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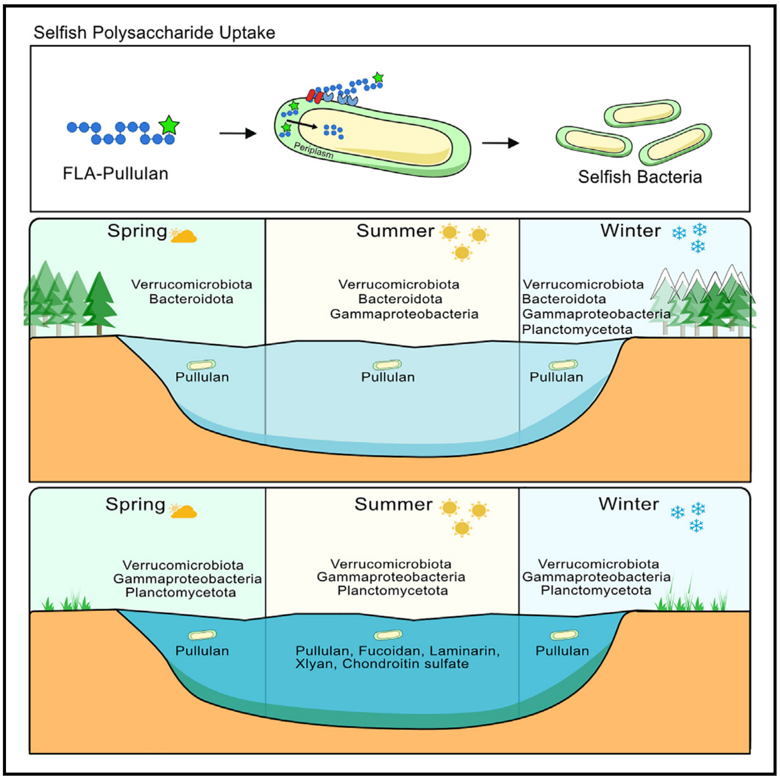
Selective heterotopic bacteria can selfishly process polysaccharides in freshwater lakes

Graphical abstract



Highlights

- Selfish polysaccharide uptake found in freshwater lakes, varying by season and trophic status
- Pullulan shows highest selfish uptake, highlighting substrate-specific bacterial strategies
- Seasonal DOM inputs drive selfish activity, peaking in winter and summer in different lakes
- Bacteroidota, Planctomycetota, and Verrucomicrobiota show dynamic substrate uptake behaviors

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In brief

Čačković et al. reveal selfish polysaccharide uptake by bacteria in lakes, shaped by trophic status and seasonal DOM. Bacteroidota, Planctomycetota, and Verrucomicrobiota show substrate-specific activity, with pullulan being a key substrate. These findings advance the understanding of bacterial roles in carbon cycling.

Čačković et al., 2025, Cell Reports *44*, 115415
April 22, 2025 © 2025 The Author(s). Published by Elsevier Inc.
https://doi.org/10.1016/j.celrep.2025.115415

Cell Reports

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Selective heterotopic bacteria can selfishly process polysaccharides in freshwater lakes

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https://doi.org/10.1016/j.celrep.2025.115415

SUMMARY

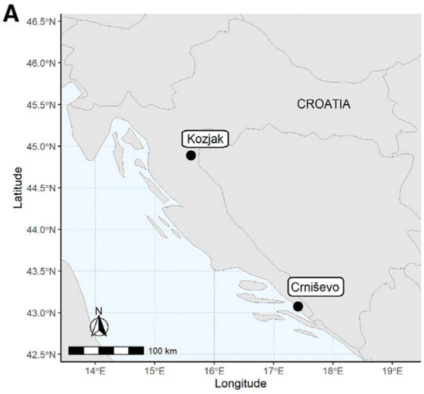
Polysaccharides, one of the main components of dissolved organic matter, are utilized by bacteria through three foraging enzymatic mechanisms: scavenging, sharing, and selfish. Our research aimed to identify selfish polysaccharide utilization by bacteria in freshwater ecosystems by examining spatial and seasonal variations in two lakes. The results of our fluorescently labeled substrate incubations revealed selfish activity in both lakes, with a larger proportion of the community showing selfish uptake of pullulan. The mesotrophic lake showed greater microbial diversity and ability for selfish uptake, particularly during a phytoplankton bloom. On the other hand, the oligotrophic lake had higher selfish activity during periods of increased terrestrial influence. Fluorescence *in situ* hybridization (FISH) staining combined with microbial diversity analysis revealed the selfish activity of phyla Bacteroidota, Planctomycetota, and Verrucomicrobiota as well as class Gammaproteobacteria, with different genera active depending on lake, season, and substrate. This research provides a basis for future interpretation of nutrient cycling in freshwater ecosystems.

