Nutrient availability and organic matter quality shape bacterial community structure in a lake biofilm

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ABSTRACT: Heterotrophic bacteria play a key role in ecosystem processes, but little is known about the factors that shape bacterial community structure in aquatic biofilms, especially in lakes. We used molecular techniques (16S rRNA) to evaluate resource controls on biofilm bacterial community structure in an oligotrophic subalpine lake. We manipulated nutrients (nitrogen and phosphorus; NP) and glucose (G) on inorganic (rock) and organic (wood) substrates under light and dark conditions (i.e. with and without autotrophy, respectively) in a full factorial design using nutrient diffusing substrates *in situ* for 20 d. Distinct patterns of separation in community structure between treatments with nutrients (NP, NP+G) and without nutrients (control, G-only) indicated that community structure was more strongly influenced by nutrients than organic matter irrespective of substrate type or light availability. Further separation in community structure between treatments with nutrients only (NP) and nutrients with glucose (NP+G) on both organic and inorganic substrates indicated that once nutrient limitation was alleviated, organic matter quality played an important role in shaping community structure. Differences in the relative abundance of 6 phyla, 3 classes, and 19 genera among treatments revealed (1) contrasting taxa-specific resource requirements, (2) the influence of interspecific interactions on composition, and (3) the potential for individual taxa to participate in the decomposition of recalcitrant organic matter. Our findings provide insight into the role that nutrients and organic matter quality play in shaping bacterial community structure, which is a critical step in bridging the knowledge gap between microbial composition and ecosystem function within aquatic environments.

KEY WORDS: Benthic · Carbon · Heterotrophic bacteria · Lake · Substrate · 16S rRNA

1. INTRODUCTION

Benthic biofilms are an ecologically important component of aquatic ecosystems (Vadeboncoeur et al. 2002, Battin et al. 2016). Biofilms are widely recognized as hotspots of biodiversity and metabolic activity, especially in shallow environments where sufficient sunlight reaches the bottom (Battin et al. 2016). Along with microalgae (including cyanobacteria) and fungi, heterotrophic bacteria represent an impor-

tant component of benthic biofilms and are largely responsible for ecosystem processes such as community respiration and organic matter decomposition (Battin et al. 2008). Bacterial communities are typically diverse, and many of the biogeochemical processes carried out by bacteria are widely considered to be associated with taxonomic composition (Fuhrman 2009, Graham et al. 2016, Hall et al. 2018). Bacterial communities can also be sensitive to environmental change (Logue et al. 2015), and environmental per-

turbations that alter community diversity may indirectly alter their role in ecosystem function (Zeglin 2015). Despite improvements in the characterization of freshwater bacterial diversity (Logue & Lindström 2008, Newton et al. 2011, Veach et al. 2016) and known differences in function related to taxonomic composition (Zwart et al. 2015), work is needed to evaluate the factors that regulate the taxonomic composition of bacterial communities in freshwater biofilms (Besemer 2016).

Organic matter availability and composition are among the most important factors regulating heterotrophic bacteria in aquatic ecosystems (Eiler et al. 2003, Logue & Lindström 2008). Bacteria use organic carbon to fuel growth and metabolism, but their capacity to use different organic carbon compounds is strongly related to substrate quality (del Giorgio & Cole 1998, Hofmann & Griebler 2018). Autochthonous sources of carbon (e.g. algal-derived) are more easily degraded and therefore preferentially used by bacteria (Wyatt & Rober 2020) but are rarely produced in sufficient quantity to maintain metabolic demand (Rier & Stevenson 2002, Wyatt et al. 2012, Ward et al. 2017). Allochthonous forms of carbon (e.g. wood and terrestrially derived plant matter) tend to be more abundant but are composed of high-molecular weight compounds that are more difficult to degrade (i.e. recalcitrant) (Wilkinson et al. 2013). Owing to variable growth rates of different bacterial groups on different carbon substrates (Cottrell & Kirchman 2000), it stands to reason that organic matter quality also influences bacterial community composition (Kritzberg et al. 2006). Evidence that bacterial community structure is shaped by carbon quality derives primarily from studies of planktonic bacteria (Crump et al. 2003, Kritzberg et al. 2006, Jones et al. 2009, Ruiz-González et al. 2015, Muscarella et al. 2016). Relatively little is known about the effects of organic matter quality on bacterial community structure in aquatic biofilms (Olapade & Leff 2006, Besemer 2016).

The processing and fate of organic carbon by heterotrophic bacteria is frequently linked to nutrient availability (e.g. nitrogen [N] and phosphorus [P]). Heterotrophic bacteria require N and P to build biomass, and demand for nutrient assimilation often increases as the availability or quality of organic carbon decreases (Castillo et al. 2003, Nelson & Carlson 2011, Ghosh & Leff 2013). Consequently, the ability of bacteria to process more refractory forms of organic matter (e.g. wood) is often mediated by the availability of nutrients (Zhao et al. 2017, Jones et al. 2019). Likewise, nutrient uptake can be limited by

the quantity or quality of organic carbon (Stets & Cotner 2008, Rofner et al. 2017, Hofmann & Griebler 2018), which can influence the ability of bacteria to compete for nutrients (Olapade & Leff 2005, Leflaive et al. 2008, Pepe-Ranney & Hall 2015). Complex interactions among bacterial assemblages for available resources may be particularly evident in benthic biofilms, which are uniquely positioned to intercept resources at the interface of aquatic and terrestrial environments. For example, heterotrophic bacteria in benthic biofilms can access limiting resources through atmospheric deposition and surface-water runoff (Holtgrieve et al. 2011), reciprocal relationships with primary producers (Wyatt et al. 2019), or from the substrates to which they are attached (Romaní et al. 2004, Jones et al. 2019). Nevertheless, few studies have experimentally examined how changes in nutrient availability or carbon quality influence bacterial communities within biofilms, which is a critical step in understanding the relationship between community structure and ecosystem processes (Graham et al. 2016, Louca et al. 2018).

Evaluating how nutrients and organic matter shape benthic bacterial communities may be especially important in lakes because lakes play a critical role in the global carbon budget (Tranvik et al. 2009) and are sensitive to environmental change (Adrian et al. 2009). Connections between microbial diversity and the cycling of carbon and other limiting nutrients suggest that ecosystem-level responses to changing environmental conditions are mediated by the resident microorganisms (Nelson & Carlson 2011, Zeglin 2015). Surveys of bacterial diversity in lakes have largely focused on the pelagic zone (Newton et al. 2011, Zwart et al. 2015), despite the fact that the majority of the world's lakes are shallow with an abundance of benthic habitat (Downing et al. 2006). As a consequence, many of the bacterial taxa participating in critical ecosystem processes within benthic environments of lakes remain poorly described (Parfenova et al. 2013). Understanding these compositional dynamics has important implications for whole-lake metabolism and linkages between benthic and pelagic environments (Vadeboncoeur et al. 2002).

Our goal in this study was to evaluate the independent and interactive effects of nutrients and organic matter on bacterial community structure in a lake biofilm. To do this, we used a full factorial enrichment of nutrients and glucose on inorganic and organic substrates using nutrient diffusing substrates (NDS) and examined bacterial community structure with 16S rRNA based metabarcoding (Illumina MiSeq). Each treatment was replicated in both

light and dark conditions to evaluate bacterial community structure with and without photosynthesis, respectively. In a concurrent study, we evaluated how the availability of nutrients and organic matter interact to influence the relative biomass of autotrophic (algae) and heterotrophic (bacteria and fungi) components of the biofilm (Wyatt et al. 2019). Here, we evaluated the effects of resource composition and quality on bacterial community structure. We predicted that different bacterial taxa within the biofilm would exhibit variation in their response to available resources and that shifts in the relative abundance of taxa among treatments would allow us to infer differences in underlying functional properties (e.g. Ruiz-González et al. 2015).

2. MATERIALS AND METHODS

2.1. Site description

This study was conducted in Castle Lake, an oligotrophic subalpine lake in the Siskiyou Mountains of Northern California (41° 13′ N, 122° 22′ W; elevation: 1657 m). Approximately 54% of the lake surface area (0.2 km^2) is littoral zone with a depth of 3–5 m surrounding a basin with a maximum depth of 35 m (Vander Zanden et al. 2006). Water-column dissolved nutrient concentrations are typically <10 µg l^{-1} for N (NO$_3$-N + NH$_4$-N) and P (PO$_4$-P) (Higley et al. 2001). The high transparency of the lake during the ice-free season (Secchi depth range: 9–13 m) permits photosynthesis at depths up to 25 m (Higley et al. 2001). The extensive littoral habitat coupled with oligotrophic clear-water conditions provided a unique opportunity to evaluate the independent and interactive effects of nutrients and organic matter on bacterial community structure in an ecosystem with little anthropogenic disturbance.

