Multifunctionality and Diversity in Bacterial Biofilms

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Abstract

Bacteria are highly diverse and drive a bulk of ecosystem processes. Analysis of relationships between diversity and single specific ecosystem processes neglects the possibility that different species perform multiple functions at the same time. The degradation of dissolved organic carbon (DOC) followed by respiration is a key bacterial function that is modulated by the availability of DOC and the capability to produce extracellular enzymes. In freshwater ecosystems, biofilms are metabolic hotspots and major sites of DOC degradation. We manipulated the diversity of biofilm forming communities which were fed with DOC differing in availability. We characterized community composition using molecular fingerprinting (T-RFLP) and measured functioning as oxygen consumption rates, the conversion of DOC in the medium, bacterial abundance and the activity of five specific enzymes. Based on assays of the extracellular enzyme activity, we calculated how the likelihood of sustaining multiple functions was affected by reduced diversity. Carbon source and biofilm age were strong drivers of community functioning, and we demonstrate how the likelihood of sustaining multifunctionality decreases with decreasing diversity.

Introduction

In the face of a global decline in biodiversity, several recent reviews and meta-analyses reported a positive, but saturating relationship between biodiversity and individual ecosystem functions [1,2], like biomass accrual [3], resource utilization [4] and temporal stability [5]. Saturation of functioning at high levels of diversity suggests the presence of functionally redundant species. Microbes encompass the highest diversity of all life forms on Earth [6,7] and they drive a bulk of ecosystem functions. However, the roles microbial diversity play in controlling ecosystem functioning remain largely overlooked [8]. Microbial diversity is commonly neglected in biogeochemical models, which implicitly assume that large-scale ecosystem functions are independent from microbial diversity [9].

While early studies about biodiversity and ecosystem functioning focused mainly on single ecosystem processes [8,10], Hector and Bagchi [11] addressed the importance of simultaneous effects of biodiversity on multiple ecosystem functions. They found that the number of species required to sustain ecosystem functioning increased with the number of processes considered. Gamfeldt and coauthors [12] show that analyzing a single response variable overestimates the amount of functional redundancy. Functional redundancy for a single ecosystem function occurs if several species perform the same function. When multiple functions are performed at the same time, complementarity of species across functions is expected to reduce multifunctional redundancy [12]. This concept requires trade-offs for species to perform different functions [8]. Such trade-offs could arise because some functions conflict with other functions (e.g. e.g. productivity and stress tolerance [13]). Therefore, a lesser degree of redundancy is expected to emerge when more functions are addressed. Bacterial communities are often assumed to be functionally redundant due to their high abundance, immense diversity, high dispersal capacity, physiological versatility and horizontal gene transfer. However, there are only few empirical tests of the relationship of biodiversity and multifunctionality [13,14].

In this study, we address the relationships between bacterial diversity and the activities of extracellular enzymes degrading dissolved organic carbon (DOC). DOC is a key component of the carbon cycle of inland waters [15]. The degradation of DOC is mediated primarily by heterotrophic bacteria, which in streams are located predominantly in biofilms [16,17]. DOC comprises a diverse mixture of different components [18] and its degradation requires a range of hydrolytic and oxidative enzymes [19]. Bacterial extracellular enzymes accumulate in biofilms [20], where they transform high molecular weight compounds to lower molecular weight compounds which are ultimately assimilated by heterotrophic microbes [21]. These enzymatic reactions constitute different ecosystem functions and their interplay is of crucial importance for overall ecosystem functioning.

Here, we manipulated the diversity of heterotrophic bacterial biofilms in laboratory-based bioreactors and measured the activity of a range of extracellular enzymes and the degradation of DOC as ecosystem functions. We hypothesized that individual ecosystem functions are affected by the loss of diversity and we expected to find a strong effect of the manipulation on ecosystem multifunctionality. This was addressed as the likelihood of sustaining multiple extracellular enzymes above a threshold of the maximal activity. We provided two different substrates and measured activity. We provided two different substrates and measured activity.

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biofilm composition and function at an early and late stage of biofilm development. We expected a dependency of multifunctionality on substrate availability and that the age of biofilms plays an important role for the establishment of species interactions and the maintenance of multifunctionality.