INTRODUCTION

Lakes are highly biodiverse ecosystems that play a crucial role in the global carbon cycle. They actively facilitate the transport, reactivity, and storage of organic carbon. In lakes, organic carbon is primarily present as dissolved organic matter (DOM).[1] The primary components of DOM are polysaccharides, primarily carbohydrates, which traditionally comprise about 5%–20% of the total DOM composition.[2] However, recent studies suggest that carbohydrate concentrations can be even higher in lake ecosystems. For example, a study on lake DOM composition revealed that carbohydrates make up 29%–39% of DOM across lakes with various trophic states.[3] Specifically, in mesotrophic lakes, carbohydrates account for approximately 20% of DOM,[4] while in eutrophic lakes, this percentage can increase to 30%.[5] The sources of polysaccharides in lakes are diverse and of variable origin, such as local autotrophic and heterotrophic processes and allochthonous inputs from terrestrial sources.[6] The degradation of polysaccharides in lakes is an important biogeochemical process facilitated by enzymes produced by archaea, heterotrophic bacteria, or fungi.[7] The degradation of DOM components, including polysaccharides, in lakes differs depending on the lake's trophic status. Oligotrophic and mesotrophic lakes with lower DOM concentrations rely on microbe-produced extracellular enzymes for nutrient cycling,[8,9] while eutrophic lakes depend on internal regeneration through microbial decomposition and chemical desorption.[10]

Bacteria utilize polysaccharides via three different mechanisms.[11] They can produce surface-associated or extracellular enzymes that break down polysaccharides into oligo- and monosaccharides. Polysaccharide hydrolysis products are freely available in the extracellular environment and can be scavenged by bacteria that do not produce hydrolyzing enzymes, referred to as scavenging or cheating.[12] Consequently, bacteria that produce enzymes only partially benefit from their activity.[12] Additionally, bacteria can use a selfish mechanism, which was originally discovered in the gut microbiome[13] and involves the binding of polysaccharides to the bacterial cell surface, partially breaking down the polysaccharides to oligosaccharides, and subsequently directly importing the oligosaccharides into the periplasm for further hydrolysis. Selfish activity results in little to no loss of hydrolysis products to the external environment. Selfish bacteria have been identified in the marine environment,[14,15] the human gut,[13] and the rumen,[16] but their role in freshwater lakes remains unexplored.

The selfish mechanism is crucial in bacterial polysaccharide degradation in the marine environment, accounting for 25% to 60% of bacterial polysaccharide hydrolysis activity.[11,17] The



B

LAKE	Sampling season	t (°C)	O_2 (mg/L)	DOC (mg/L)
KOZJAK	Spring	10.02	12.54	1.80
	Summer	17.58	14.25	1.80
	Winter	4.27	12.11	6.20
CRNIŠEVO	Spring	9.47	10.28	2.04
	Summer	15.64	13.07	3.63
	Winter	8.24	10.52	4.50

Figure 1. Study area and environmental conditions

(A) Geographical position of sampled lakes in Croatia.

(B) Changes in temperature, O_2, and DOC concentrations over sampling seasons in lakes.

relative contributions of selfish uptake and extracellular hydrolysis to polysaccharide degradation vary by the location, season, and type of polysaccharides involved.[17–19] For example, selfish activity is high in the early stages of phytoplankton bloom, but as the bloom progresses, there is a shift to predominantly external hydrolysis.[20] Similarly, polysaccharide utilization patterns have been shown to differ between lakes of differing trophic status, with higher trophic status lakes exhibiting more externally hydrolyzed polysaccharides.[21] However, a detailed understanding of the role of selfish mechanisms in the degradation and uptake of polysaccharides from allochthonous vs. autochthonous sources in freshwater lakes remains incomplete, specifically an understanding of the contribution of the different polysaccharide degradation mechanisms over time (early vs. late bloom stages) and across different lake types (oligotrophic vs. mesotrophic). This includes examining seasonal variations and spatial differences to fully comprehend polysaccharide utilization in lakes.

Here, we explore the presence, prevalence, and seasonal dynamics of selfish polysaccharide utilization in freshwater lakes, investigated across three different seasons in two lakes. The study was carried out in two Croatian lakes (Kozjak and Crniševo), which differ in their trophic status, climatic conditions, and DOM compositions,[22] where we examined the selfish utilization of several polysaccharides with distinct origins across three seasons. We specifically address the effects of lake trophic status on polysaccharide origin (autochthonous vs. allochthonous) on seasonal dynamics of polysaccharide degradation rates via selfish uptake in two different lakes.