2.2. Experimental design

NDS were used in a full factorial design with and without nutrients (N and P in combination; NP), with and without glucose (G), and with and without sunlight (light and dark conditions) to evaluate the effect of resource availability on heterotrophic bacterial community structure on both inorganic (rock) and organic (wood) substrates (n = 4 for each treatment combination). A detailed description and schematic of the experimental setup are presented elsewhere (Wyatt et al. 2019). Briefly, we filled 60 ml NDS canisters (LA Container) with either agar only (control treatment), 0.5 M glucose (G treatment), 0.5 M KNO$_3$ + 0.5 M KH$_2$PO$_4$ (NP treatment), or a combination of nutrients and glucose (NP+G treatment) in agar (Rier & Stevenson 2002, Tank et al. 2017). Our goal with enrichments was to alleviate resource limitation of the biofilm, and we assumed that previously reported diffusion rates (Rugenski et al. 2008, Wyatt et al. 2015) would be growth-saturating because they exceeded levels known to be growth limiting to biofilms (Wyatt & Turetsky 2015). Also, diffusion rates exceeded background concentrations of dissolved nutrients (25.6 ± 8.24 µg l^{-1} NO$_3$; 3.53 ± 0.27 µg l^{-1} PO$_4$) and organic carbon (6.80 ± 0.11 mg l^{-1}) in the lake at the time of the study. Glucose was selected as a labile source of organic carbon because it is readily used by heterotrophic bacteria (Olapade & Leff 2005, Wyatt & Turetsky 2015) and has been shown to comprise a large fraction of the total carbohydrate pool released by algal biofilms during photosynthesis (Wyatt et al. 2012). We covered NDS with either a sterilized fritted glass disc (i.e. rock) (Tank et al. 2017) or a 1 mm thick untreated wood veneer disc (Tank & Dodds 2003) to examine differences in community structure on inorganic and organic substrates, respectively (Wyatt et al. 2019). Rock and wood substrates were selected because they are both prevalent in the littoral zone of the lake. Additionally, drought-induced tree mortality has increased across western North America (Young et al. 2017), resulting in greater inputs of dead woody debris to alpine lakes that could alter substrate for biofilm development. Discs were secured by a tight-fitting cap that had a 2.5 cm diameter hole cut from the center to allow for biofilm colonization.

NDS were attached to 4 planks and anchored to concrete blocks that were submerged 25–30 cm below the surface along the southeast-facing shore, approximately 10 m from the shoreline. Each set of NDS were positioned 30 cm apart and arranged so that each treatment was represented only once per plank, as illustrated by Wyatt et al. (2019). Light-transparent (L) NDS (n = 32) were placed upright to receive ambient sunlight and dark (D) NDS (n = 32) were suspended upside down and loosely skirted with shade cloth that blocked >99% of incoming light to inhibit algal photosynthesis (Wyatt et al. 2019). The biofilm was allowed to colonize substrates undisturbed for 20 d during the summer growing season starting 28 June 2017. Access to either L or D NDS by consumers was not prohibited during the study and any consumptive effects were assumed to have occurred in all samples, which would be accounted for in final bacterial community composition.

2.3. DNA extraction and MiSeq library preparation

At the end of the experiment, we carefully removed NDS discs with forceps and stored them at −80°C in sterile 50 ml centrifuge tubes until they could be processed. To isolate heterotrophic bacteria within biofilms, we followed established procedures (Veach et al. 2016). Molecular grade water was added to the 50 ml centrifuge tubes containing the NDS discs, and established biofilms were removed from each substrate using a Branson 2800 sonicating water bath (Branson Ultrasonics) at maximum oscillation for 10 min to aid separation (Richter-Heitmann et al. 2016, Veach et al. 2016), followed by vortexing, and finally by physically scrapping any remaining biofilm into water using sterile, single-use scalpels. The detached biofilm slurry was centrifuged at 4°C (Sorvall Legend X1R; ThermoScientific) at $5000 \times g$ for 15 min to pellet biofilms after which the supernatant was discarded and samples were further centrifuged at $10\,000 \times g$ for 10 min. Excess supernatant was removed, pellets were resuspended in 250 µl molecular grade sterile water, and the entire volume was transferred to extraction tubes (DNeasy® PowerSoil® Kit; QIAGEN) and extracted following the manufacture's protocol. We included a negative control (molecular grade water instead of cell pellet) and extracted as above to control for reagent or laboratory contaminants. While it may be possible that some biofilm-associated lineages could have been under- or over-represented in our data due to methodological or amplification biases, all samples were processed similarly and any inadvertent biases would be systemic and not likely to affect the community-wide differences in taxonomic abundance seen here. Extracted DNA was quantified with a NanoPhotometer N60 (Implen) and normalized to a concentration of 2 ng µl^{-1} prior to PCR amplification.

The bacterial amplicon library was generated by amplifying the V4 region of the 16S rRNA gene repeat using a 2-step amplification process following Brown et al. (2018). The V4 region was amplified with the primers nexF-N[3-6]-515f and nexR-N[3-6]-806r, where 515f and 806r are universal bacterial primers, N[3-6] represents 4 identical primers with 3–6 ambiguous bps mixed to equal molarity, and nexF and nexR are the Nextera forward and reverse sequencing primers (Caporaso et al. 2012). Primary PCRs consisted of 50 µl reactions with 10 µl DNA template (20 ng), 10 µl 5× Phusion High-fidelity Buffer, 200 µM each deoxynucleoside triphosphate (dNTP), 0.5 µM of each forward and reverse primer, 0.5 µl Phusion HotStart II DNA Polymerase, and 15 µl molecular grade water. The PCR conditions were 98°C for 30 s, 25 cycles of 98°C for 20 s, 52.5°C for 30 s, and 72°C for 40 s, followed by a final extension at 72°C for 10 min; all ramp rates were set to 1°C s^{-1}. This resulted in a final primary PCR construct of nexF-N[3-6]-515f-{V4}-806r-N[3-6]-nexR. Amplification was confirmed using gel electrophoresis (1.5% agarose w:v in tris-borate-EDTA [TBE]).

Secondary PCRs were done in 25 µl reactions using forward and reverse primers that include the P5-i5-overlap and P7-i7-overlap, where P5 and P7 are the Illumina Adaptor sequences, i5 and i7 are 8 bp unique dual barcodes, and the overlap consists of partial nexF and nexR sequences that acts as the annealing sites for the secondary PCRs. The forward and reverse barcoded secondary primers were combined to generate unique dual barcoded primers at a working concentration of 10 µM (5 µM primer^{-1}). The secondary PCR reactions consisted of 2.5 µl of primary PCR product (template), 5 µl 5× Phusion High-fidelity Buffer, 200 µM each dNTP, 0.5 µM of mixed primers, 0.25 µl Phusion HotStart II DNA Polymerase, and 14.75 µl molecular grade water with the PCR conditions of 98°C for 30 s, 8 cycles of 98°C for 20 s, 50°C for 30 s, and 72°C for 40 s, followed by a final extension at 72°C for 10 min. This produced the final amplicon constructs of P5-i5-nexF-N[3-6]-515f-{V4}-806r-N[3-6]-nexR-i7-P7 with a total of 32 cycles. Secondary PCR products were cleaned using AMPure XP beads (Beckman Coulter) with a modification from the protocol whereby a 1:1 product:bead solution to volume ratio was used to maximize retention of larger PCR fragments (Brown & Jumpponen 2014). In addition to experimental samples, we included 2 different controls (molecular grade water prior to extraction and molecular grade water as template DNA for PCR) throughout and including sequencing. These remained free of visual amplification and largely free of any obtained sequences (negative controls were removed during bioinformatic processing suggesting that our sequences remained free of contaminants). Amplicons were quantified with Qubit 3.0 fluorometric assays (dsDNA HS Assay Kit) and pooled to equal concentrations and cleaned once more as above. The library was sequenced in one reaction of Illumina MiSeq (v2, 250PE) at the Kansas State University Integrated Genomics Facility in Manhattan, KS, USA. Demultiplexing of the raw data was conducted based on sequences of the i5 and i7 barcodes and individual paired 'fastq' files were retained (Table S1 in the Supplement at www.int-res.com/articles/suppl/a085p001_supp.pdf).

