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RESEARCH ARTICLE

Microhabitats are associated with diversity–productivity relationships in freshwater bacterial communities

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ABSTRACT

Eukaryotic communities commonly display a positive relationship between biodiversity and ecosystem function (BEF) but the results have been mixed when assessed in bacterial communities. Habitat heterogeneity, a factor in eukaryotic BEFs, may explain these variable observations but it has not been thoroughly evaluated in bacterial communities. Here, we examined the impact of habitat on the relationship between diversity assessed based on the (phylogenetic) Hill diversity metrics and heterotrophic productivity. We sampled co-occurring free-living (more homogenous) and particle-associated (more heterogeneous) bacterial habitats in a freshwater, estuarine lake over three seasons: spring, summer and fall. There was a strong, positive, linear relationship between particle-associated bacterial richness and heterotrophic productivity that strengthened when considering dominant taxa. There were no observable BEF trends in free-living bacterial communities for any diversity metric. Biodiversity, richness and Inverse Simpson's index, were the best predictors of particle-associated production whereas pH was the best predictor of free-living production. Our findings show that heterotrophic productivity is positively correlated with the effective number of taxa and that BEF relationships are associated with microhabitats. These results add to the understanding of the highly distinct contributions to diversity and functioning contributed by bacteria in free-living and particle-associated habitats.

Keywords: diversity-productivity; biodiversity-ecosystem function; bacterial communities; microhabitats; particle-associated; limnology; heterotrophic productivity

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INTRODUCTION

In freshwater ecosystems, heterotrophic bacteria, despite their tiny size, regulate the freshwater carbon cycle by processing globally significant amounts of carbon (Cotner and Biddanda 2002; Tranvik *et al.* 2009; Cole 2013; Biddanda 2017). Therefore, it is essential to understand the factors that control microbial activity—the underlying context for why freshwater ecosystems serve as hot spots of carbon cycling. One of these factors is biodiversity, which has resulted in hundreds of studies that have evaluated the relationship between biodiversity and ecosystem function (BEF). Studies have found that there is an even larger biodiversity effect in natural ecosystems compared to controlled experiments (Duffy, Godwin and Cardinale 2017). BEF relationships focusing on the number of species (i.e. species richness) as the biodiversity value are generally positive and asymptotic and thus biodiversity loss causes a small change in ecosystem function at first and then, at some tipping point, a dramatic decrease in function (Cardinale *et al.* 2012; Tilman, Isbell and Cowles 2014). While this field was originally developed with a focus of on the local and global species loss of eukaryotic organisms, bacterial species can also go extinct (Louca *et al.* 2018) and species numbers have been found to be decreasing at local scales within the human gut (Blaser 2014) and terrestrial ecosystems (Singh *et al.* 2014). Of particular concern is the loss of the number of bacterial guilds responsible for key geochemical transformations, such as methane oxidation (Levine *et al.* 2011) that controls rates of methane emissions. Elucidating the relationship between bacterial biodiversity and its impact on ecosystem functioning can help inform the potential impact of species loss or decreased biodiversity on bacterial ecosystem function.

An extrapolation from eukaryotic relationships would predict there to be no richness-ecosystem function relationships for bacterial communities because they are generally composed of an order of magnitude more taxa than the communities in most eukaryotic BEF studies. Several studies have indicated no relationship between species richness with broad functional processes, such as heterotrophic respiration or biomass production, that are performed by many taxa (see Fig. 5 in Langenheder, Lindström and Tranvik 2006; Levine *et al.* 2011; Delgado-Baquerizo *et al.* 2016). Yet, other studies on broad processes such as denitrification (Philippot *et al.* 2013) and on narrow metabolic processes that are catalyzed by few bacterial taxa, such as methanotrophy (Levine *et al.* 2011), and the degradation of triclosan and microcystin (Delgado-Baquerizo *et al.* 2016) did find evidence of bacterial richness and ecosystem function relationships. In addition to the number of species, there are several other components of biodiversity including functional trait or gene richness (i.e. abundance-unweighted) (Reich *et al.* 2004; Flynn *et al.* 2011; Evans *et al.* 2017), species or functional dominance or evenness (i.e. abundance-weighted) (Wilsey and Potvin 2000; Wilsey and Polley 2004; Kirwan *et al.* 2007; Wittebolle *et al.* 2009) and phylogenetic diversity metrics (Flynn *et al.* 2011). Plant community evenness has been shown to impact plant productivity more than richness (Wilsey and Potvin 2000; Wilsey and Polley 2004; Kirwan *et al.* 2007) and the initial evenness of microbial microcosms has been found to be a key factor in functional stability, even under the selective stressors of temperature and salt stress (Wittebolle *et al.* 2009).

Phylogenetic relatedness, while having received less attention than richness and evenness, may also influence BEF relationships. Indeed, some studies show relationships across different ecosystems between phylogenetic diversity and ecosystem functions (Cadotte, Cardinale and Oakley 2008; Jiang, Tan and Pu 2010). However, research with freshwater green algae (Fritschie *et al.* 2014; Venail *et al.* 2014) did not find this relationship. A more recent study found the opposite result by reporting that closely related green algal species had weaker competition and more facilitation than distantly related species, thus resulting in higher productivity (Narwani *et al.* 2017). Relationships between phylogenetic relatedness among community members and ecosystem function have been assessed in bacterial systems (Tan *et al.* 2012; Galand, Salter and Kalenitchenko 2015; Roger *et al.* 2016), though it is worthwhile to expand these findings to more natural communities.

The nature of BEF relationships and the mechanism(s) that underpins them may depend on habitat structure or heterogeneity. Increasing habitat heterogeneity has been found to enhance the strength of BEF relationships (Tylianakis *et al.* 2008), presumably due to a greater role for niche complementarity effects in heterogeneous environments (Cardinale 2011). Species sorting dominates community assembly over stochastic forces with increased environmental heterogeneity because of more available niches (Lagenheder and Lindström 2019), which could facilitate complementary interactions within heterogeneous environments and thus, increase the strength of BEF relationships. While habitat heterogeneity contributes to increased diversity within bacterial populations and communities (Shade, Jones and McMahon 2008; Zhou *et al.* 2008), the influence of habitat heterogeneity on BEF relationships remains unknown for bacterial systems.