**Methods**

**Bioreactors**

A set of 36 bioreactors was installed in a constant temperature room (20°C) and kept dark. The bioreactor consisted of glass columns (approx. 200 mL) filled with sintered borosilicate glass beads (90,000:1 surface to volume ratio) providing a large area for the growth of biofilm [22]. Three-way valves at the in- and outflow of the bioreactors were used as sampling ports and peristaltic pumps supplied the bioreactors with medium at a flow rate of 1 mL min⁻¹. Substrate composition was manipulated by providing two types of medium - carbon-free, artificial lake water medium amended with (i) 15 ppm of a mixture of labile and recalcitrant carbon (termed “labile”) or (ii) 15 ppm of recalcitrant carbon only (termed “recalcitrant”). The artificial lake water was prepared as described in Bastviken et al. [23] and for the labile treatment amended with a mix of 7.5 ppm of aged stream water DOC and 7.5 ppm of an artificial labile carbon source (3.75 ppm each of cellobiose and the dipeptide leucine-proline, both Sigma-Aldrich, St. Louise, MO, USA). For the recalcitrant treatment, 15 ppm of aged stream water DOC was added to the artificial lake water. The stream water was sampled from a small tributary to Fihyån, Sweden and aged for 2 month at 20°C. It contained 110.8 ppm DOC. Bioreactors were assembled steriley, and all sampling and handling was conducted under aseptic conditions. The glass beads were muffled at 450°C for 4 h, while the glass reactors, pump tubing (Tygon) and silicone connection tubing were steam autoclaved (121°C for 20 min). During the entire term of the experiment, we monitored bacterial abundance in the outflow as an indicator of community stability in the bioreactors. Retention time for the medium was estimated in a separate reactor by changing from pure water to a saline solution (100 ppt) in the inflow and measuring conductivity in the outflow. Retention time was estimated to be approximately 300 minutes.

**Preparation of the inoculum**

A gradient in diversity was established in pre-cultures using a dilution-to-extinction approach [24]. The bacterial community was collected from a stream (Fihyån, Sweden, N 59° 53’ 7” E 17° 20’ 43”). Sediment and water were sampled, mixed and filtered (GF/F, Whatman, Maidstone, Kent, UK) to eliminate predatory eukaryotes. The number of cells in the inoculum was adjusted to be between 10⁷ and 10⁸ cells using step-wise dilution with sterile stream water. The number of cells in the inoculum was filtered (GF/F, Whatman, Maidstone, Kent, UK) to eliminate predatory eukaryotes. The number of cells in the inoculum was adjusted to be between 10⁷ and 10⁸ cells using step-wise dilution with sterile stream water. 100 mL batch cultures with particle filters (GF/F, Whatman, Maidstone, Kent, UK) were used as sampling ports and peristaltic pumps supplied the bioreactors with medium at a flow rate of 1 mL min⁻¹. Substrate composition was manipulated by providing two types of medium - carbon-free, artificial lake water medium amended with (i) 15 ppm of a mixture of labile and recalcitrant carbon (termed “labile”) or (ii) 15 ppm of recalcitrant carbon only (termed “recalcitrant”). The artificial lake water was prepared as described in Bastviken et al. [23] and for the labile treatment amended with a mix of 7.5 ppm of aged stream water DOC and 7.5 ppm of an artificial labile carbon source (3.75 ppm each of cellobiose and the dipeptide leucine-proline, both Sigma-Aldrich, St. Louise, MO, USA). For the recalcitrant treatment, 15 ppm of aged stream water DOC was added to the artificial lake water. The stream water was sampled from a small tributary to Fihyån, Sweden and aged for 2 month at 20°C. It contained 110.8 ppm DOC. Bioreactors were assembled steriley, and all sampling and handling was conducted under aseptic conditions. The glass beads were muffled at 450°C for 4 h, while the glass reactors, pump tubing (Tygon) and silicone connection tubing were steam autoclaved (121°C for 20 min). During the entire term of the experiment, we monitored bacterial abundance in the outflow as an indicator of community stability in the bioreactors. Retention time for the medium was estimated in a separate reactor by changing from pure water to a saline solution (100 ppt) in the inflow and measuring conductivity in the outflow. Retention time was estimated to be approximately 300 minutes.

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**Acclimation phase**

After inoculating the bioreactors, the cells were allowed to colonize the surface of the glass beads for 4 to 7 weeks. We monitored DOC concentration and oxygen consumption (see below for a description of the methods). During the acclimation phase, the medium was recycled, but we replaced approximately half of the medium (4 L) on a weekly basis.