2.4. Bioinformatics

Sequences were processed with the program mothur v.1.39.5 (Schloss et al. 2009), following Kozich et al. (2013) with modifications. The forward and reverse reads were conjugated and sequences with any ambiguous bases were culled and trimmed to remove primer sequences. Retained sequences were aligned (SILVA release 132; www.arb-silva.de) and filtered to exclude non-16S V4 regions or poorly aligned reads. Sequences were pre-clustered (following Huse et al. 2010 as implemented in mothur), screened for chimeric reads (mothur implemented VSEARCH; Rognes et al. 2016), and identified putative chimeras were culled. Sequences were taxonomically classified using a mother-implemented naïve Bayesian classifier against the ribosomal database project training set (v.16; Wang et al. 2007). Non-target lineages were culled (including cyanobacteria) and a pairwise distance matrix for bacteria was created. Sequences were clustered into operational taxonomic units (OTUs) using OptiClust (3% dissimilarity; Westcott & Schloss 2017). OTUs with fewer than 10 global sequences were removed as their veracity is suspect (Brown et al. 2015). This removal of rare OTUs represented fewer than 1% of total sequence loss and each culled OTU represented less than $1.5 \times 10^{-7}\%$ total abundance and were not observed to be conditionally rare. However, to be certain that elimination of rare, potentially spurious OTUs would not drastically impact obtained results, we confirmed adequate community coverage with our subsampling depth (2455 sequences sample^{-1} with 1000 iterations) where Good's coverage (Good 1953) estimated an average (± SD) of 99.97% (± 1.00%) of entire community captures and Boneh estimates (Boneh et al. 1998) suggest that if we were to double our sequencing depth, we would only obtain an additional 3.2 OTUs. All negative controls were eliminated during sequence quality control. Demarcated OTUs were assigned taxon affinities based on the most representative sequence of the OTU (centroid). After all-sequence quality control, our library sequencing resulted in $\sim 6.2 \times 10^6$ high-quality reads.

2.5. Statistical analyses

Bacterial OTUs were iteratively subsampled 1000 times at a sequence depth of 2445 sequences using the 'phyloseq' package in R (McMurdie & Holmes 2013) to control for biases associated with unequal variance and to minimize loss of samples. Subsam-pling excluded one sample from a rock substrate in the control light treatment and a wood substrate in the G light treatment, leaving n = 3 for each of these treatment combinations. Non-metric multidimensional scaling (nMDS) was used to visualize communities across treatment combinations (light and dark were visualized separately) and was performed with 1000 iterations using the 'vegan' package (Oksanen 2018) based on Bray-Curtis dissimilarity values in R (R Core Team 2016). To test if communities differed among treatments, we used 999 permutations of pairwise comparisons using a permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) with Bonferroni's post hoc test using the 'ecodist' (Goslee & Urban 2017), 'vegan' (Oksanen 2018), and 'pairwiseAdonis' (Martinez 2019) packages in R. Relative observed OTU richness (S), the complement to Simpson's diversity ($1 - D$), and Simpson's evenness (E_D) were estimated across all treatment combinations using the 'phyloseq' (McMurdie & Holmes 2013) and 'microbiome' (Lahti et al. 2017) packages in R.

Four-way general linear models (GLMs) with Tukey's post hoc comparison of means tests were used to evaluate the effects of nutrients (with, without), G (with, without), substrate type (organic, inorganic), and light (light, dark) on diversity estimates. Four-way MANOVA and Tukey's post hoc comparison of means tests were performed to evaluate differences in the relative abundances of bacterial phyla and proteobacterial classes (α, β, γ-Proteobacteria). Only phyla and proteobacterial classes that were present at ≥5% relative abundance in at least one treatment were included in analysis, which included 6 phyla (*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Armatimonadetes*, *Deinococcus-Thermus*, *Gemmatimonadetes*) and 3 classes (α, β, γ-Proteobacteria). The relative abundance of genera that comprised ≥1% of all sequences were analyzed using 4-way MANOVA as described above and included 17 genera (*Aquabacterium*, *Aquitalea*, *Armatimonas/Armatimonadetes* Gp1, *Azorhizobium*, *Burkholderia*, *Chryseolinea*, *Curvibacter*, *Deinococcus*, *Dyella*, *Herbaspirillum*, *Lactococcus*, *Lactovum*, *Leuconostoc*, *Massilia*, *Pseudomonas*, *Rhizobium*, *Yersinia*) and 2 unclassified members of the order *Rhizobiales* and the family *Comamonadaceae*. When MANOVA indicated significant differences among treatments, Tukey's post hoc comparison of means tests were used to discriminate between treatments. Both GLM and MANOVA tests were performed using SPSS v.20. We also used linear discriminant analysis (LDA) effect size (LEfSe analysis) (Segata et al. 2011) to identify biomarker OTUs that were most likely to

explain differences among treatment combinations using the Galaxy web application (Blankenberg et al. 2010). Only the 50 most abundant OTUs were used for LEfSe analysis, and treatment combinations in light and dark conditions were analyzed separately.

3. RESULTS

3.1. Distribution of bacterial communities among treatments

The nMDS ordination analysis reflected patterns of variation in bacterial community structure among treatments (Fig. 1). We observed clear segregation in bacterial community composition between treatments with nutrients (NP, NP+G) and without nutrients (control, G-only) (PERMANOVA, pseudo-F = 3.26, p = 0.03; Table 1a). Nutrients alone explained 27% of the variation in bacterial community composition in the light (Table 1b) and 53% in the dark (Table 1c). Community composition was further separated between NP and NP+G treatments (PERMANOVA, p = 0.006), but there was no separation in bacterial community structure in the absence of nutrients (control, G-only) (PERMANOVA, p = 0.78; Fig. 1). Patterns of segregation were more pronounced in the dark (without autotrophy) than in the light, but light alone explained little of the variation in community composition (PERMANOVA, R^2 = 0.04; Table 1a). There was no segregation in bacterial community structure between rock and wood substrates in the light (PERMANOVA, pseudo-F = 2.05, p = 0.11; Table 1b, Fig. 1a), and minimal segregation in the dark (PERMANOVA, R^2 = 0.08, Table 1c, Fig. 1b).

3.2. Estimates of bacterial community diversity and evenness

Measures of S, $1 - D$, and E_D were highest in the absence of nutrient enrichment (control and G-only treatments) and declined in the presence of nutrients (with or without G), but were lowest in the NP+G
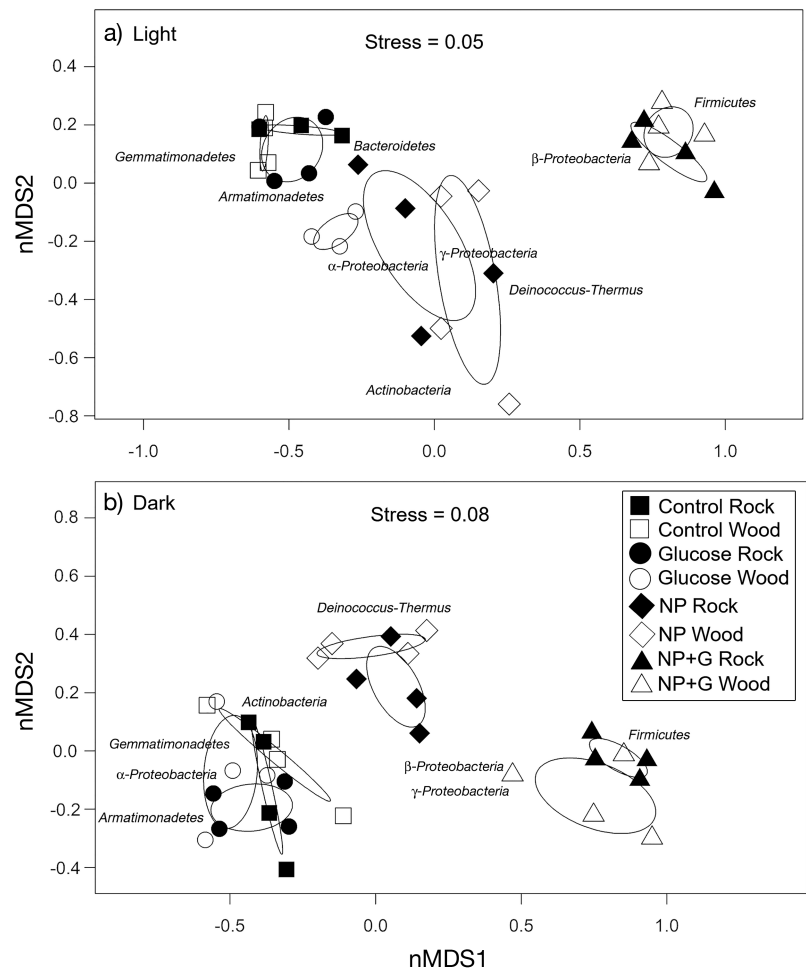