In this study, we hypothesized that bacterial diversity would be positively correlated with bacterial heterotrophic production, and that this relationship would be stronger in more heterogeneous environments. We simultaneously surveyed free-living and particle-associated surface water bacterial communities. These habitats have been extensively studied for their ability to sustain distinct bacterial communities and ecosystem processes (Crump, Armbrust and Baross 1999; Bižić-Ionescu *et al.* 2014; Mohit *et al.* 2014; Schmidt, White and Denef 2016; Wang *et al.* 2019). In addition, studies on model aggregates had 3-fold higher bacterial protein production and two orders of magnitude higher protease activity (Grossart *et al.* 2007), indicating particles can be an important place for microbial activity in aquatic ecosystems. Particulate matter comprises a variety of types and sizes of particles with some particles also harboring physicochemical gradients (Simon *et al.* 2002), and hence represents a more heterogeneous habitat than the surrounding water. We tested BEF relationships using a variety of the Hill numbers (^{q}D), which represent the effective numbers of taxa or lineages in a community and include richness (^{0}D), Shannon (^{1}D) and Simpson (^{2}D) diversity and their phylogenetic decompositions (i.e. ^{0}PD, ^{1}PD, ^{2}PD; Chao, Chiu and Jost 2010). We focused on heterotrophic bacterial production as our measure of ecosystem function, as it is a key process affecting freshwater bacterial growth that in turn fuels the eukaryotic food web (Cotner and Biddanda 2002).

METHODS

Lake sampling and sample processing

Surface water samples were collected at 1 meter depth from 4 long-term sampling stations (Steinman *et al.* 2008) in mesotrophic Muskegon Lake (Figure S1), which is a freshwater estuarine lake connecting the Muskegon River on its east side and Lake Michigan on its west side. These stations included the mouth of the Muskegon River (43.2501, −86.2557), the channel to Bear Lake (43.2387, −86.2992; a hypereutrophic lake), channel to Lake Michigan (43.2333, −86.3229; oligotrophic lake) and the deepest basin of Muskegon Lake (43.2239, −86.2972; max depth = 24 m).

Samples were collected during the morning to early afternoon of 3 days in 2015 (May 12, July 21 and September 30) aboard the *R/V W.G. Jackson*. All water samples were

collected with vertical Van Dorn samplers. Additionally, a vertical profile of temperature (T), pH, specific conductivity (SPC), oxidation-reduction potential (ORP), chlorophyll (Chl*a*), total dissolved solids (TDS) and dissolved oxygen (DO) was constructed at each station to characterize the water column using a calibrated YSI 6600 V2–4 multiparameter water quality sonde (Yellow Springs Instruments Inc.). Total Kjeldahl nitrogen (TKN), ammonia (NH_3), total phosphorus (TP) and alkalinity (Alk) were processed from whole water while nitrate (NO_3), phosphate (PO_4) and chloride (Cl^-) were hand filtered using a 60 mL syringe fitted with Sweeny filter holder with a 13 mm diameter 0.45 μm pore size nitrocellulose filters (Millipore) and were determined by standard wet chemistry methods in the laboratory (EPA 1993).

Bacterial abundance by epifluorescence microscopy

Lake surface water samples were processed within 2–6 hr of their collection for determination of heterotrophic bacterial abundance. Unfiltered lake water samples (5 mL) were preserved with 2% formalin and 1 mL subsamples were stained with acridine orange stain and filtered onto black 25 mm 0.2 μm pore size polycarbonate filters (Millipore) at a maximum pressure of 0.1 Bar or 1.5 PSI. Prepared slides were stored frozen until manual enumeration by standard epifluorescence microscopy at 1000x magnification under blue light excitation (Hobbie, Daley and Jasper 1977). Bacteria within the field of view (100 μm x 100 μm) that were not associated with any particles were counted as free-living bacteria, whereas bacteria that were on particles were counted as particle-associated. A total of 20 fields of view were counted for each sample. Sample filtration may bias counts due to free-living or particle-associated cells being hidden on the underside of particles, free-living bacteria settling on top of particles, or particle-associated cells dislodging. In the absence of any quantitative studies that have rigorously addressed this issue, we have assumed the net effect of these opposing methodological biases to be negligible in the present study.

Heterotrophic bacterial production measurements

Community-wide heterotrophic bacterial production was measured using [^{3}H] leucine incorporation into bacterial protein (Kirchman, K'nees and Hodson 1985; Simon and Azam 1989). Quadruplicate 1 m water samples were incubated in the dark under in situ temperatures for 1 hr with a 20 nM final concentration of [^{3}H]-leucine. One 50% trichloroacetic acid (TCA)-killed control was run for every three live incubations of the same sample. At the end of the incubation with [^{3}H]-leucine, cold TCA-extracted samples were filtered onto 3 μm filters that represented the leucine incorporation by particle-associated bacteria (>3.0 μm). Each filtrate was collected and filtered onto 0.2 μm filters and the activity therein represented incorporation of leucine by free-living bacteria (3-0.22 μm). The rate of uptake was linear over a 2 hr incubation period and the controls accounted for 0.5–6% of the [^{3}H]-leucine found in live treatments. On the basis of such repeatable linear uptake measurements over the representative period of the incubations, we presumed there was no measurable recirculation of incorporated [^{3}H] back into solution. The timeline for our incubations (1 hr), as well as the sensitivity of the [^{3}H] method, were insufficient to distinguish between the production rates of r- versus k-selected taxa. However, longer incubations would have likely led to problems of non-linear uptake and recirculation of the incorporated [^{3}H] (Kirchman, K'nees and Hodson 1985). Thus, we chose to run the incubations over the short time of 1 hr where bacterial community production measurements were most reliable. Measured leucine incorporation during the incubation was converted to bacterial carbon production rate using a standard theoretical conversion factor of 2.3 kg C per mole of leucine (Simon and Azam 1989). Per-capita heterotrophic production was estimated by dividing heterotrophic production by the cell counts measured in each fraction.