RESULTS

Sampling and environmental parameters of Lake Kozjak and Lake Crniševo

Water samples were collected in the spring and summer of 2022 and the winter of 2023 from two natural karst lakes in Croatia, Kozjak and Crniševo (Figure 1A), at a 10 m depth. The spring samples were taken between spring and summer phytoplankton blooms, the summer samples during the summer phytoplankton bloom, and the winter samples at the end of winter, before the spring phytoplankton bloom (Table S1). Lake water temperatures peaked in summer (16°C in Kozjak and 18°C in Crniševo) and dropped to winter minimums (4°C and 8°C, respectively; Figure 1B). Dissolved oxygen concentrations (O_2) followed this trend, peaking over 13 mg L^{-1} in summer, and were generally higher in Kozjak (Figure 1B). Dissolved inorganic carbon (DOC) concentrations were highest in winter (6 mg L^{-1} in Kozjak and 4 mg L^{-1} in Crniševo; Figure 1B). Crniševo had higher conductivity, salinity, Cl^-, SO_4^{2-}, and Na^+ concentrations, peaking in summer, while Kozjak had higher Ca^{2+} concentrations in spring and winter. Mg^{2+} and NO_3^- concentrations, along with pH values, were similar across both lakes and stable across seasons (Table S1).

Total cell counts

The total cell numbers over time in the fluorescently labeled substrate incubations were determined based on nucleic acid stain (4′,6-diamidin-2-phenylindol, DAPI) signal enumeration (see STAR Methods for details; Figure S1) after the exclusion of Cyanobacteria (see STAR Methods for details; Figure S2). In Kozjak, average cell abundances were 1.4×10^6 cell mL^{-1} across seasons, peaking in winter at 4.5×10^6 cell mL^{-1} (Figure 2A). Crniševo had higher initial cell abundances, averaging 6.3×10^6 cell mL^{-1}, with significant seasonal and incubation-related fluctuations (Figure 2B). In Kozjak's summer incubations, cell counts peaked on days 3 and 6 and then stabilized at an average of 1.7×10^6 cell mL^{-1}. Winter incubations showed an increase from days 6 to 12, reaching up to 3.7×10^6 cell mL^{-1} across all incubations, with a further increase up to 4.5×10^6 cell mL^{-1} by day 12, except in xylan, where cell counts dropped (Figure 2A).

In Crniševo, spring cell counts initially varied widely (2.8×10^6–9.9×10^6 cell mL^{-1}), fluctuating but generally decreasing over time (2.4×10^6–5×10^6 cell mL^{-1}). Summer initial counts also varied (1.4×10^7–4.8×10^6 cell mL^{-1}), dropping by day 3 and then rising by day 6, especially in pullulan and xylan incubations, before declining by day 18, except in fucoidan. In contrast, winter counts started uniformly (average of 6.3×10^6 cell mL^{-1}), peaked at day 6 or 12 (average 6.4×10^6 cell mL^{-1}), and then decreased significantly (average of 6.3×10^6 cell mL^{-1}; Figure 2B).

Changes in selfish utilization mechanism

The proportion of the community showing selfish uptake was quantified by the abundance of cells showing fluorescently labeled polysaccharide (FLAPS) uptake over time (Figure 2C). Selfishly active bacteria were detected in both lakes during all sampling periods (Figures 3A and 3B).

In both lakes, the proportion of the community showing selfish uptake was highest for pullulan, especially peaking in