Fig. 1. Non-metric multidimensional scaling (nMDS) ordinations based on Bray-Curtis dissimilarly values illustrating bacterial community structure on nutrient diffusing substrates enriched with either agar only (control), glucose (G), nitrogen + phosphorus (NP), or combination of all 3 (NP+G) on inorganic (rock) and organic (wood) substrates in (a) light and (b) dark conditions. Ellipses represent the 95% CI of the mean surrounding each treatment combination. Stress values for light and dark ordinations show representation of the bacterial community in 3 dimensions and ranged from good (≤0.05) to acceptable (<0.1)

treatment (Fig. 2). Observed richness of OTUs was greater in the control (agar-only) and G-only treatments compared to NP or NP+G treatments (GLM, p ≤ 0.02; Fig. 2a). There was no effect of substrate type on observed OTU richness (GLM, p = 0.27) and observed OTU richness was lower in the dark compared to the light, but the magnitude of the difference decreased as observed richness declined (Fig. 2a). Taxonomic diversity was similar among control, G-only, and NP-only treatments but declined in the NP+G treatment and was not affected by light or substrate type (GLM, p ≥ 0.14; Fig. 2b). The evenness of OTUs in nutrient enriched treatments (NP or NP+G) was similar to the control treatment (GLM, p ≥ 0.07)

Table 1. Permutational multivariate analysis of variance results comparing bacterial community composition among treatments enriched with either agar only (control), glucose (G), nitrogen + phosphorus (NP), or combination of all 3 (NP+G) on inorganic (rock) and organic (wood) substrates. Analyses were conducted for (a) light and dark conditions combined, (b) light only, and (c) dark only. Pseudo-F: F-value by permutation; R^2 for Bray-Curtis distance matrices values were calculated using 999 permutations; **bold** indicates significant differences in taxonomic composition with $p < 0.05$ (lowest p-value possible: 0.001)

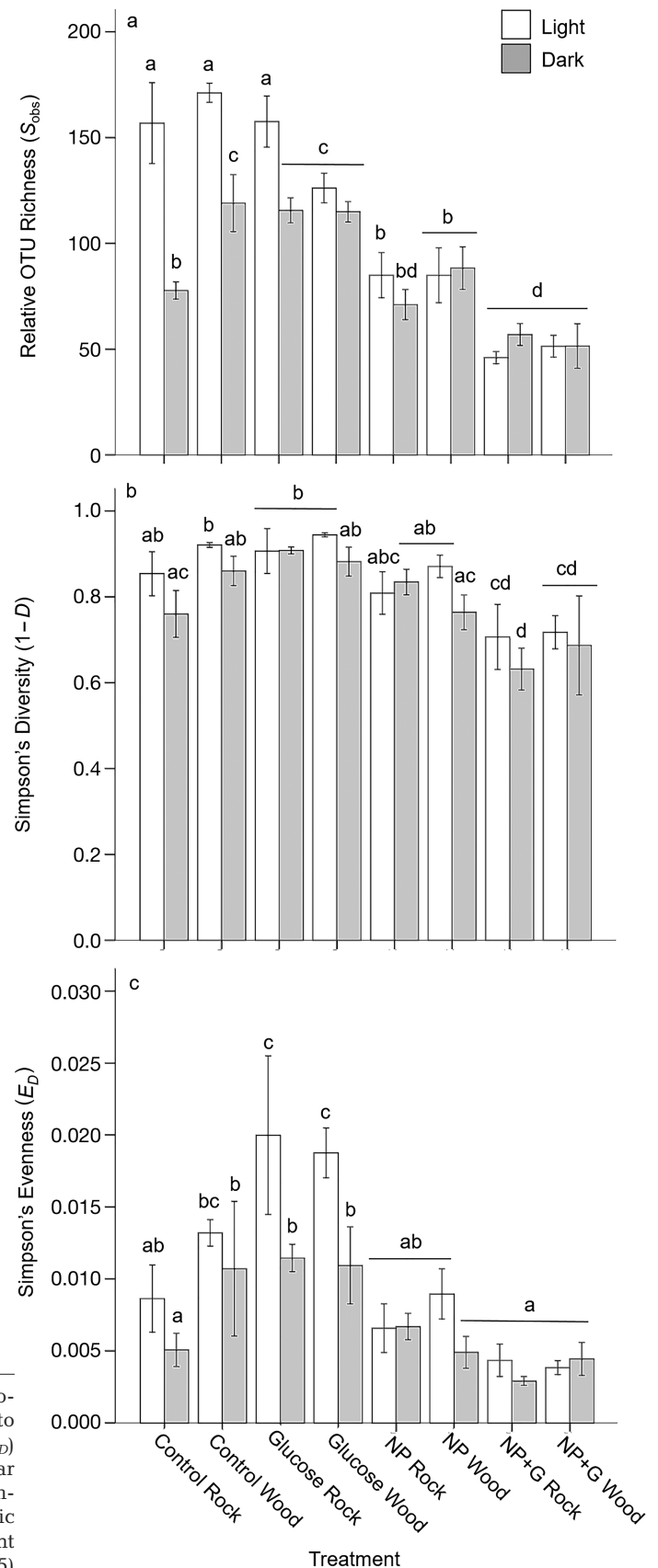| Source	df	SS	Pseudo-F	R^2	p
(a) Light and dark combined					
Substrate	1	0.26	5.94	0.03	**0.002**
Light	1	0.32	7.56	0.04	**0.002**
G	1	0.66	15.3	0.08	**0.001**
NP	1	2.62	60.9	0.33	**0.001**
Substrate × light	1	0.16	3.77	0.02	**0.013**
Substrate × G	1	0.06	1.32	0.01	0.243
Substrate × NP	1	0.13	3.03	0.02	**0.033**
Light × G	1	0.21	4.79	0.03	**0.008**
Light × NP	1	0.39	9.14	0.05	**0.001**
G × NP	1	0.69	15.9	0.09	**0.001**
Substrate × light × G	1	0.02	0.39	0.00	0.810
Substrate × light × NP	1	0.03	1.41	0.01	0.217
Substrate × G × NP	1	0.04	0.90	0.00	0.438
Light × G × NP	1	0.11	2.66	0.01	0.060
Substrate × light × G × NP	1	0.14	3.26	0.02	**0.032**
Residuals	46	1.98		0.25	
Total	61	7.84		1.00	
(b) Light only					
Substrate	1	0.10	2.05	0.03	0.109
G	1	0.57	11.9	0.16	**0.001**
NP	1	0.96	19.9	0.27	**0.001**
Substrate × G	1	0.05	1.02	0.01	0.387
Substrate × NP	1	0.16	3.25	0.04	**0.027**
G × NP	1	0.62	12.8	0.17	**0.001**
Substrate × G × NP	1	0.10	2.07	0.03	0.095
Residuals	22	1.07		0.29	
Total	29	3.63		1.00	
(c) Dark only					
Substrate	1	0.32	8.30	0.08	**0.002**
G	1	0.33	8.75	0.08	**0.002**
NP	1	2.09	55.0	0.53	**0.001**
Substrate × G	1	0.02	0.49	0.00	0.680
Substrate × NP	1	0.01	0.19	0.00	0.880
G × NP	1	0.16	4.30	0.04	**0.020**
Substrate × G × NP	1	0.08	2.04	0.02	0.120
Residuals	24	0.91		0.23	
Total	31	3.91		1.00	