Preservation of bacterial filters in the field

Microbial cells for DNA extraction were collected by sequential in-line filtration onto a 3 μm (> 3 μm) isopore polycarbonate filter (TSTP, 47 mm diameter, Millipore, Billerica, MA, USA) for the particle-associated fraction and a 0.22 μm (3–0.22 μm) Express Plus polyethersulfone membrane filter (47 mm diameter, Millipore, MA, USA) for the free-living fraction. We used 47 mm polycarbonate in-line filter holders (Pall Corporation, Ann Arbor, MI, USA) and an E/S portable peristaltic pump with an easy-load L/S pump head (Masterflex®, Cole Parmer Instrument Company, Vernon Hills, IL, USA). The total volume filtered varied from 0.8–2.2 L with a maximum filtration time of 16 minutes per sample. Filters were submerged in RNAlater (Ambion) in 2 mL cryovials, frozen in liquid nitrogen and transferred to a −80°C freezer until DNA extraction.

DNA extraction, sequencing and preprocessing

DNA extractions were performed using an optimized method based on the AllPrep DNA/RNA/miRNA Universal kit (Qiagen; McCarthy *et al.* 2015; details in supplementary methods). Extracted DNA was prepared for sequencing based on Illumina's protocol for MiSeq libraries (15 039 740 Rev. D). DNA was sequenced using Illumina MiSeq V2 chemistry 2 × 250 (500 cycles) of dual index-labelled primers that targeted the V4 hypervariable region of the 16S rRNA gene (515F/806R) (Caporaso *et al.* 2012; Kozich *et al.* 2013) at the Microbial Systems Laboratories at the University of Michigan Medical School in July 2016. RTA V1.17.28 and MCS V2.2.0 software were used to generate data. Fastq files were submitted to NCBI sequence read archive under BioProject accession number PRJNA412984. We analyzed the sequence data using MOTHUR V.1.38.0 (seed = 777; Schloss *et al.* 2009) based on the MiSeq standard operating procedure accessed on 3 November 2015 and modified with time (see data accessibility and supplemental methods). A combination of the Silva Database (release 123; Quast *et al.* 2013) and the freshwater TaxAss 16S rRNA database and pipeline (Rohwer *et al.* 2018, accessed August 18, 2016) were used for classification of operational taxonomic units (OTUs). All non-bacterial and chloroplast sequences were pruned out of the dataset and replicate samples were merged by summing sample sequencing read counts using the *merge_samples* function (phyloseq). A batch script for our protocol can be found in this project's GitHub page in https://github.com/DenefLab/Diversity_Productivity/blob/master/data/mothur/mothur.batch.taxass. After primary filtering, 7806 OTUs remained. Finally, OTUs with two sequences or less throughout the entire dataset were removed as these are more prone to be artefacts originating from sequencing errors or the OTU clustering algorithm. The remaining 2979 OTUs were used in the biodiversity analysis. Representative sequences of each remaining OTU were collected from the aligned fasta file produced within mothur, and header names in the mothur output fasta file were modified using bbmap (Bushnell 2016) to only include the OTU name. A phylogenetic tree was created with FastTree using the GTR+CAT (general time reversible) model (Price, Dehal and Arkin 2010). Mismatches between the species

community data matrix and the phylogenetic tree were checked with the *match.phylo.comm* command (picante).

Estimating biodiversity

We measured the within-sample (alpha) diversity of particle-associated and free-living communities in two ways. First, we estimated diversity with the Hill numbers (^qD) using the iNEXT package, which interpolates and extrapolates through rarefaction and prediction (Hsieh, Ma and Chao 2016; Figure S2A). The Hill numbers are ideal for comparing across studies because they differentially weight species richness and relative abundances with an effective number of species in a sample for a given order, q. As q increases, species abundances are weighted more and therefore the impact of rare taxa are decreased. We calculated the Hill numbers for three orders ($q = 0, 1, 2$) with the equation $^qD = (\sum_{i=1}^{S} p_i^q)^{\frac{1}{1-q}}$ where S is the number of species in the sample and p_i is the relative abundance of the ith species. ^{0}D represents the 'effective number of observed species' or observed richness, whereas ^{1}D indicates the 'effective number of typical species' or Shannon's diversity (i.e. exp(Shannon Entropy)) and ^{2}D marks the 'effective number of dominant species' or the Inverse Simpson's index (Chao, Chiu and Jost 2016).

Second, we built off of the first diversity metrics mentioned above by using a series of phylogenetic Hill numbers proposed by Chao, Chiu and Jost (2010), which consider the topology of a phylogenetic tree, the relative branch lengths and the relative abundance of each branch. Thus, the phylogenetic Hill numbers indicates the 'effective number of lineages' and will always be lower than the OTU Hill diversity numbers (Chao, Chiu and Jost 2010, 2016). We calculated the phylogenetic Hill numbers using the hill_phylo() function within the hillR (Li 2018) R Package for $q = 0$, 2: $^qPD(T) = (\sum_{i \in B_T} L_i (\frac{a_i}{T})^q)^{\frac{1}{(1-q)}}$ or for $q = 1$, $^1PD(T) = exp(-\sum_{i \in B_T} \frac{L_i}{T} a_i log a_i)$ where T is the tree depth, B_T is the total branch length for T depth, L_i is the length of branch i in the set of branches in B_T and a_i is the sum of relative abundances of all OTUs descended from branch i. When $q = 0$, the equation simplifies to Faith's phylogenetic diversity for 'the effective total number of lineages'. When $q = 1$, the equation can be interpreted as the 'effective number of common lineages' that is similar to Allen's H_p whereas $q = 2$ represents the 'effective number of dominant lineages' that is similar to Rao's Q (Chao, Chiu and Jost 2016; Alberdi and Gilbert 2019). As such, correlations between the Hill numbers and the phylogenetic Hill numbers are especially strong for $q = 0$ (Figure S2B) and become weaker as the order increases (Figure S2C–D). For two communities that have equal abundance and relative abundances, the community with a deeper branching tree will have higher phylogenetic diversity (see Fig. 4 in Alberdi and Gilbert 2019).