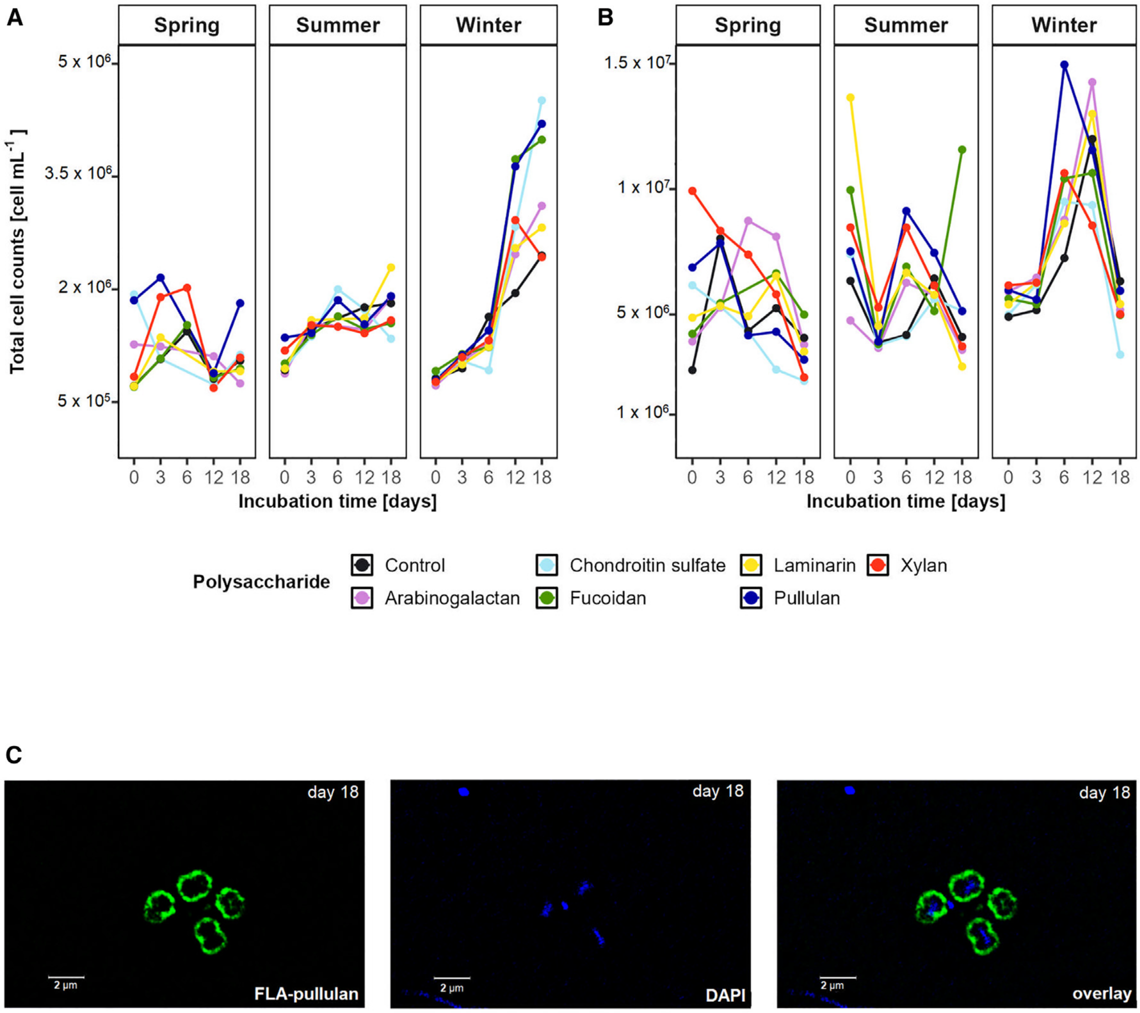


Figure 2. Total cell counts and selfish polysaccharide uptake

(A and B) Total cell counts over the incubation time in control samples and with added polysaccharides in different seasons in Kozjak (A) and Crniševo (B). Different colors represent added polysaccharides, and the control sample is colored black.

(C) Super-resolution images (SR-SIM) of cells stained by fluorescently labeled pullulan (FLA-pullulan) and nucleic acid stain (DAPI) and an overlay of both signals in Lake Kozjak during the summer incubation of pullulan on the 18th day. Data are represented as mean values. White scale bar: 2 µm.

Kozjak during summer up to 12% (Figure 3A) and in Crniševo during winter up to 7% (Figure 3B). Xylan showed the second highest activity within both Kozjak and Crniševo, with up to 5% stained cells in winter and summer, respectively (Figures 3A and 3B). In Kozjak, selfish uptake was variable over spring and summer (Figure 3A). In spring, fucoidan and laminarin were selfishly taken up during the first 3 days of incubation (more than 1%), while pullulan was taken up the most on day 12 (10%). Comparatively, Crniševo showed very consistent selfish activity in spring and winter, with pullulan and xylan showing the most selfish activity in both seasons (Figure 3B). Interestingly, during winter in Kozjak and summer in Crniševo, the selfish activity of all substrates was highly consistent, with the same abundance of cells showing staining at each time point over 18 days, which has not been seen previously in a substrate's incubations.[23]

Microbial community composition

Microbial richness was generally higher in Crniševo than in Kozjak, with lower richness in Kozjak during summer and winter (Table S2). Alpha diversity peaked in spring for Kozjak and in winter for Crniševo, with both lakes showing the lowest diversity in summer (Table S2).

In both lakes, temperature and O_2 significantly influenced summer microbial communities, while DOC and Ca^{2+} influenced winter communities in Crniševo. Salinity, Cl^{-}, and SO_4^{2-} influenced Crniševo's summer community, and NO_3^{-} impacted Lake Kozjak's spring community (Figure 3). Both lakes' microbial communities were initially dominated by Actinobacteriota, Bacteroidota, Cyanobacteria, and Proteobacteria, with minor seasonal shifts (Figure 4A). In Kozjak, Actinobacteriota and Proteobacteria peaked in summer and Bacteroidota in spring, and Actinobacteriota and rare taxa (<1% relative abundance)