**Tests of ecosystem function**

We performed two sets of tests of ecosystem functioning, lasting for 10 days each. The first test was performed after 4 weeks of acclimation (termed “young biofilms”), while the second was performed after 7 weeks of acclimation (termed “old biofilms”). In each test we included the three diversity levels each with six bioreactors, three each of to the labile and recalcitrant carbon treatment. We measured DOC concentration, absorbance at 250 nm, and oxygen in the in- and the outflow of the bioreactors and calculated respiration in each bioreactor by difference. After 10 days, the corresponding bioreactors were opened (destructive sampling) and the glass beads were sampled to measure the activity of five different enzymes (see below). During the colonization period, differences in community composition might have established along the flow path in the bioreactors, hence we sampled the beads along a gradient, with samples from the inflow, the mid section and the outflow of the bioreactors. A subsample of the beads was stored at −80°C for molecular community composition using Terminal Restriction Fragment Length Polymorphism (T-RFLP). Another subsample was covered with 3.7% formaldehyde and stored at 4°C for enumeration of cell abundance on the beads using flow cytometry.

**DNA extraction and T-RFLP**

Cells were harvested by sonication 25 mL of beads in MQ water and filtering the supernatant onto 0.2 µm membrane filters (Supor, Pall, Port Washington, NY, USA), which were stored at −80°C. DNA was extracted using the UltraClean Soil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA). DNA extracts were used as templates for PCR amplification of the 16S rRNA genes (see Text S1). Reactions of the PCR product with the restriction enzymes hai III and hind I were incubated at 37°C for 16 h [25]. Terminal fragments were sized by electrophoretic separation and detection on a capillary sequencer (ABI 96, Applied Biosystems, Carlsbad, CA, USA). Size and quantity (peak height) of terminal restriction fragments were analyzed using GeneMarker (ver. 1.7.7) software.

**Bacterial abundance**

Bacterial abundance was measured by flow cytometry of Syto13 (Molecular Probes, Invitrogen, Carlsbad, CA, USA) stained cells [26]. Cells in the outflow were sampled daily, fixed with 3.7% formaldehyde and stored at 4°C. Bacterial abundance on the beads was estimated after short sonication at intermediate power (12 Watts, Misonix XL, Misonix, Farmingdale, NY, USA) of beads covered with 3.7% formaldehyde solution. The samples were analyzed with a Cyflow Space (Partec, Görlitz, Germany) flow cytometer equipped with a 96 well-plate autosampler (see Text S2).

**Dissolved Organic Carbon**

Samples for DOC analysis were taken daily, from the in- and outflow of the bioreactors into muffled 17 mL vials. DOC concentration was analyzed immediately using a Sievers 900 TOC analyzer (GE Healthcare, Boulder, CO, USA) with an
accuracy range of ±0.5 ppb. At the beginning and the end of the two tests, absorbance at 250 nm was measured using a spectrophotometer (Lambda 40, Perkin Elmer, Waltham, MA, USA) and a 1 cm quartz cuvette. Carbon-specific absorbance at 250 nm (abs250:DOC) was employed to indicate the proportion of humic substances [27].

**Oxygen consumption**

Oxygen consumption was measured four times during each test period. Reactor-wide oxygen consumption was estimated as the difference in oxygen concentration between the in- and outflow of each bioreactor normalized for retention time of the medium. A flow-through optode probe (FTC, PreSens, Regensburg, Germany) connected via an optical fiber to a Fibox 3 oxygen meter (PreSens) was used. The measurements were temperature compensated and excess medium was discarded.

**Extracellular enzyme activities**

We measured the activity of five extracellular enzymes produced by natural biofilm communities (see Table S1). The activity of β-glucosidase, β-xylosidase, cellobiohydrolase and leucine-aminopeptidase, which are involved in the last steps of the hydrolysis of polymeric compounds (cellulose, hemicellulose, peptides), was determined spectrophotometrically by using fluorescent-linked artificial substrates (Sigma-Aldrich). The activity of phenoloxidase, which is involved in the oxidation of lignin was measured using L-3,4 dihydroyphenylalanine (Sigma-Aldrich) following the method of Sinsabaugh et al. [28]. All enzymatic activities were measured under saturating conditions (see Text S3). Fluorescence was measured at 360/465 nm excitation/emission using a plate reader (Ultra 384, Tecan, Switzerland). Blanks were subtracted from the samples to correct for abiotic hydrolysis of the substrate and fluorescent substances in the medium.