Further, we calculated the abundance-unweighted and abundance-weighted mean pairwise phylogenetic distance (or MPD). The MPD measures the average phylogenetic distance between all combinations of two taxa pulled from the observed community and compares it with a null community of equal richness pulled from the gamma diversity of all the samples (*see supplemental methods for more details*). We estimated the standardized effect size (SES) MPD with the ses.mpd() function in the Picante R package (Kembell *et al.* 2010) using null.model = 'independentswap'. Values higher than zero indicate phylogenetic overdispersion (longer phylogenetic distance between two taxa) while values less than zero indicate phylogenetic clustering (shorter phylogenetic distance between two taxa) or that species are more closely related than expected according to the null community (Kembel 2009). Two communities of different diversities can have similar values and thus, the SES_{MPD} does not represent a true diversity metric. Rather, the SES_{MPD} is a community property signifying how phylogenetically clustered a community is.

Statistical analysis

Data analysis was performed using R version 3.6.2 (R Core Team 2019), specifically with the phyloseq (McMurdie and Holmes 2013), stats (R Core Team 2019) and broom (Robinson 2017) R packages. All main figures were made using the ggplot2 R package (Wickham 2009). To assess a statistical difference in particle-associated and free-living cell abundances, production rates and biodiversity metrics, a Wilcoxon rank sum test (stats::wilcox.test() function) was performed. We evaluated whether diversity metrics or environmental variables predicted heterotrophic production rates using ordinary least squares (OLS) linear regression (stats::lm() function) and accessed specific regression variables with broom::glance() (i.e. logLik, AIC, adjusted R^2). *P*-values were corrected for multiple inferences using the Benjamini–Hochberg method to control the False Discovery Rate (stats::p.adjust(method = 'fdr'). As the regressions had the same number of parameters (i.e. one), we selected the best performing OLS regression by choosing the model with the highest log(likelihood) (Hilborn and Mangel 2013).

To test which variable(s) were the best predictors of bacterial heterotrophic production, we performed variable selection via a lasso regression. We ran lasso regressions using the glmnet R package (alpha = 1 and lambda.1se as the tuning parameter; Friedman, Hastie and Tibshirani 2010) on all of the environmental, biodiversity and PC1 and PC2 from a redundancy analysis (RDA). Variables for RDA analysis had no missing data and were scaled to a mean of 0 and a standard deviation of 1 (stats::scale(); R Core Team 2019). RDA analysis was performed with stats::rda() and plotted with stats::biplot().

Data and code availability

Original fastq files can be found on the NCBI sequence read archive under BioProject accession number PRJNA412984. Processed data and code can be found on the GitHub page for this project at https://deneflab.github.io/Diversity_Productivity/ with the main analysis at https://deneflab.github.io/Diversity_Productivity/Analysis.html.

RESULTS

Free-living communities had more cells/mL but particle-associated communities had higher per-capita heterotrophic production

On average, we observed an order of magnitude more cells per milliliter in the free-living (FL) fraction than compared to the particle associated (PA) fraction (FL: 734,522 ± 86,601 cells/mL; PA: 41,169 ± 7418 cells/mL; $P = 1 \times 10^{-6}$, Fig. 1A). Community-wide heterotrophic production was ∼2.5 times higher in the free-living fraction than the particle-associated fraction (FL: 24.1 ± 5.06 μgC/L/day; PA:9.96 ± 2.38 μgC/L/day; $P = 0.024$, Fig. 1B). However, when calculated per-capita, particle-associated bacteria were an order of magnitude more productive on average than free-living bacteria (with $\log_{10}$ values of −7.56 ± 0.114 in the FL and −6.73 ± 0.160 in the PA; $P =$

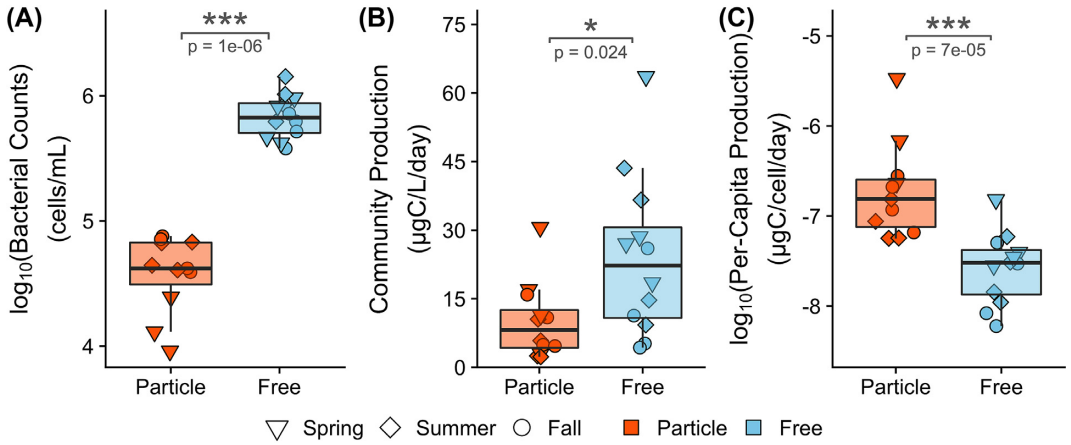

Figure 1. Bacterial counts, community-wide and per-capita heterotrophic production differ between microhabitats. Particle-associated and free-living samples were taken from four stations within Muskegon Lake during 2015 in May, July and September. (**A**) Free-living bacteria were an order of magnitude (10^5 cells/mL) more abundant compared to particle-associated bacteria (10^4 cells/mL). (**B**) Free-living bacteria were more heterotrophically productive compared to particle-associated bacteria. (**C**) Particle-associated bacteria were disproportionately heterotrophically productive per cell (expressed in $\log_{10}$(per-capita production)) compared to free-living bacteria.