Fig. 2. Mean (±1 SE) relative observed operational taxonomic unit (OTU) (a) richness (S) (b) the complement to Simpson's diversity ($1 - D$), and (c) Simpson's evenness (E_D) on nutrient diffusing substrates amended with either agar (control), glucose (G), nitrogen + phosphorus (NP), or combination of all 3 (NP+G) on inorganic (rock) and organic (wood) substrates in light and dark conditions. Significant differences are indicated by different letters (α = 0.05)
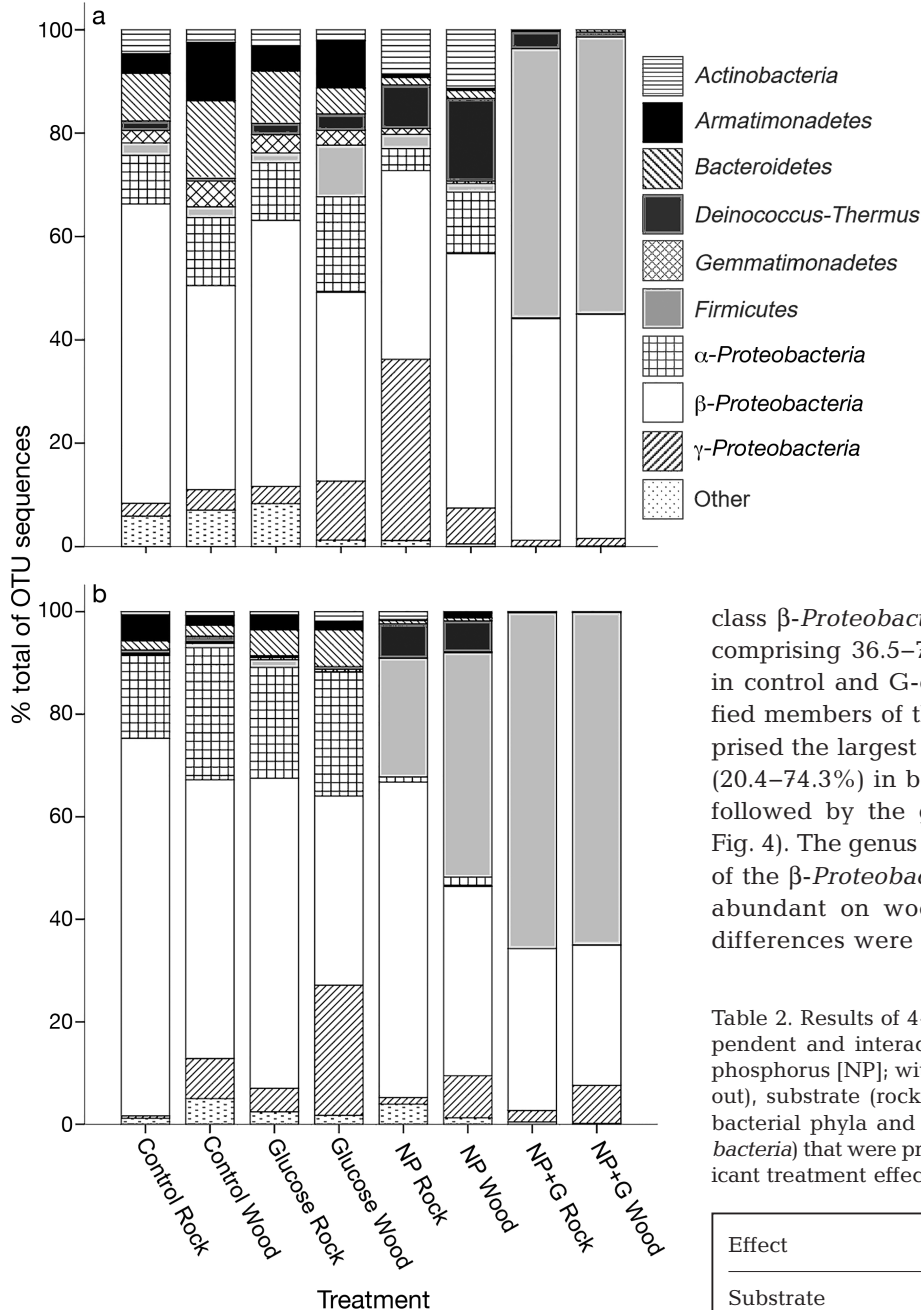
Fig. 3. Percent of total operational taxonomic unit (OTU) sequences of bacterial phyla and proteobacteria classes (α, β, γ-*Proteobacteria*) present at ≥5% relative abundance in at least one treatment combination on nutrient diffusing substrates amended with either agar (control), glucose (G), nitrogen + phosphorus (NP), or combination of all 3 (NP+G) on inorganic (rock) and organic (wood) substrates in (a) light and (b) dark conditions

class β-*Proteobacteria* was the most abundant taxa, comprising 36.5–73.6% of the bacterial community in control and G-only treatments (Fig. 3). Unclassified members of the family *Comamonadaceae* comprised the largest proportion of the β-*Proteobacteria* (20.4–74.3%) in both control and G-only treatments followed by the genus *Aquabacterium* (6.6–23.9%; Fig. 4). The genus *Curvibacter* comprised 22.0–41.7% of the β-*Proteobacteria* in the control and was more abundant on wood than rock substrates, though differences were only significant in the dark (MA-

Table 2. Results of 4-way MANOVA to determine the independent and interactive effects of nutrients (nitrogen and phosphorus [NP]; with or without), glucose (G; with or without), substrate (rock or wood), and light (light or dark) on bacterial phyla and proteobacterial classes (α, β, γ-*Proteobacteria*) that were present at >5% relative abundance. Significant treatment effects (p < 0.05) are indicated by **bold** font

Effect	df	F	p
Substrate	9	1.75	0.11
Light	9	7.19	**<0.0001**
G	9	9.53	**<0.0001**
NP	9	34.9	**<0.0001**
Substrate × light	9	2.31	**0.03**
Substrate × G	9	1.23	0.31
Substrate × NP	9	1.86	0.09
Light × G	9	3.40	**0.003**
Light × NP	9	9.64	**<0.0001**
G × NP	9	10.2	**<0.0001**
Substrate × light × G	9	0.87	0.56
Substrate × light × NP	9	2.52	**0.02**
Substrate × G × NP	9	1.41	0.22
Light × G × NP	9	1.40	0.22
Substrate × light × G × NP	9	1.93	0.08
Error	40		

but lower than the G-only treatment on rock and wood substrates in both light and dark conditions (GLM, p ≤ 0.01; Fig. 2c).

3.3. Bacterial community composition in the absence of nutrients

Bacterial community composition was similar between control (agar-only) and G-only treatments on rock and wood substrates regardless of light condition (MANOVA, $F_{9,40} = 0.87$, p = 0.56; Table 2). The
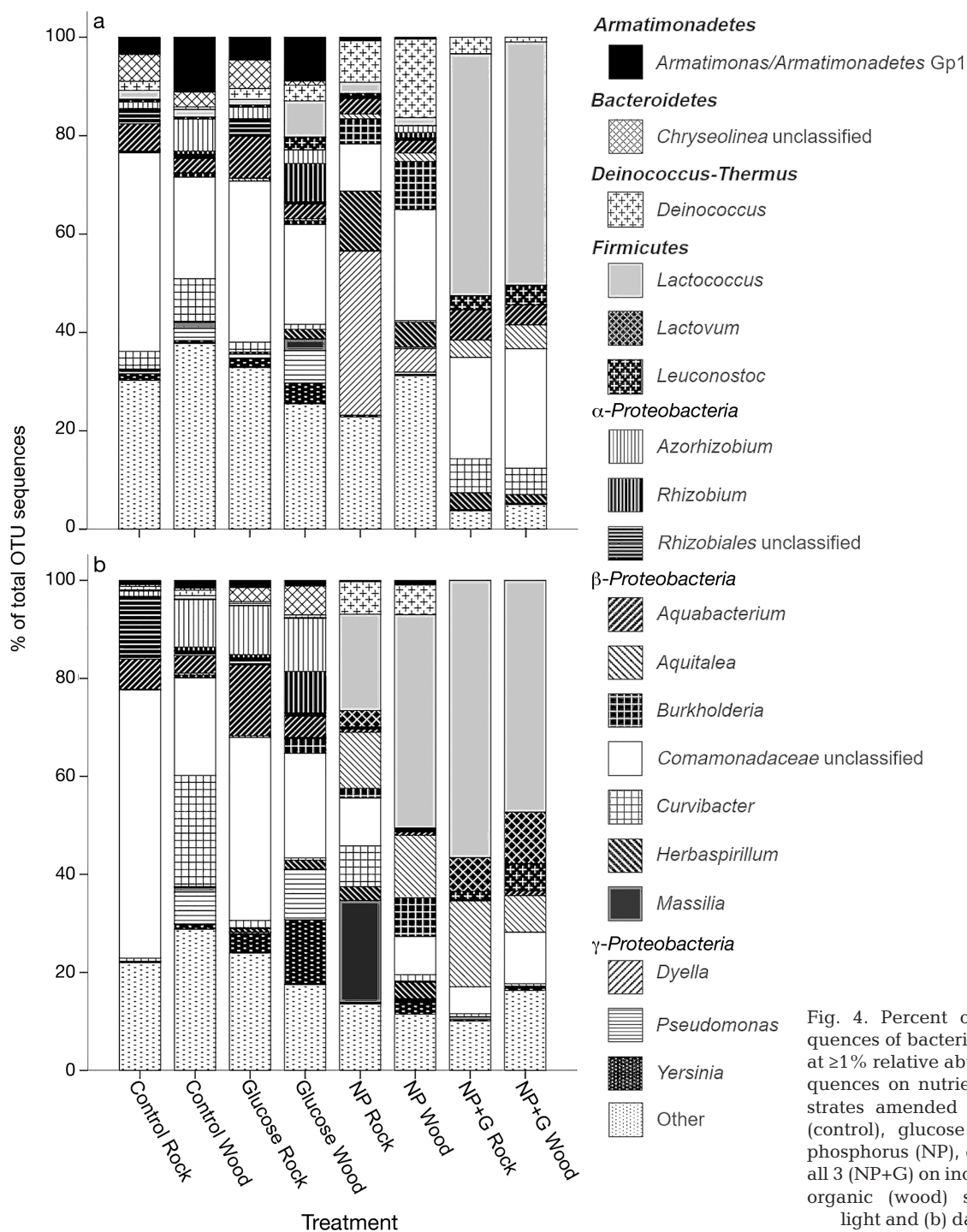
Fig. 4. Percent of total OTU sequences of bacterial genera present at ≥1% relative abundance of all sequences on nutrient diffusing substrates amended with either agar (control), glucose (G), nitrogen + phosphorus (NP), or combination of all 3 (NP+G) on inorganic (rock) and organic (wood) substrates in (a) light and (b) dark conditions