**Data analysis**

To investigate bacterial community composition, non-metric multidimensional scaling (nMDS) using Bray-Curtis similarities and Principal Component Analysis (PCA) were calculated using PAST (ver. 2.01) [29]. Both analyses are based on relative abundance data obtained by T-RFLP. Differences in abundance on the beads, number of OTUs and oxygen consumption rates were analyzed using ANOVA. Variability in the enzymatic activity measurements (β-glucosidase, β-xylosidase, cellobiohydrolase, leucine-aminopeptidase, and phenoloxidase) was analyzed by multivariate analyses of variance (MANOVA, SPSS for Windows, ver. 12.0). SIMCA-P+ (ver. 12.0.1.0, Umetrics, Sweden) was used for PLS modeling (projections of latent structures by means of partial least squares) to display community composition, while differences in community composition from the in- to the outflow of the bioreactors (Fig. 1A). Separate analysis of the high, medium or low diversity treatments revealed the impact of age of the biofilms and of carbon source composition, while differences in community composition from the in- to the outflow were not obvious. Under high diversity, the outlines of the treatments overlap considerably, although they appear clearly separated by age (Fig. 1B). The separation of communities by carbon source composition and age became more evident when diversity was reduced (Fig. 1C and 1D).

**Functioning**

The absolute activities of the five enzymes were strongly affected by the age of the bioreactors, with old biofilms showing greater activities than young biofilms (MANOVA, P<0.001, Fig. 2). We could also observe pronounced differences among the carbon and diversity treatments and along the gradient from the in- to the outflow of the bioreactors (Fig. 2). For all enzymes, the activities were generally greater in the labile than in the recalcitrant carbon treatment, with the exception of phenoloxidase which was rather low and similar for each substrate. In the recalcitrant treatment, we generally found a decrease in extracellular enzyme activities from high to low diversity. This pattern differed in the labile treatment. For example, old biofilms with medium diversities achieved similar enzyme activities as the high diversity treatment (see Text S3 for a more detailed comparison of results obtained by the enzyme activity assays).

The change in the ratio between absorbance at 250 nm and DOC (Figure S1) during the 10 d periods of both test runs was positive in bioreactors fed with labile carbon. In bioreactors fed with recalcitrant carbon, this ratio remained unchanged in young biofilms and was negative in old ones. The diversity treatment did not influence these patterns of DOC utilization.
application of labile carbon and the change in the abs250:DOC ratio. For peptidase, glucosidase and xylosidase activity, as well as for oxygen consumption, the presence of labile carbon was important for the diversity treatment and community composition (PCA scores) of the 16S rRNA genes community profiles. Figure 1. Non-metric multidimensional scalings (nMDS) of the 16S rRNA genes community profiles. Panel (A) shows the entire dataset. Panel (B) shows the high diversity treatment, panel (C) the medium and panel (D) the low diversity treatments. In panel (A), outlines indicated the diversity treatments: + high diversity, o medium diversity and Δ low diversity. In panel B, C and D the outlines indicate the treatments: o labile/young, + labile/old, x recalcitrant/young and Δ recalcitrant/old. doi:10.1371/journal.pone.0023225.g001

Reactor-wide oxygen consumption rates in the beginning of the test periods ranged between 0.10 and 0.50 mg O2h−1 in bioreactors fed with labile carbon and between 0.04 and 0.15 mg O2h−1 in bioreactors fed with recalcitrant carbon. Oxygen consumption rates dropped during the two test periods in the labile carbon treatment but remained similar in the recalcitrant carbon treatment (Figure S2). Diversity did not influence oxygen consumption in the labile (ANOVA MS 0.07, d.f. 8 P = 0.50) and the recalcitrant (ANOVA MS 0.01, d.f. 8 P = 0.31) treatment.

Two significant components were identified using PLS (Figure 3), which yielded a cumulative R2F of 0.66 and a Q2F of 0.60. Variable influence on projections (VIP) indicated that carbon source and age were overall important X variables (VIP≥1), while the diversity treatment and community composition (PCA scores) were less important (VIP<0.5) in predicting Ys. Based on the VIP, the presence of labile carbon was important for peptidase, glucosidase and xylosidase activity, as well as for oxygen consumption and the change in the abs250:DOC ratio. For cellobiohydrolase and phenoloxidase, however, age was relatively more important.