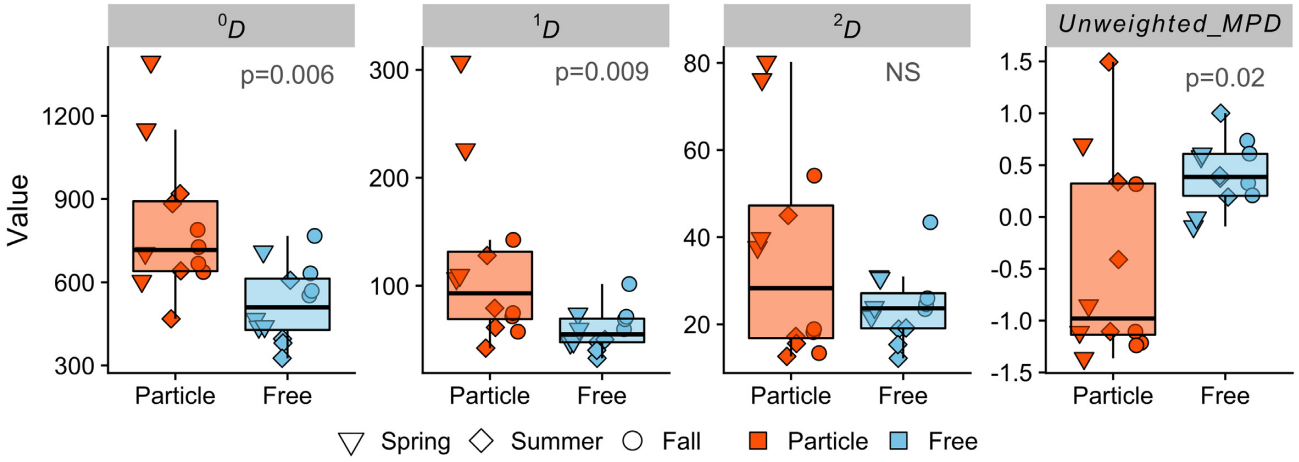

Figure 2. Particle-associated diversity tends to be higher than free-living communities, except in ^2D (inverse Simpson's index). The y-axis represents the Hill number (^qD) or the unweighted mean pairwise distance (MPD) (grey headers). As the order, q, increases in ^qD from 0 to 2, total, common and dominant taxa are weighted more, respectively. When the unweighted MPD is negative, the total community is phylogenetic clustered whereas if its positive, the community is more phylogenetically overdispersed. ^0D = OTU richness; ^1D = Shannon diversity; ^2D = inverse Simpson's index. *P*-values were corrected for multiple inferences using the Benjamini-Hochberg method from pairwise Wilcoxon rank sum tests.

7×10^{-5}, Fig. 1C). Particle-associated and free-living cell abundances in samples taken from the same water sample did not correlate (Figure S3A). Heterotrophic production between corresponding free-living and particle-associated fractions from the same water sample were positively correlated for both community (Adjusted $R^2 = 0.40$, $P = 0.017$; Figure S3B) and per-capita production rates (Adjusted $R^2 = 0.60$, $P = 0.003$; Figure S3C).

Particle-associated communities tended to be more diverse

Particle-associated bacterial communities tended to be more diverse than free-living communities across all Hill numbers, except ^{2}D (i.e. inverse Simpson's index; Fig. 2) and phylogenetic Hill number (Figure S4). There was a larger difference between particle-associated and free-living ^{0}D and ^{1}D in the spring, especially at the Bear and River stations (Figs 2, S5 and S6). When assessed by station, particle-associated ^{0}D and ^{2}PD tended to be higher than free-living communities and was maintained across the four sampling stations in the lake (Figures S5 and S6A). Particle-associated samples near inputs to Muskegon Lake (i.e. River and Bear Lake stations) were on average more OTU-rich than the outlet to Lake Michigan and the Deep stations (Figures S5 and S6A). Additionally, the particle-associated samples at the river station had almost twice the inverse Simpson's diversity compared with all other lake stations (Mean inverse Simpson Indices: Outlet = 23.8 ± 8.2; Deep = 23.8 ± 7.0; Bear = 35.6 ± 20.3; River = 59.8 ± 10.6; Figure S6A).

Particle-associated communities were more phylogenetically clustered than free-living communities based on unweighted mean pairwise distance (MPD) ($P = 0.02$, Figs 2 and S5). Compared to other particle-associated samples, the outlet station that connects to oligotrophic Lake Michigan had a larger unweighted phylogenetic diversity, indicating phylogenetic overdispersion (Figure S6A). There was no difference between particle-associated and free-living weighted MPD (Figure S4). However, all communities became less phylogenetically clustered moving east to west from the river to outlet stations (Figure S6A). Nevertheless, no sample across the entire dataset differed significantly from the null model with a significance threshold *P*-value of 0.05 for either unweighted or weighted MPD.

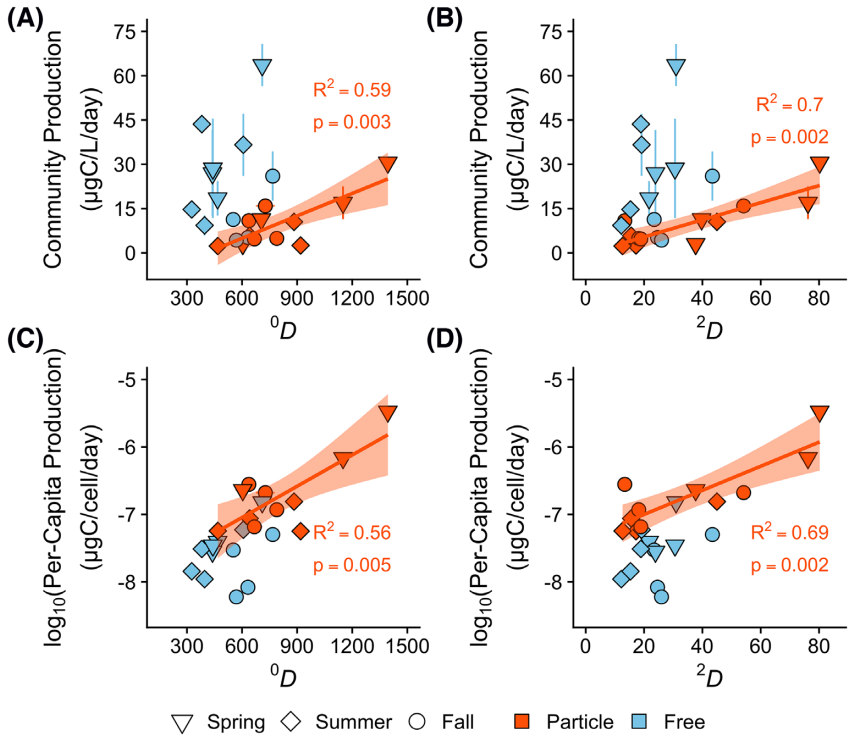

Figure 3. ^0D (OTU richness; left) and ^2D (inverse Simpson; right) correlate with heterotrophic productivity. **Top panel:** Biodiversity and community-wide heterotrophic production (μgC/L/day) relationships with **(A)** ^0D and **(B)** ^2D. **Bottom panel:** Biodiversity and $\log_{10}$(per-capita heterotrophic production) (μgC/cell/day) relationships with **(C)** ^0D and **(D)** ^2D. Solid lines represent ordinary least squares regression models for significant linear regressions for particle associated (orange) communities. All R^2 values represent the adjusted R^2. *P*-values were corrected for multiple inferences using the Benjamini–Hochberg method.