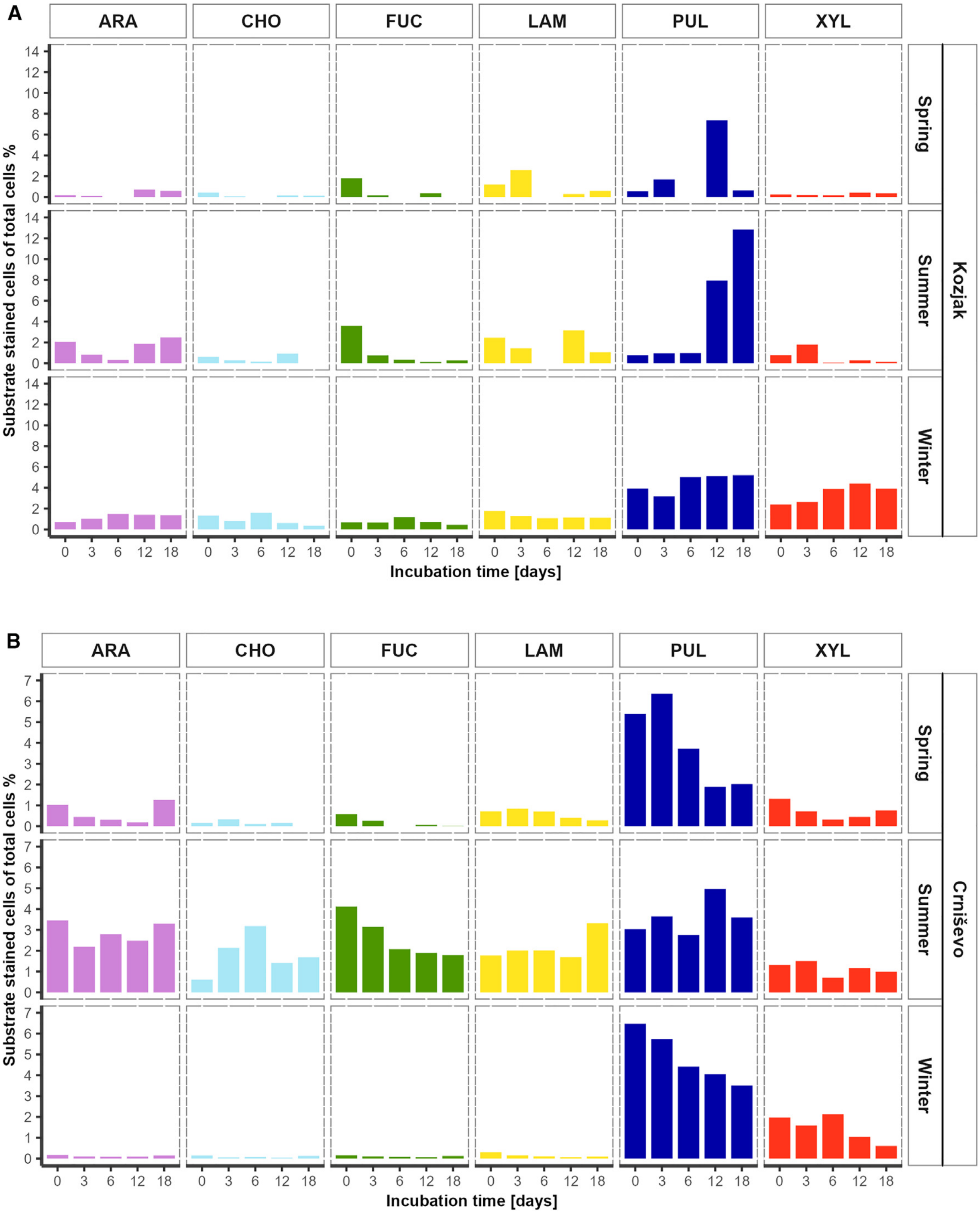


Figure 3. Proportion of the community showing selfish uptake

Relative abundance of substrate-stained cells (%) in incubations with different polysaccharides (CON, control; ARA, arabinogalactan; CHO, chondroitin sulfate; FUC, fucoidan; LAM, laminarin; PUL, pullulan; XYL, xylan) in different seasons in Kozjak (A) and Crniševo (B). Data are represented as mean values.

increased in winter. In Crniševo, Proteobacteria dominated in spring, Cyanobacteria in summer, and Bacteroidota with Actinobacteriota and Proteobacteria in winter. Archaea and autotrophic Cyanobacteria were excluded from further analyses due to FLAPS uptake focused on heterotrophic bacteria and potential primer biases for archaeal detection.[24]

Bacterial community composition differed significantly between lakes (analysis of similarity [ANOSIM]: R = 0.68, p = 0.001) and

