	-1.33	-0.58	0.57
NH_4^+-N	1.42	3.64	< 0.01
Verrucomicrobia			
Intercept	41.05	8.88	< 0.01
NO_3^--N	0.92	2.09	0.04
Water	-1.86	-2.27	0.03
pH	-4.56	-7.59	< 0.01
Fungi			
Basidiomycota			
Intercept	-0.86	-1.3	0.20
NO_3^--N	-0.17	-2.13	0.04
TN	0.35	3.49	< 0.01
Chytridiomycota			
Intercept	0.11	3.04	< 0.01
pH	-0.01	-2.61	0.01
Zygomycota			
Intercept	-2.69	-4.71	< 0.01
Water	-0.71	-2.76	< 0.01

Appendix B - Chapter 4 Supplementary Files

This section contains supplementary tables and figures for Chapter 4.

Supplementary Table 4.1 Abiotic site characteristics (temperature, dissolved inorganic nitrogen, and turbidity) collected during sampling times. Multiple linear regression models indicate that no abiotic characteristics differed over time or across sites. Any missing values are denoted by n.a.

	Sampling time					
	2	4	8	16	35	64
Temperature						
Site 1	12.4	15.3	10.2	12	14.7	16.5
Site 2	13	16.5	9.9	12.3	14.7	17.5
Site 3	11.7	14.1	11.4	14.3	14.7	17.7
NO_3-N (μg/L)						
Site 1	52.9	250.8	121.1	188.7	37.7	75.1
Site 2	26.6	n.a.	69.4	139.5	29.1	29.6
Site 3	82.1	87.5	214.5	216.6	31.1	93.1
NH_4-N (μg/L)						
Site 1	5.3	91	n.a.	227.2	143.2	105.6
Site 2	70.5	41.9	83.7	66.9	59.1	89.4
Site 3	98.8	68.8	59	99.4	50.8	157.6
Turbidity (NTU)						
Site 1	396.8	n.a.	3.2	0	1.3	4.2
Site 2	563.1	n.a.	136.5	5.4	5.5	2.6
Site 3	3.1	n.a.	1.1	0.6	8.8	2.6

Supplementary Table 4.2 The 12 bp Multiplexing Identifier tags (MIDs) for each sample across time (2, 4, 8, 16, 35, 64 d), site (1, 2, 3), and replicate within a site (1, 2, 3) used in the secondary PCR.

Sample ID			
Time	Site	Replicate	MID Sequence
2	1	1	TACAGCGCATAC
2	1	2	TAACGTGTGTGC
2	1	3	CGAGCAATCCTA
2	2	1	CAACTCCCGTGA
2	2	2	GAGCCATCTGTA
2	2	3	GGCCAGTTCCTA
2	3	1	TATACCGCTGCG
2	3	2	ACTTCCAACTTC
2	3	3	ATGGGTTCCGTC
4	1	1	ACCGGTATGTAC
4	1	2	CATTATGGCGTG
4	1	3	AGTCGTGCACAT
4	2	1	TTGCGTTAGCAG
4	2	2	TTGGGTACACGT
4	2	3	GATGTTCGCTAG
4	3	1	AGTTGAGGCATT
4	3	2	CTCACCTAGGAA
4	3	3	TAGGCATGCTTG
8	1	1	AATTGTGTCGGA
8	1	2	CCAATACGCCTG
8	1	3	GTATCTGCGCGT
8	2	1	TACGAGCCCTAA
8	2	2	AAGGCGCTCCTT
8	2	3	CTATCTCCTGTC
8	3	1	ACAATAGACACC
8	3	2	GTGTTGTCGTGC
8	3	3	AACTAGTTCAGG
16	1	1	TGCATACACTGG
16	1	2	GATCTGCGATCC
16	1	3	CGAGGGAAAGTC
16	2	1	CACTACGCTAGA
16	2	2	TAATACGGATCG
16	3	1	ACTCACAGGAAT

16	3	2	CGGTCAATTGAC
35	1	1	AGTCGAACGAGG
35	1	2	CAGCTCATCAGC
35	1	3	CAAATTCGGGAT
35	2	1	TGCAGTCCTCGA
35	2	2	TCGGAATTAGAC
35	2	3	ATGATGAGCCTC
35	3	1	GTGGAGTCTCAT
35	3	2	TATCGACACAAG
35	3	3	AGCATGTCCCGT
64	1	1	ACCAGTGACTCA
64	1	2	CAAACAACAGCT
64	1	3	AGATTGACCAAC
64	2	1	ACCATAGCTCCG
64	2	2	TGTGAATTCGGA
64	2	3	GTCGACAGAGGA
64	3	1	GCTCGAAGATTC
64	3	2	GATTCCGGCTCA
64	3	3	GTACGATATGAC

Supplementary Table 4.3. Multiple regression model statistics for bacterial groups which did not change in relative sequence abundance over successional time after Bonferroni-correction. Note that full regression model's α must be ≤ 0.003 to be deemed statistically significant.

	Full model statistics			
	F-value	DF	Adj. R^2	P-value
Acidobacteria	1.54	13	0.08	0.25
Actinobacteria	4.65	13	0.39	0.02
Bacteroidetes	6.89	13	0.51	0.004
Betaproteobacteria	6.43	13	0.49	0.005
Chloroflexi	0.24	13	0.16	0.87
Deltaproteobacteria	2.06	13	0.16	0.51
Gammaproteobacteria	3.01	13	0.26	0.07

Supplementary Figure 4.1 The amount of precipitation received in Manhattan, KS during the study period (location of gage: Manhattan Airport). Site of precipitation data collection (courtesy of weather underground, www.wunderground.com) is approximately 7 miles northwest of Konza Prairie. Thirteen days after tiles were placed in stream, a 38.4 mm rainfall event caused sites to become temporarily connected.