Diversity–Productivity relationships were observed in particle-associated but not free-living communities

We analyzed BEF relationships for both community-wide and $\log_{10}$(per-capita) production due to the distinct patterns of these two measures of heterotrophic production (Fig. 1). A strong, positive, linear BEF relationship between both community-wide and $\log_{10}$(per-capita) production was present in the particle-associated communities. This relationship was significant for all Hill numbers and low order phylogenetic Hill numbers (Figs 3, S7 and S8). No BEF relationships were observed for the free-living communities in any case. The ^{2}D, inverse Simpson's index, explained the most amount of variation in community-wide heterotrophic production (Fig. 3B; Adjusted $R^2 = 0.70$, $P = 0.002$) and per-capita (Fig. 3D; Adjusted $R^2 = 0.69$, $P = 0.002$). When the two data points with the highest inverse Simpson's index and heterotrophic production were removed from the regression (Fig. 3B), the relationship was still significant (Adjusted $R^2 = 0.38$; $P = 0.036$), though not with richness (Fig. 3A; Adjusted $R^2 = -0.08$; $P = 0.59$). These results are also robust across a range of minimum OTU abundance filtering thresholds (see *Sensitivity Analysis of Rare Taxa* in the supplemental methods and Figure S9) and hold up for all threshold levels in ^{1}D and ^{2}D whereas it only held up for richness until the removal of OTUs observed 25 times (community-wide heterotrophic production) and 15 times (per-capita heterotrophic production).

BEF relationships were stronger as dominant taxa were weighted more for the Hill numbers ^{0}D,^{1}D and ^{2}D (Figs 3 and S7), however, the opposite trend existed for the phylogenetic Hill numbers. The strongest BEF relationship was with ^{0}PD, a weak relationship with ^{1}PD (which was insignificant after *P*-value adjustment for multiple tests) and no relationship with ^{2}PD, a

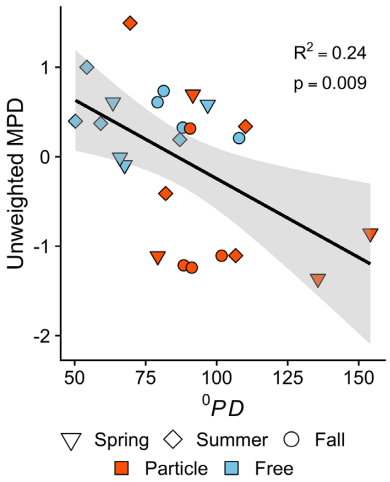

Figure 4. Communities with more total lineages (^0PD or Faith's phylogenetic diversity) tended to be more phylogenetically clustered based on the unweighted mean pairwise distance (MPD). When the unweighted MPD is negative, the total community is phylogenetic clustered whereas if its positive, the community is more phylogenetically overdispersed. R^2 value represents the adjusted R^2 from an ordinary least squares regression.

pattern for both community-wide and $\log_{10}$(per-capita) production (Figure S7). To delve deeper into these ^{q}PD BEFs relationships and the phylogenetic structuring of the communities, we evaluated the relationship between the phylogenetic Hill numbers (^{0}PD, ^{1}PD, ^{2}PD) and the mean pairwise distance (MPD). There was a negative, linear correlation (Fig. 4; Adjusted $R^2 = 0.24$, $P = 0.009$), indicating that (particle-associated) communities with

Table 1. The variables selected that best describe production in particle-associated and free-living fractions for community wide and $\log_{10}$(Per-Capita Production).

	Community-Wide Production	Log_{10}(Per-Capita Production)
Particle	Richness & Inverse Simpson	Richness & Inverse Simpson & Temperature
Free	pH	pH

more effective total lineages tended to be more phylogenetically clustered whereas (free-living) communities that had lower total lineages tended to be more phylogenetically overdispersed (Fig. 2). There was no relationship between ^{1}PD and ^{2}PD and weighted MPD (Figure S10).

Diversity, and not environmental variation, was the best predictor of particle-associated heterotrophic production

To identify variables that best predicted community-wide and $\log_{10}$(per-capita) heterotrophic production (i.e. remove variables that were collinear with each other and/or uninformative), we performed lasso regression with particle-associated and free-living samples (Table 1). For prediction of community-wide heterotrophic production, only biodiversity variables were chosen by the lasso including richness (^{0}D) and the inverse Simpson's index (^{2}D). An environmental variable, pH, was the sole variable selected for free-living samples. In contrast, for $\log_{10}$(per-capita) heterotrophic production, richness (^{0}D), inverse Simpson's index (^{2}D) and temperature were selected for particle-associated samples. Again, pH was the only predictor for free-living samples. Therefore, the best model for heterotrophic production in particle-associated microhabitats always included biodiversity variables, richness and inverse Simpson's index, whereas free-living samples only included an environmental variable, pH (Table 1).

To further verify that there were no confounding impacts of seasonal and environmental variables on community-wide and per-capita heterotrophic production, we separately performed ordinary least square (OLS) regressions and a dimension-reduction analysis of the environmental variables through a redundancy analysis (Table S1 and S2; Figure S11). Specifically, the first 2 environmental axes explained ~70% of the environmental variation in the sampling sites (Figure S11). Next, we predicted community-wide and per-capita heterotrophic production with all environmental variables and the first two components as predictor variables with individual particle-associated and free-living samples (Table S1 and S2). The best single predictor of community-wide heterotrophic production was inverse Simpson for particle-associated samples (logLik = -34.12; Adjusted $R^2 = 0.70$, FDR.p.value = 0.013; Table S1) and pH for the free-living samples (logLik = 98.43; Adjusted $R^2 = 0.49$, $P = 0.006$, FDR.p.value = 0.18). Whereas, the best single predictor of per-capita heterotrophic production was inverse Simpson for particle-associated samples (logLik = -1.11; Adjusted $R^2 = 0.69$, FDR.p.value = 0.026) and pH for the free-living samples (logLik = 4.20–2.39; Adjusted $R^2 = 0.78$, FDR.p.value = 0.002) (Table S2). Thus, the results from the OLS regressions agree with the lasso regressions.