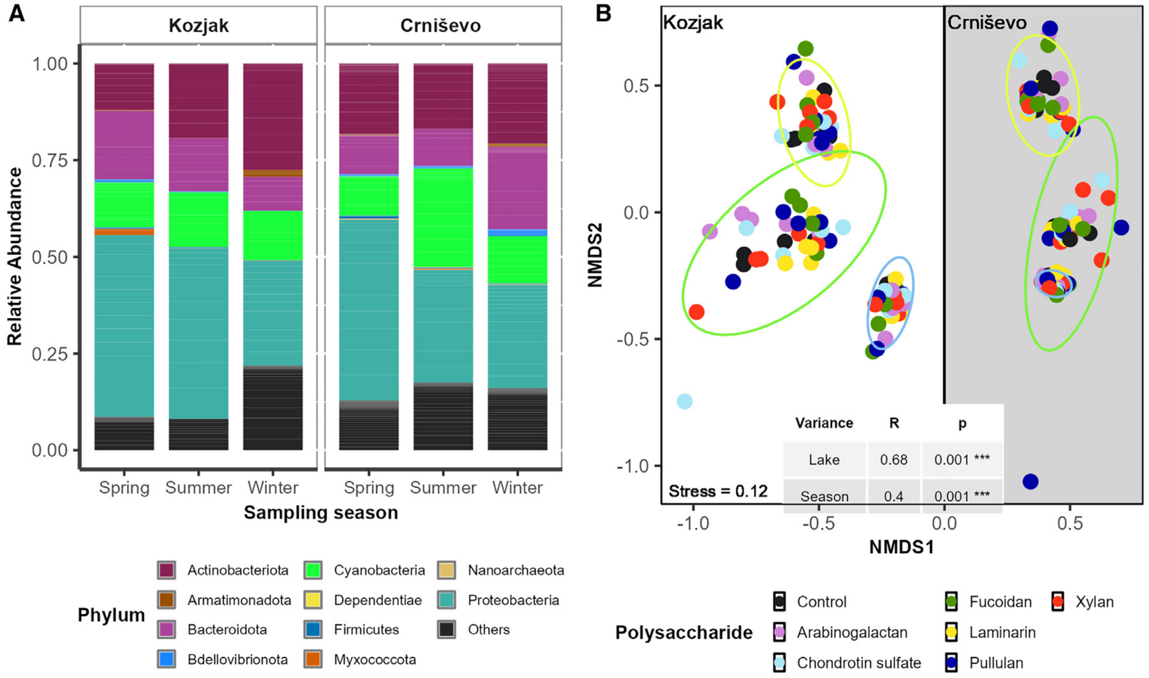


Figure 4. Significant difference in microbial communities between Lakes Kozjak and Crniševo in several seasons

(A) Average relative abundance of the microbial community structure (phylum level) in all added polysaccharide incubations from day 0 throughout sampling seasons. Plots are divided by lake. Taxa are colored by phylum. The “other” group contains phyla with a relative abundance less than 1%.

(B) Non-metric multi-dimensional scaling (NMDS) ordination showing Bray-Curtis dissimilarity in microbial community compositions across lakes (Kozjak and Crniševo) and color coded by added polysaccharides (see legend). Communities in lakes cluster by sampling seasons that are connected by color-coded ellipses (green: spring, yellow: summer, and blue: winter). Analysis of similarity (ANOSIM) of bacterial community compositions based on Bray-Curtis dissimilarities of relative read abundance. The table shows ANOSIM test results, including R values indicating the strength of community separation and p values assessing statistical significance.

seasons within each lake (Kozjak: ANOSIM: R = 0.77, p = 0.001; Crniševo: ANOSIM: R = 0.90, p = 0.001; Figure 4B). Incubation time had a weaker but significant effect (Kozjak: ANOSIM: R = 0.13, p = 0.001; Crniševo: ANOSIM: R = 0.04, p = 0.03), while polysaccharides had no significant impact (Kozjak: ANOSIM: R = −0.02, p = 0.82; Crniševo: ANOSIM: R = −0.03, p = 0.97; Figure 4B).

Response of bacteria to FLAPS incubation

We analyzed the change in bacterial relative abundance across the incubations as a percentage of change in abundance. The analysis reveals bacterial clades that show a positive response (increase in abundance compared to T0) to specific substrates (Figures 5 and S4).

In Kozjak, Planctomycetota increased by the end of the incubations but showed no specific substrate response in winter (Figure S4A). Verrucomicrobiota increased in spring and summer but decreased in winter as Bacteroidota became more prevalent. Gammaproteobacteria slightly increased in arabinogalactan and xylan incubations during spring and were present in all incubations, along with Alphaproteobacteria (Figure S4A). Key genera included *Pirellula* (Planctomycetota), *Luteolibacter* (Verrucomicrobiota), *Dinghuibacter* and *Sediminibacterium* (dominant in summer) with *Emticicia* or *Flavobacterium* (dominant in spring and winter), and seasonal presences like *Candidatus Methylopumilus* (all seasons), *Acinetobacter* (spring), and *Simplicispira* (winter; Figure 5A).