NOVA, p ≤ 0.0001; Fig. 4). The class α-*Proteobacteria* comprised 9.4–25.8% of the bacterial community in control and G-only treatments and was significantly greater in dark (control$_D$ and G$_D$) than light (control$_L$ and G$_L$) treatments (MANOVA, p ≤ 0.01; Fig. 3), which was primarily driven by an increase in the genus *Azorhizobium* (Fig. 4). The phylum *Armatimonadetes* (genus *Armatimonas*) comprised 1.9–11.3% of the community in control and G-only treatments and was more abundant on wood than rock substrates in the light (control$_L$ and G$_L$) (MANOVA, p ≤ 0.02; Fig. 3). *Bacteroidetes* comprised 1.8–15% of the community on rock and wood substrates in both control and G-only treatments and was more abundant in the light (control$_L$ and G$_L$) than the dark (control$_D$ and G$_D$) (MANOVA,

$p \leq 0.06$; Fig. 3). The class γ-*Proteobacteria* comprised <10% of the community on rock and wood substrates in the control under both light and dark conditions (Fig. 3). G enrichment increased the relative abundance of γ-*Proteobacteria* 3 fold on wood substrates compared to the control, particularly in the dark (G_D) (MANOVA, $p \leq 0.01$; Fig. 3). Patterns of γ-proteobacterial abundance in the control treatment were driven by the genus *Pseudomonas*, whereas a combination of *Pseudomonas* and *Yersinia*

explained the increase in the relative abundance of γ-*Proteobacteria* in the G-only treatment, especially on wood substrates in the dark (G_D) (Fig. 4). Our LEfSe analysis identified biomarkers that differentiated control and G-only treatments, which included members of α-*Proteobacteria* (*Azorhizobium*, *Rhizobium*, *Sphingomonas*, *Xanthobacter*), *Gemmatimonadetes* (*Gemmatimonas*), β-*Proteobacteria* (*Ideonella*), and γ-*Proteobacteria* (*Pseudomonas*; Tables 3 & 4).

Table 3. Biomarker operational taxonomic units (OTUs) identified by linear discriminant analysis (LDA) effect size (LEfSe) in the light condition only. Listed biomarker OTUs were statistically significant at the α = 0.05 level. G: glucose; NP: nitrogen + phosphorus

Resource × substrate Biomarker OTU	LDA score (log 10)	Phylum/Class	Genus
Control × rock			
Otu00005	4.937	β-*Proteobacteria*	*Curvibacter*
Otu00015	4.977	*Armatimonadetes*	*Armatimonas/Armatimonadetes* Gp1
Otu00022	4.935	*Bacteroidetes*	*Sphingomonas*
Otu00023	4.670	*Gemmatimonadetes*	*Gemmatimonas*
Otu00037	4.284	α-*Proteobacteria*	*Caulobacter*
Otu00038	4.290	γ-*Proteobacteria*	*Pseudomonas*
Control × wood			
Otu00002	5.548	β-*Proteobacteria*	*Comamonadaceae* unclassified
Otu00031	3.013	β-*Proteobacteria*	*Paludibacterium*
Otu00033	4.288	α-*Proteobacteria*	*Xanthobacter*
Otu00039	4.188	*Armatimonadetes*	*Armatimonas/Armatimonadetes* Gp1
Otu00042	4.267	*Bacteroidetes*	*Chryseolinea*
G × rock			
Otu00014	4.490	α-*Proteobacteria*	*Rhizobiales* unclassified
Otu00035	3.795	α-*Proteobacteria*	*Asticcacaulis*
Otu00046	4.409	Unclassified bacterium	Unclassified bacterium
G × wood			
Otu00021	4.578	α-*Proteobacteria*	*Sphingomonas*
Otu00027	4.505	β-*Proteobacteria*	*Duganella*
Otu00028	4.663	α-*Proteobacteria*	*Rhizobium*
Otu00030	4.558	γ-*Proteobacteria*	*Pseudomonas*
Otu00049	4.315	*Bacteroidetes*	*Hymenobacter*
NP × rock			
Otu00012	5.069	β-*Proteobacteria*	*Herbaspirillum*
Otu00017	5.523	γ-*Proteobacteria*	*Dyella*
NP × wood			
Otu00008	5.181	*Deinococcus-Thermus*	*Deinococcus*
Otu00019	4.972	β-*Proteobacteria*	*Burkholderia*
Otu00026	4.109	γ-*Proteobacteria*	*Salmonella*
Otu00032	4.936	*Actinobacteria*	*Curtobacterium*
Otu00048	4.322	*Actinobacteria*	*Microbacteriaceae* unclassified
NP+G × rock			
Otu00004	4.675	β-*Proteobacteria*	*Aquitalea*
Otu00018	4.540	β-*Proteobacteria*	*Comamonadaceae* unclassified
Otu00024	4.702	β-*Proteobacteria*	*Aquabacterium*
NP+G × wood			
Otu00001	5.693	*Firmicutes*	*Lactococcus*
Otu00003	4.843	β-*Proteobacteria*	*Comamonadaceae* unclassified

Table 4. Biomarker operational taxonomic units (OTUs) identified by linear discriminant analysis (LDA) effect size (LEfSe) in the dark condition only. Listed biomarker OTUs were statistically significant at the α = 0.05 level. G: glucose; NP: nitrogen + phosphorus

Resource × substrate Biomarker OTU	LDA score (log 10)	Phylum/Class	Genus
Control × rock			
Otu00002	5.548	β-*Proteobacteria*	*Comamonadaceae* unclassified
Otu00003	5.207	β-*Proteobacteria*	*Comamonadaceae* unclassified
Otu00014	5.102	α-*Proteobacteria*	*Rhizobiales* unclassified
Otu00016	4.356	β-*Proteobacteria*	*Aquabacterium*
Otu00020	4.746	β-*Proteobacteria*	*Thauera*
Otu00036	4.617	*Armatimonadetes*	*Armatimonadetes* unclassified
Otu00045	4.337	β-*Proteobacteria*	*Uliginosibacterium*
Otu00049	3.687	*Bacteroidetes*	*Hymenobacter*
Control × wood			
Otu00005	5.353	β-*Proteobacteria*	*Curvibacter*
Otu00015	3.776	*Armatimonadetes*	*Armatimonas/Armatimonadetes* Gp1
Otu00037	4.542	α-*Proteobacteria*	*Caulobacter*
Otu00038	4.592	γ-*Proteobacteria*	*Pseudomonas*
Otu00039	3.884	*Armatimonadetes*	*Armatimonas/Armatimonadetes* Gp1
Otu00050	3.585	β-*Proteobacteria*	*Aquabacterium*
G × rock			
Otu00007	4.753	β-*Proteobacteria*	*Comamonadaceae* unclassified
Otu00011	5.023	β-*Proteobacteria*	*Aquabacterium*
Otu00021	4.339	α-*Proteobacteria*	*Sphingomonas*
Otu00022	3.707	*Bacteroidetes*	*Mucilaginibacter*
Otu00023	3.617	*Gemmatimonadetes*	*Gemmatimonas*
Otu00024	4.019	β-*Proteobacteria*	*Aquabacterium*
Otu00025	4.206	β-*Proteobacteria*	*Ideonella*
Otu00035	4.538	α-*Proteobacteria*	*Asticcacaulis*
Otu00042	4.107	*Bacteroidetes*	*Chryseolinea*
G × wood			
Otu00006	5.035	α-*Proteobacteria*	*Azorhizobium*
Otu00010	5.122	γ-*Proteobacteria*	*Yersinia*
Otu00027	4.487	β-*Proteobacteria*	*Duganella*
Otu00028	4.639	α-*Proteobacteria*	*Rhizobium*
Otu00030	4.693	γ-*Proteobacteria*	*Pseudomonas*
Otu00032	3.283	*Actinobacteria*	*Curtobacterium*
Otu00034	4.673	γ-*Proteobacteria*	*Pseudomonas*
Otu00040	4.580	α-*Proteobacteria*	*Rhizobium*
Otu00044	4.614	*Bacteroidetes*	*Chryseolinea*
Otu00048	3.853	*Actinobacteria*	*Microbacteriaceae* unclassified
NP × rock			
Otu00009	5.316	β-*Proteobacteria*	*Massilia*
Otu00018	4.705	β-*Proteobacteria*	*Comamonadaceae* unclassified
NP × wood			
Otu00008	4.751	*Deinococcus-Thermus*	*Deinococcus*
Otu00012	4.482	β-*Proteobacteria*	*Herbaspirillum*
Otu00019	4.798	β-*Proteobacteria*	*Burkholderia*
NP+G × rock			
Otu00001	5.750	*Firmicutes*	*Lactococcus*
Otu00004	5.239	β-*Proteobacteria*	*Aquitalea*
NP+G × wood			
Otu00026	4.566	γ-*Proteobacteria*	*Salmonella*
Otu00031	4.677	β-*Proteobacteria*	*Paludibacterium*
Otu00041	4.669	*Firmicutes*	*Leuconostoc*