DISCUSSION

We examined bacterial biodiversity-ecosystem function (BEF) relationships in relation to two microhabitats within freshwater lakes: particulate matter and the surrounding water. First, free-living bacteria had higher community-wide production whereas particle-associated bacteria had higher per-capita production. Second, particle-associated communities were more diverse based on all (phylogenetic) Hill numbers, except with the inverse Simpson's index (^{2}D). Third, community-wide and per-capita heterotrophic productivity of particle-associated, but not free-living bacterial communities, displayed a positive, linear BEF relationship with both richness and evenness contributing. Finally, particle-associated heterotrophic production was better explained by biodiversity than by environmental parameters. Next, we sought to answer: Why do biodiversity–ecosystem function relationships only exist in particle-associated communities?

Microbes have a large diversity of metabolisms and the choice of which to focus on may inherently affect the BEF relationship. Indeed, 'narrow' metabolic processes that are catalyzed by a small subset of taxa within bacterial communities, such as some nitrogen and sulfur cycling, have been found to display BEF relationships (Levine *et al.* 2011; Delgado-Baquerizo *et al.* 2016). In contrast, for 'broad' processes that are performed by the majority of taxa within a bacterial community, such as heterotrophic production (i.e. focus of the present study) and respiration, functional redundancy appears to weaken or remove the presence of BEF relationships (Griffiths *et al.* 2000; Langenheder, Lindström and Tranvik 2006; Wertz *et al.* 2006; Levine *et al.* 2011; Peter *et al.* 2011, Galand, Salter and Kalenitchenko 2015). These findings are in line with the absence of a BEF relationship for free-living bacterial communities in our study, which are likely to be more prone to functional redundancy due to stability and nutrient limitation of the bulk environmental conditions.

However, the above results and hypotheses surrounding narrow and broad processes are in conflict with the strong BEF relationship we observed in particle-associated bacterial communities. As such, our study signifies that microhabitats or perhaps habitat heterogeneity (or complexity) can influence bacterial BEF relationships, in agreement with previous research in eukaryotic systems across a variety of ecosystems (Tylianakis *et al.* 2008; Cardinale 2011; Zeppilli *et al.* 2016). A study using controlled stream mesocosms by Cardinale (2011) found that niche complementarity effects are particularly important in more heterogeneous environments. For example, in more heterogeneous streams, algal populations used different nutrients and avoided direct competition for resources, resulting in coexisting unique species occupying distinct and local microhabitats (Cardinale 2011). An experimental study by Gravel *et al.* (2011) showed that BEFs depend on the legacy of previous evolutionary events. Specifically, they found that after several hundred generations of evolution on a variety of carbon substrates, generalist bacteria were more productive because of their more efficient exploitation of the environmental heterogeneity. Similarly, Enke and Datta *et al.* (2019) found that generalists could successfully attach to particles, regardless of the substrate. Finally, a recent study on freshwater lake bacterial communities found a positive correlation between OTU evenness and the number of dissolved organic matter (DOM) components, suggesting that

DOM resource heterogeneity may increase the diversity of bacterial communities by creating equity among bacterial species (Muscarella *et al.* 2019). Thus, it is likely that particles could provide sustenance for a large generalist population with diverse metabolisms. Additionally, the substrate (and metabolic) heterogeneity could promote communities with more dominant species (i.e. higher evenness).

Though our observational study could not directly test the role of niche complementarity effects, our analysis hints that this could be the potential mechanism. The best predictor for heterotrophic production in particles was the inverse Simpson's index. Therefore, communities with higher production have more dominant taxa, or higher evenness, supporting niche complementarity alone or in combination with species selection as the mechanism. Communities that are more even have an increased likelihood for complementary species to neighbor each other. Neighboring cells are more likely to interact, creating the opportunity for facilitation and thus, increasing the likelihood for niche complementarity (Battin *et al.* 2016). Moreover, we also found that particle-associated bacteria had higher per-capita production, similar to previous studies (Anesio, Abreu and Biddanda 2003), which could be strongly impacted by close proximity to cells.

In our study, there are several reasons why heterogeneity of particulate matter may allow for niche complementarity to occur. First, particles have a 2-fold layer of heterogeneity as they (A) may be composed of different substrates such as organic matter from terrestrial or aquatic environments and either heterotrophically or photosynthetically derived (Grossart 2010), and (B) each particle may comprise physicochemical gradients as well (Simon *et al.* 2002). Second, microbial interactions are more likely to occur between cells aggregated on particles as the interaction distances are usually much shorter (Cordero and Datta 2016) compared to free-living bacterial cells. In fact, genes mediating social interactions, such as motility, adhesion, cell-to-cell transfer, antibiotic resistance, mobile element activity and transposases, have been found to be more abundant in marine particles than compared to the surrounding water (Ganesh *et al.* 2014). Third, dense patches of bacterial cells on model marine chitin particles promoted cross-feeding of oligosaccharides when particles were recalcitrant (Ebrahimi, Schwartzman and Cordero 2019). Further, we found that particles in the spring from the Bear and River stations were especially diverse (i.e. ^{0}D and ^{2}D) and productive (Figs 2, 3, S4 and S5). These stations have a closer proximity to external inputs, which is primarily terrestrial sources in spring (Crump *et al.* 2003) that are more likely to be recalcitrant. Diversity in these stations are also more likely to be impacted by mass effects of terrestrial taxa to the lake whereas species sorting will influence communities in the Deep and Outlet stations (Crump, Amaral-Zettler and Kling 2012; Doherty *et al.* 2017).