**Multifunctionality**

The probability of finding all five enzymes active above a certain threshold was strongly affected by diversity (Figure 4). In general, at higher diversities, the probability of retaining multifunctionality was much greater than to treatments where diversity was reduced. As expected, likelihoods decreased drastically when the threshold level was increased, however, the high diversity treatment performed above the 0.5 threshold in all scenarios. Bioreactors fed with the labile carbon source were less affected by the loss of diversity. In bioreactors exclusively fed with recalcitrant carbon, the effects of diversity on multifunctionality were very pronounced, with a reduction of the likelihood to sustain enzyme activities from 1 at highest diversity to 0.2 at lowest diversity (threshold level 0.5). Old biofilms had a reduced likelihood of all enzymes being active, especially when diversity was low.

**Discussion**

An ongoing debate in ecology revolves around how bacterial diversity and ecosystem function are related. Here we present experimental estimates of the effects of loss of diversity on multifunctionality (Figure 4). In natural biofilms, the interplay of several enzymes determines the processing of organic carbon. Insights into how the outcome of complex interactions of these enzymes and their substrates depends on diversity, requires a multifunctional perspective. Ecosystem functioning is the joint effects of multiple constituent functions and cannot be expressed as the average of individual functions, because a decline in one function cannot be compensated by an increase in another function [12]. These relationships can be assessed by defining a specific threshold for each function, and when a function drops below this threshold, overall ecosystem functioning is no longer sustained [13]. Moreover, there might be metabolic and/or stoichiometric constraints on the synthesis of extracellular enzymes [31]. A threshold relative to the maximal enzyme activity reduces the risk of a bias introduced by such constraints. We chose to investigate a range of thresholds, from values where the high diversity treatment sustained multifunctionality (0.5) to more stringent conditions (0.9). The selection of thresholds was based on Gamfeldt et al. [12] and the results of a recent meta-analysis [4] which reports an average diversity effect size corresponding to a 40% reduction in functioning. The likelihoods of sustaining multifunctionality converged under stringent conditions (threshold: 0.9), which could reflect the importance of individual species performing extraordinarily well [4].

When functional redundancy among species occurs, single ecosystem functions tend to saturate at relatively low levels of diversity [5]. However, more species are needed to sustain a higher number of functions, i.e. redundancy decreases when more functions are considered. In an analysis of published data, Gamfeldt and co-authors [12] found that, compared to plant communities, bacterial communities feature a high degree of multifunctional redundancy. However, these results were derived from biomass, consumer biomass and decomposition measurements. In the present study, multifunctionality of bacterial communities with respect to the activity of the studied extracellular enzymes was lost with decreasing diversity to an extent that implies a relatively low level of multifunctional redundancy. Multifunctional redundancy is conceptually based on the idea that trade-offs exist for species performing several functions [8,12]. Our results could reflect that functional trade-offs might be stronger between more specialized functions than in functions like biomass or respiration. Our observation is in line with the study of He et al. [14] who found a strong coupling of microbial diversity and multifunctionality in soils. They used backward statistical selection to extract OTUs with a positive effect on specific functions and estimated the effects of diversity on multiple functions. The statistical selection procedure does not account for compensatory growth or physiological adaptations, which might be of pivotal importance to natural communities which encounter a loss of diversity. Our experimental approach corroborates the findings of He et al. [14], while at the same time taking into account the possibility that fast growth rates and physiological versatility of the bacterial community might have enabled compensation for the loss of functions along with the loss.
of certain taxa. This is reflected in our finding that substrate composition influences the way multifunctionality is maintained (Fig. 4). Multifunctionality was less impaired by reduced diversity in the labile medium compared to the recalcitrant medium, where only the highest diversity was likely to sustain multiple functions at the same time. This reflects the ability of bacterial species to adjust to their environment, which consequently affects multifunctional redundancy.

We found that bacterial biofilms differ in their composition and function on short temporal and small spatial scales. Multifunctionality was less affected in young compared to old biofilms, which might reflect that communities in later successional stages may establish more complex structures [17]. Biodiversity effects on functioning are known to become stronger over time [8,32] and old biofilms may be richer in facilitative interactions among species. Also, with increasing duration more biomass might accumulate in the biofilms and affect functions like respiration rate. However, we found that abundance on the beads rather decreased during the experiment (Text S6).