3.4. Bacterial community composition with nutrients only

Bacterial community structure in the NP-only treatment was significantly different from treatments without nutrients (control, G-only) regardless of substrate type or light condition (MANOVA, $F_{9,40}$ = 34.9, p ≤ 0.0001; Table 2). Similar to the control, β-*Proteobacteria* was the most abundant taxa, comprising 36.5–61.5% of the bacterial community in NP_L and NP_D treatments (Fig. 3). The proportion of β-*Proteobacteria* was similar between rock and wood substrates in the light, but was 24.6% greater on rock than wood substrates in the dark (MANOVA, p = 0.02; Fig. 3). In contrast to the control, the β-*Proteobacteria* in the NP-only treatment were dominated by the genera *Burkholderia*, *Herbaspirillum*, *Aquitalea*, and *Massilia* (Fig. 4).

In the dark, NP enrichment promoted a 21.7–43.6% increase in the relative abundance of *Firmicutes* (genus *Lactococcus*) compared to the control (MANOVA, p ≤ 0.01), and the increase was 20% greater on wood than rock substrates (MANOVA, p ≤ 0.01; Figs. 3b & 4b). However, *Firmicutes* was significantly lower in the NP_L treatment compared to the NP_D treatment, comprising only 1.7–2.8% of the community on both rock and wood substrates (MANOVA, p ≤ 0.01; Fig. 3). The phyla *Deinococcus-Thermus* (genus *Deinococcus*) and *Actinobacteria* each comprised ≤16% of the bacterial community in the NP treatment, but were more elevated compared to the control (MANOVA, p ≤ 0.04), with each phylum comprising a greater proportion of the community in the NP_L compared to the NP_D, particularly on wood substrates (Fig. 3). The γ-*Proteobacteria* (genus *Dyella*) increased 3 fold on rock substrates in the NP_L treatment compared to the control (MANOVA, p ≤ 0.01), comprising 35% of the community but remained ≤10% in all other NP treatment combinations (Fig. 3). Biomarkers that characterized the NP treatment included members of β-*Proteobacteria* (*Herbaspirillum*, *Burkholderia*), γ-*Proteobacteria* (*Dyella*), and *Deinococcus-Thermus* (*Deinococcus*; Tables 3 & 4).

3.5. Bacterial community composition with nutrients and glucose in combination

Bacterial community structure in the NP+G treatment was different from all other treatment combinations regardless of substrate type or light condition (MANOVA, $F_{9,40}$ = 10.2, p ≤ 0.0001; Table 2) and

≥92% of the community was comprised of *Firmicutes* and β-*Proteobacteria* (Fig. 3). The increase of *Firmicutes* was primarily driven by the genus *Lactococcus*, which comprised 47.3–56.5% of the community on rock and wood substrates in both $NP+G_L$ and $NP+G_D$ treatments (Fig. 4). The greater relative abundance of *Firmicutes* in the $NP+G_D$ treatment compared to the $NP+G_L$ was due in part to an increase in the genus *Lactovum* (6.9–10.5%), which was absent in the $NP+G_L$ treatment (Fig. 4). Biomarkers that explained differences between the NP+G treatment and all other treatment combinations included members of *Firmicutes* (*Lactococcus*, *Leuconostoc*), and β-*Proteobacteria* (*Aquitalea*; Tables 3 & 4).

4. DISCUSSION

4.1. Distribution of bacterial communities among treatments

The goal of this study was to evaluate the independent and interactive effects of nutrients and organic matter on bacterial community structure in a lake biofilm. We observed clear separation in bacterial community structure between treatments with nutrients (NP, NP+G) and without nutrients (control, G-only) irrespective of light or substrate type. Given that bacterial community structure was similar between control and G-only treatments but was markedly different in the presence of nutrients alone, we concluded that community composition was more strongly influenced by nutrients than organic matter. This response was not unexpected given the oligotrophic nutrient status of the lake (Higley et al. 2001) and may explain why our findings differ from previous research showing that carbon availability, particularly low-molecular weight forms (e.g. glucose), has a stronger influence on bacterial community structure than nutrients in aquatic ecosystems (Eiler et al. 2003, Ruiz-González et al. 2015, Rofner et al. 2017). Yet, bacterial community structure was also distinct between treatments with nutrients only (i.e. NP) and treatments with combined nutrients and G (NP+G), whereas there was little effect of wood (recalcitrant carbon) on community structure with or without nutrients. This finding suggests that once nutrient limitation was alleviated, organic matter quality played an important role in shaping bacteria community structure. Previous research has shown that resource availability and substrate quality regulate bacterial abundance and metabolism (Cottrell & Kirchman 2000, Castillo et al. 2003, Eiler et al. 2003,

Olapade & Leff 2006, Battin et al. 2016). Our findings show that nutrients and organic matter quality also play a key role in structuring bacterial communities in lake biofilms.

4.2. Bacterial community composition in the absence of nutrients

Taxonomic richness of the bacterial community was most elevated in the absence of nutrients. The presence of the phyla *Bacteroidetes* (*Chryselolinea*) and *Armatimonadetes* (*Armatimonas*) and classes α-*Proteobacteria* (*Azorhizobium* and *Rhizobiales*), β-*Proteobacteria* (*Aquabacterium*, *Comamonadaceae*, and *Curvibacter*), and γ-*Proteobacteria* (*Pseudomonas* and *Yersinia*) in low nutrient conditions is consistent with studies of bacterioplankton (Eiler et al. 2003, Hutalle-Schmelzer & Grossart 2009) and freshwater biofilms (Battin et al. 2016, Veach et al. 2016). Although we did not identify specific functional genes, many of the identified LEfSe biomarker OTUs were members of the α-*Proteobacteria* (*Azorhizobium*, *Rhizobium*, *Sphingomonas*, *Xanthobacter*), *Gemmatimonadetes* (*Gemmatimonas*) and γ-*Proteobacteria* (*Pseudomonas*) in control and G-only treatments. It has been shown that several of these taxa have *nifD* or *nifH* genes that code for enzymes involved in N-fixation (Gaby & Buckley 2014). The potential for these taxa to fix atmospheric N may explain their presence in low-nutrient conditions in our study. However, we found lower overall bacterial biomass in the absence of nutrients (i.e. control and G-only treatments) in a concurrent study (Wyatt et al. 2019), indicating that even if the taxa were capable of performing specific functions (e.g. N-fixation), they were not doing so at a rate that would generate a significant increase in biomass.

4.3. Bacterial community diversity and evenness in response to nutrient enrichment

Estimates of bacterial diversity (e.g. observed OTU richness) declined with shifts in community composition in response to nutrient enrichment. Lower diversity estimators in both NP and NP+G treatments, irrespective of light or substrate type, is suggestive of community homogenization within these treatments, possibly due to competitive exclusion or priority effects (Brown & Jumpponen 2015). Such reductions in bacterial community diversity and evenness likely have implications for lake ecosystem function (e.g.

biofilm productivity, stability, and resilience) (Naeem 2009). The decline in observed richness and evenness was coupled with an increase in members of the *Firmicutes* phylum, which was surprising since *Firmicutes* are rarely numerically dominant in freshwater ecosystems (Van der Gucht et al. 2005, Newton et al. 2011, Battin et al. 2016). While we did not measure the diversity of functional genes, previous research has shown that extreme dominance of a few taxa, as we observed following nutrient enrichment, decreases the likelihood that the remaining members of the community can perform the same functions, thereby reducing the potential for the community to resist environmental stress (Wittebolle et al. 2009). These findings may be especially applicable to similar undisturbed lakes in mountain regions owing to growing pressure on remote freshwaters from global changes, such as the deposition of atmospheric N (Holtgrieve et al. 2011) as well as drought-induced tree mortality (Young et al. 2017), which may alter the composition of substrates available for biofilm colonization (Czarnecka 2016).