In Crniševo, Planctomycetota were abundant in summer but absent in winter, briefly appearing in certain spring incubations (Figure S4B). Verrucomicrobiota declined over time in summer, with low abundances in winter and spring, with a brief increase during spring fucoidan incubation. Bacteroidota dominated in winter but was low in spring, while Gammaproteobacteria and Alphaproteobacteria dominated pullulan and xylan incubations in spring, with Alphaproteobacteria present alone in summer. Key genera included *Pirellula* and *Blastopirellula* (Planctomycetota), *Prosthecobacter* and *LD29* (Verrucomicrobiota, summer only), *Luteolibacter* (Verrucomicrobiota, other seasons), and *Flavobacterium* and *Sediminibacterium* (Bacteroidota, winter). *Candidatus Aquirestis* appeared only in summer, while *Candidatus Methylopumilus* was present in both spring and summer.

FISH-based quantification of bacterial abundance in FLAPS incubations

Fluorescence *in situ* hybridization (FISH) staining was performed on samples from pullulan and xylan incubations from both lakes, plus all summer incubations from Crniševo, as these samples had the highest substrate-stained cells (Figures 3A and 3B). Probes EUB-I, EUB-II, EUB-III, and CF319a targeted remaining bacteria (EUB-I), Planctomycetota (EUB-II), Verrucomicrobiota