The importance of niche complementarity in microbial communities can also be deduced from findings in the field of microbiology, which have shown widespread metabolic interdependence among bacterial community members. First, a 2016 study that reconstructed 2540 draft genomes of microbes found that most bacteria specialize in one particular step in sulfur and nitrogen pathways and 'hand-off' their metabolic byproducts to nearby organisms (Anantharaman *et al.* 2016). It is likely that metabolic hand-offs, a specific form of bacterial facilitation, will occur more in particle-associated compared to free-living communities. Indeed, some taxa on model marine chitin particles are incapable of breaking down particles and instead rely on carbon produced by primary degraders to thrive in later phases of particle degradation (Datta *et al.* 2016), a repeatable result for three other polysaccharide substrates (Enke and Datta *et al.* 2019). Second, Lilja and Johnson (2016) demonstrated that different microbial cell types eliminate inter-enzyme competition by cross feeding, which increases substrate consumption by allowing intracellular resources to go towards a single enzyme, rather than having two enzymes that perform two separate reactions compete for nutrients within a cell. Third, some bacteria are unable to grow in laboratory cultures unless they are in co-culture with other organisms, which may be due to metabolic hand-offs or growth factors such as siderophores or catalases (Stewart 2012).

Considering that (i) closely related taxa share more genes and metabolic pathways than distantly related bacterial taxa (Konstantinidis and Tiedje 2005; Kim *et al.* 2014) and (ii) bacteria commonly have incomplete metabolic pathways, it may be possible that closely related bacteria are most likely to exchange their metabolic byproducts. This may be why we found that particle communities with higher ^{0}PD also tend to be more phylogenetically clustered. In other words, as new taxa were added to the community, the new taxa represented taxonomic clades more similar to or already present in the community. Similarly, co-cultures of phylogenetically related freshwater algae and vascular plants were more productive (Narwani *et al.* 2017). A study of bacterial communities inhabiting Mediterranean soils found that plots containing more recently diverged lineages had higher ecosystem function levels than when more distantly related lineages were present (Goberna and Verdú 2018). However, other bacteria-focused studies found higher levels of antagonism with more closely related taxa (Russel *et al.* 2017) and more bacterial productivity (measured through colony forming units per mL) with more distantly related taxa (Venail and Vives 2013). Though, these latter studies were performed with lab grown species grown in stable conditions. Thus, it might be expected that potential interdependent relationships between bacteria are broken through the creation of lab communities or by the homogeneity of lab cultures that removes the complexity of spatial heterogeneity, environmental fluctuations and interactions with the rest of the bacterial community.

Previous studies on bacterial BEF relationships have used three approaches to manipulate bacterial diversity (Krause *et al.* 2014): (1) removal of taxa (e.g. dilution to extinction) in which complex communities are simplified by removing rare taxa (Franklin *et al.* 2001; Wertz *et al.* 2006; Peter *et al.* 2011; Philippot *et al.* 2013; see Roger *et al.* 2016 for a review of this approach; fragmentation or knockout in Bell 2019), (2) addition of taxa (e.g. manually assembled communities) in culture (Salles *et al.* 2009; Tan *et al.* 2012; invasion or coalescence in Bell 2019), or (3) natural or manipulated environmental communities (Griffiths *et al.* 2000; Levine *et al.* 2011; Galand, Salter and Kalenitchenko 2015; Rivett and Bell 2018). We took the third approach in this study. In contrast to the other two approaches, this had the benefit of (a) maintaining high diversity with both abundant and rare taxa, (b) including both r- and k-selected organisms, (c) allowing natural environmental and ecological forcings to shape the community and (d) evaluating BEF relationships in diversity and productivity ranges that reflect natural communities. Admittedly, three inherent weaknesses to our approach were that we cannot measure all the potential variables that influence heterotrophic productivity, which are especially and inherently difficult to measure within particles, we only have 24 samples for a 12 versus 12 study and our analysis is correlational and we cannot manipulate the system to unequivocally separate causes and consequences of bacterial production. For example, strong correla-

tions with heterotrophic production and pH in the free-living samples may point to pH being a consequence of, rather than a cause of, varying production levels. This is because bacterial production and bacterial respiration are positively correlated (del Giorgio and Cole 1998) and with increased respiration, pH may decrease due to CO_2 dissolution into the water.

Finally, we acknowledge that the typical sampling of bacterial communities and analysis using DNA sequencing reflects *all* bacteria present in the community and not necessarily only the *active* members of the community contributing to a given ecosystem function. In freshwater systems, up to 40% of cells from the total community have been found to be inactive or dormant (Jones and Lennon 2010). In addition, leucine incorporation is not universal across all taxa (Salcher, Posch and Pernthaler 2013). In this context, it is interesting to reflect on the richness in absence of function (i.e. x-intercept) of the observed BEF relationship, which is 406 (Fig. 3A). This could be interpreted as a baseline level of 406 particle-associated OTUs that are inactive (either dead or dormant cells or environmental DNA) or incapable of incorporating leucine. This value represents 29–87% of the total particle-associated communities and may obscure the *actual* diversity (and BEF relationship) of the community (Carini *et al.* 2016).

In conclusion, we showed that increased bacterial diversity led to increased bacterial heterotrophic production in particle-associated but not in free-living communities. As such, our findings help to further extend the principles of the impact of microhabitat on BEF relationships from Eukarya to Bacteria, contributing to current efforts to integrate macroecological theories into the field of microbial ecology (Barberán, Casamayor and Fierer 2014). Additionally, we showed that communities with higher phylogenetic diversity had higher per-capita heterotrophic production rates for ^{0}PD, which we hypothesize to be related to genome evolutionary patterns specific to bacteria that result in interdependence. The unique nature of BEF relationships across particle-associated and free-living habitats agrees with the distinct community assembly and functional partitioning previously described between these two aquatic habitats (Bižić-Ionescu *et al.* 2014; Ganesh *et al.* 2014; Mohit *et al.* 2014; Schmidt, White and Denef 2016; Balmonte, Teske and Arnosti 2018). Future studies may go beyond observations to predict changes in ecosystem function (Bell 2019) such as primary and secondary (and/or heterotrophic) productivity, carbon respiration and sequestration based on microhabitat-driven community shifts changes.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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