4.4. Bacterial community composition with nutrients alone or in combination with glucose

In treatments amended with nutrients alone, *Firmicutes* comprised between 23 and 44% of the community in the dark (on both inorganic and organic substrates) but was nearly absent in the light (<5% relative abundance). This is notable because we found elevated algal biomass in this same treatment in a concurrent study, whereas photosynthesis was absent in the dark (Wyatt et al. 2019). Thus, the elevated abundance of *Firmicutes* in the dark (in the absence of photosynthesis) suggests that interspecific interactions within the biofilm community may have played a role in shaping community structure (Veach et al. 2016).

The potential antagonistic effects of autotrophs on *Firmicutes* were reduced in the presence of combined nutrient and G amendments where *Firmicutes* comprised 52–66% of the bacterial community in both light and dark conditions. The prevalence of *Firmicutes* in the presence of nutrients (with and without G) was largely driven by the genus *Lactococcus*, providing information about its resource requirements in natural environments where it has been relatively unexplored. Additionally, the genus *Lactovum* contributed to the greater proportion of *Firmicutes* in the NP+G_D treatment, but was absent from treatments with nutrients alone (in both light

and dark conditions), suggesting that it may have higher carbon requirements than *Lactococcus*. Yet, the similar abundance of *Lactovum* on both wood and rock substrates suggests that wood alone likely could not meet the carbon requirements of this taxon. Furthermore, the absence of *Lactovum* in the NP+G_L treatment suggests that it may not have been able to acquire the necessary nutrients or G in the presence of other members of the biofilm (algae or fungi).

4.5. Influence of substrate type

While substrate type did not have a significant effect on community structure at the phyla level, several genera within the phylum *Armatimonadetes* and classes α-, β-, and γ-*Proteobacteria* were more abundant on wood than rock substrates in certain treatment combinations. The genera *Armatimonas* (*Armatimonadetes*) and *Pseudomonas* (γ-*Proteobacteria*) were only present under low nutrient conditions and were more abundant on wood than rock substrates irrespective of G availability. The ability for these taxa to degrade high molecular weight compounds (e.g. cellulose and lignin) has been well documented in soils (Sun et al. 2014, Zhao et al. 2017), but our findings suggest that they may also play a role in the breakdown of recalcitrant organic matter in oligotrophic aquatic environments. Interestingly, *Armatimonas* was more abundant in the light, indicating that it was either competitively excluded in the dark or that other parts of the autotrophic biofilm community facilitated its growth in the light.

The α-*Proteobacteria Rhizobium* and γ-*Proteobacteria Yersinia* were more abundant on wood substrates but only in the G treatment. Previous research has shown that these taxa play an important role in the degradation of terrestrially derived organic matter (Logue et al. 2016, Tláskal et al. 2017), and our results suggest that their ability to use wood as a carbon substrate may be limited by labile carbon inputs. The β-*Proteobacteria Burkholderia* only occurred on wood substrates in the NP treatment, which coincided with elevated levels of fungal biomass (Wyatt et al. 2019). This suggests that, similar to soil environments (Sun et al. 2014, Johnston et al. 2016), members of the family *Burkholderiaceae* may be closely associated with wood-decaying fungi in lake biofilms, possibly through facilitatory associations where bacteria-derived N is used for fungal generation of wood-degrading enzymes (Brown et al. 2019). Although the β-*Proteobacteria Curvibacter* was more abundant on wood than rock substrates in the control, its relative abundance was similar to the NP+G treatment where there was no effect of substrate type.

Many of the genera that were abundant on wood in one treatment were absent from another, suggesting that these taxa are not necessarily specialized in the metabolism of allochthonous carbon but were instead augmenting their metabolism with wood in the absence of a more labile source (Jones et al. 2009). The limited number of taxa that appeared to use wood substrates in this study is consistent with research showing that the ability to process allochthonous carbon is limited to certain taxonomic groups (Jones et al. 2009, Logue et al. 2016). While we have related differences in bacterial community structure to variation in organic carbon content between rock and wood substrates, we cannot rule out differences in substrate texture (e.g. roughness) between rock (i.e. fritted glass disc) and wood substrates as a potential control on colonization (Romaní et al. 2004). Nevertheless, our findings suggest that these taxa may play a role in the breakdown of allochthonous organic matter entering lake ecosystems (Zhao et al. 2017, Jones et al. 2019).

4.6. Persistent and low-abundance taxa

The persistence of the β-*Proteobacteria* across treatment types (30–70% of the community) despite variation in resource availability and quality is consistent with findings that this taxon is widely distributed across freshwater ecosystems (Urbach et al. 2001, Hutalle-Schmelzer & Grossart 2009, Besemer 2016). The β-*Proteobacteria* in the control and G-only treatments were mainly comprised of the genera *Aquabacterium* and *Curvibacter* compared to the NP and NP+G treatments which had higher relative abundance of the genera *Aquitalea*, *Burkholderia*, *Herbaspirillum*, and *Massilia*. The β-*Proteobacteria* family *Comamonadaceae* was consistently ≥10% in relative abundance across treatment combinations, suggesting that it is likely a generalist capable of using both allochthonous (i.e. wood) and autochthonous organic matter (i.e. glucose) under both high and low nutrient availability. The prevalence of OTUs within the *Comamonadaceae* in our study is consistent with previous studies from oligotrophic Crater Lake (Urbach et al. 2001), Emerald Lake (Nelson & Carlson 2011), and Adirondack Lake (Methé et al. 1998). This connection is notable as each of these lakes are located at high elevations with relatively little direct human disturbance.

Though not numerically dominant, low-abundance taxa have been shown to play an important role in maintaining taxonomic diversity and ecological function within bacterial communities (Lynch & Neufeld 2015). We found lower relative abundances (≤15%) of *Actinobacteria* and *Bacteroidetes*, which are often reported to be numerically dominant in bacterioplankton studies (Van der Gucht et al. 2005, Jones et al. 2009, Newton et al. 2011) and in stream biofilms (Zeglin 2015, Battin et al. 2016, Besemer 2016). However, it is worth noting that *Actinobacteria* were also most elevated in the NP_L treatment, which contrasts with studies showing that elevated nutrient concentrations tend to select against *Actinobacteria* in other freshwater ecosystems (Haukka et al. 2006, Nelson & Carlson 2011). Similarly, the genus *Dyella* (*γ-Proteobacteria*) comprised 35% of the bacterial community on rock substrates in the NP_L treatment but was absent from all other treatments. The greater relative abundance of both *Actinobacteria* and the *γ-Proteobacteria Dyella* in the NP_L treatment only corresponded with elevated levels of algal biomass (Wyatt et al. 2019), suggesting that these taxa may have benefitted from carbon-rich algal exudates that were stimulated by nutrient enrichment (Rier & Stevenson 2002, Wyatt & Turetsky 2015). Lastly, the greater relative abundance of the phylum *Deinococcus-Thermus* in the NP treatment, irrespective of light or substrates type, hints toward a broader ecological role of this group, as there is little information regarding their ecology in freshwater ecosystems outside of hot springs (Battin et al. 2016).

4.7. Summary

Our findings highlight unique linkages between bacterial community composition and resource availability in a lake biofilm. Given that biofilms often live at the interface of aquatic and terrestrial environments and mediate the exchange of resources between benthic and pelagic habitats, it is not surprising that the bacterial communities in this study were comprised of members from both soil and planktonic lineages (Crump et al. 2012, Veach et al. 2016). Owing to the variable nature of organic matter inputs to lake ecosystems (i.e. terrestrial and algal-derived organic matter), a diverse bacterial community representative of both aquatic and terrestrial habitats may be better able to process these different types of organic matter that can be present in the environment at the same time (Crump et al. 2003, Jones et al. 2009). Our findings further suggest that lake trophic

status (oligotrophic vs. eutrophic) may influence the ability of bacteria in benthic biofilms to respond to carbon availability. Collectively, these findings provide valuable insight into the factors that regulate benthic bacterial community composition and present an opportunity to put current knowledge of microbial diversity within an ecological context (Naeem 2009, Hall et al. 2018, Louca et al. 2018, Kayler et al. 2019).

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