The dilution of a complex community to create a gradient in diversity, as applied in this study, is successful in generating significant effects on functioning [24]. The molecular fingerprinting technique (T-RFLP), however, is restricted in the ability to reveal such gradients since it detects only the most abundant

Figure 2. Overview of extracellular enzymatic activities in the different treatments at two sampling occasions (young and old biofilms, respectively). For each level of diversity the labile (white bars) and recalcitrant (hatched bars) treatments are for samples from the inflow (G1), mid section (G2) and outflow (G3) of each bioreactor. Mean ± s.e.m bars were shown, N = 3. Abbreviations: CBH: cellobiohydrolase, β-GLUC: beta-glucosidase, XYL: xylosidase, PEP: leucine-aminopeptidase, PH-OX: phenoloxidase.
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Figure 3. PLS loading plot of community functioning. The plot depicts the correlation structure between X and Y and can be interpreted by drawing a line from any Y-variable through the origin and by identifying the position of orthogonal projections of the X-variables. Factors (X) close to and on the same side of the origin (0,0) are positively related to the response (Y), and factors more distant from the origin are more important. Y-variables close to each other are correlated. Factors are separated according to their importance (VIP). Abbreviations: CBH: cellobiohydrolase, ß-GLUC: beta-glucosidase, XYL: xyllosidase, PEP: leucine-aminopeptidase, PH-OX: phenoloxidase, abs250:DOC: change in the ratio of absorbance at 250 nm to dissolved organic carbon concentration over 10 days, PCA: scores on the first axis interpreted by drawing a line from any Y-variable through the origin.

Figure 4. Probabilities for all 5 studied enzymes to be active above a threshold level of 0.5, 0.75 or 0.9 of the maximum activities. Shown are the probabilities for bioreactors fed with labile (A and C) and recalcitrant (B and D) organic carbon. Differences in the likelihood of sustaining joint functioning for young (A and B) and old (C and D) biofilms are shown. Blue lines represent the high diversity treatment, red lines the medium diversity treatment and the green lines the low diversity treatment. Error bars indicate s.e.m. N = 3.
common and not expressed by all bacterial species [41]. However, the latter might also be more plastic in their expression, only being expressed when labile compounds are no longer available and communities forced to degrade complex substances. Such plasticity can result in patterns similar to functional redundancy [42], where several species perform the same functions and decreasing diversity can be compensated through compositional adjustments [43]. Using PLS modeling, we show that bacterial community composition (PCA scores) was relatively unimportant for the observed patterns in functioning, which rather supports the importance of plasticity.

This study demonstrates that reduced diversity impairs multifunctionality to a much larger extent than it diminishes individual functions. Hence, bacterial communities may be considerably less redundant than previously suggested [44] based on experiments and observations focusing on specific, individual functions.

Supporting Information

Figure S1 Changes in ratio of absorbance at 250 nm to DOC concentration between the start (T0) and end (T10) of the two tests (young and old biofilms) for bioreactors fed with labile and recalcitrant carbon, respectively. Positive values indicate an increase in the fraction of humic compounds relative to the total organic carbon pool. Error bars indicate ± s.e.m, N = 9.

EPS

Figure S2 Differences in oxygen concentration in the in- and outflow of the bioreactors. Results are shown for the two test periods, young biofilms (A and B) and old biofilms (C and D). Initial oxygen consumption was greater in bioreactors fed the labile (A and C) compared to bioreactors fed the recalcitrant carbon source (B and D). There were no differences between the high (△), medium (○) and low (△) diversity treatments.

EPS

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Table S1 Overview of extracellular enzyme activities measured along gradients within the bioreactors. The specific reaction performed and EC (Enzyme Commission) numbers are shown for each enzyme. The substrate analogues used for the enzyme activity measurements are also indicated (MUF: methylumbelliferone, AMC: aminomethylcoumarin).

Text S1 DNA extraction and terminal restriction fragment length polymorphism (T-RFLP) analysis. (DOCX)

Text S2 Flow Cytometry. (DOCX)

Text S3 Extracellular enzyme activities. (DOCX)

Text S4 PLS model evaluation. (DOCX)

Text S5 Extracellular enzyme activity comparisons. (DOCX)

Text S6 Abundance. (DOCX)

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Author Contributions

Conceived and designed the experiments: HP IY CG AMR SS LJH. Performed the experiments: HP IY CG. Analyzed the data: HP IY CG. Contributed reagents/materials/analysis tools: HP IY CG AMR SS LJH. Wrote the paper: HP IY CG AMR SS LJH.
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