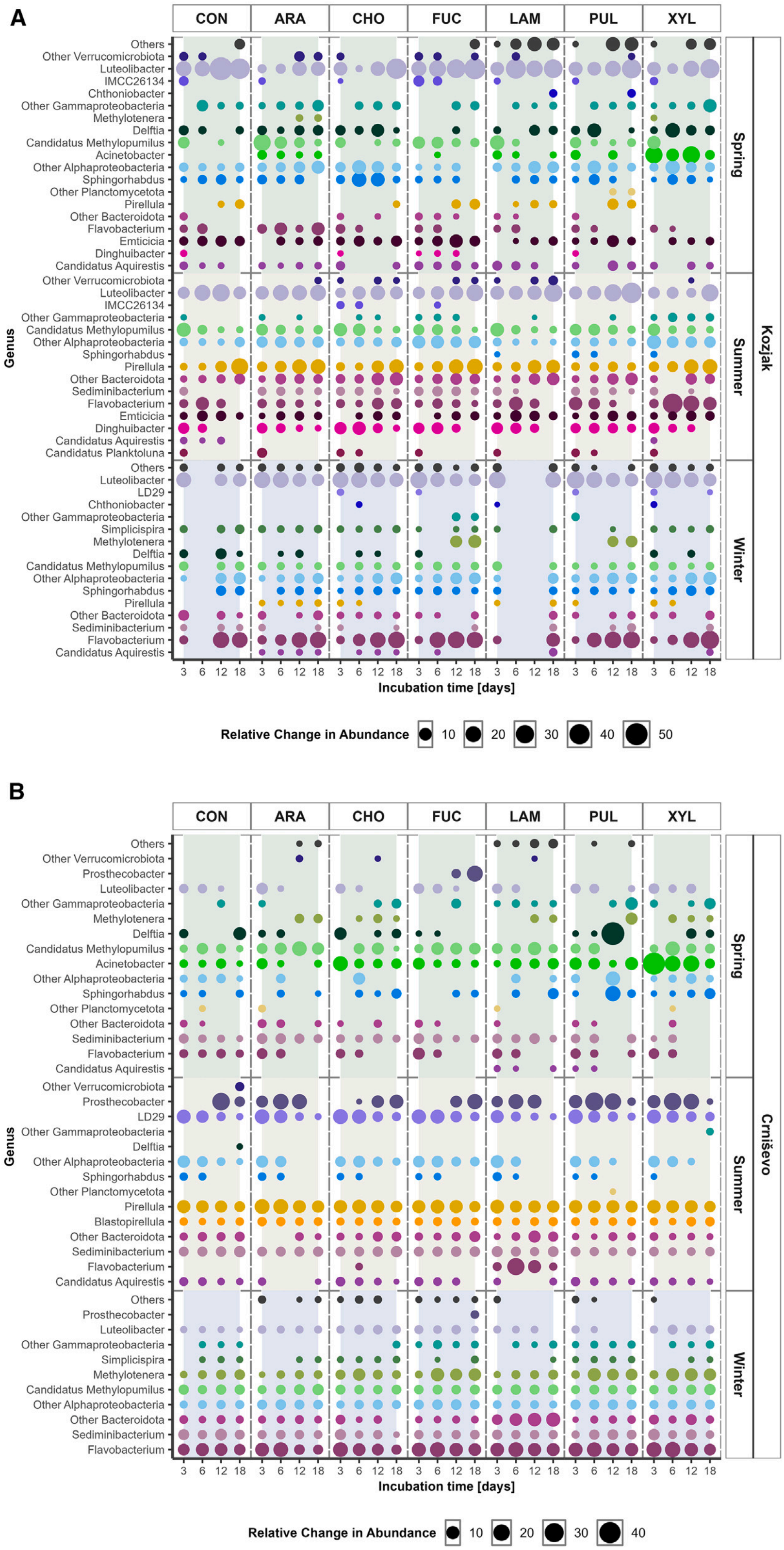
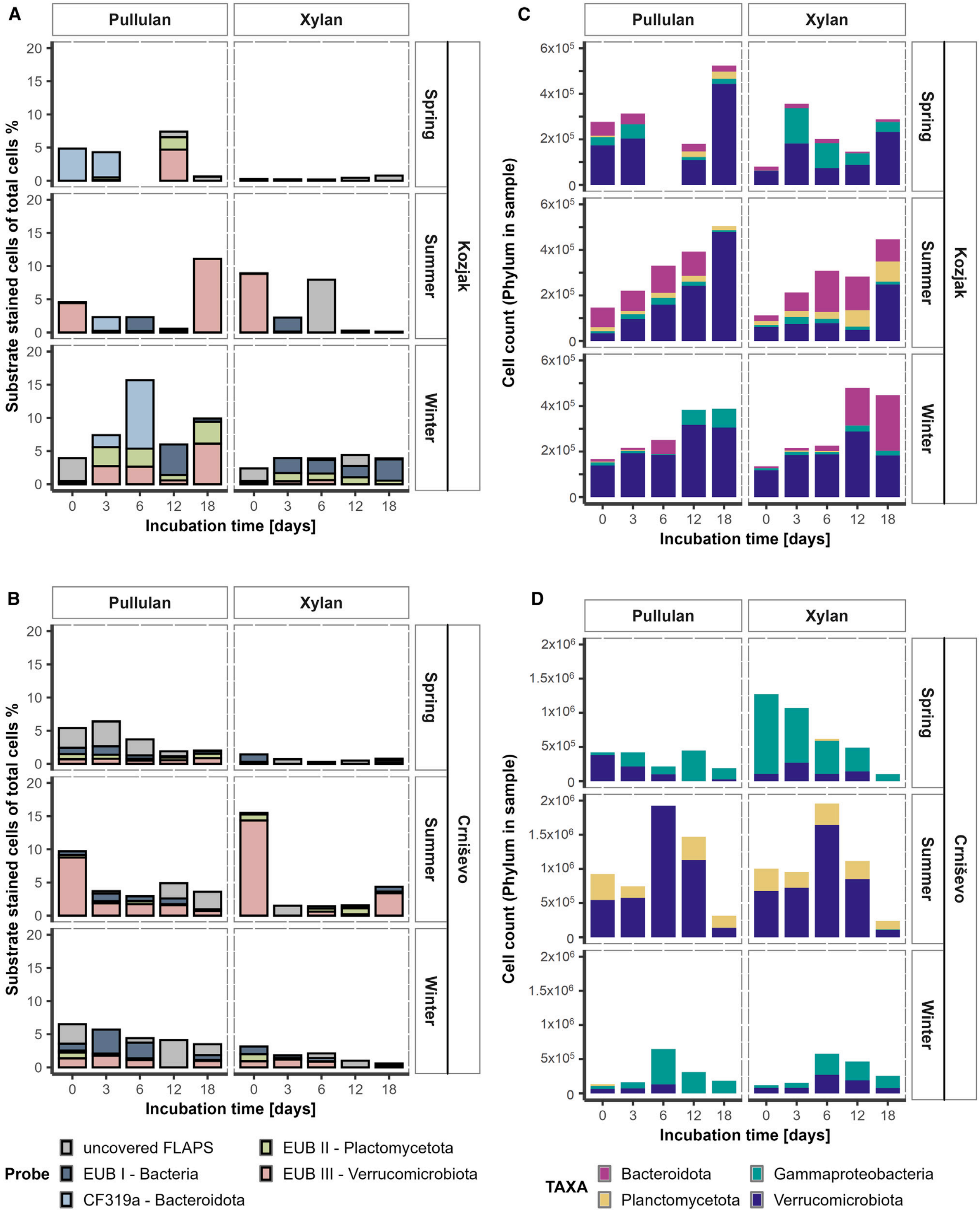


Figure 5. Bacterial community composition in Lakes Kozjak and Crniševo

Percentage change of relative abundance of the bacterial sequences compared to the initial T0 community through incubation time in Lakes Kozjak (A) and Crniševo (B) divided by season and added polysaccharide (CON, control; ARA, arabinogalactan; CHO, chondroitin sulfate; FUC, fucoidan; LAM, laminarin; PUL, pullulan; XYL, xylan). Taxa are colored by genus level.



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(EUB-III), and Bacteroidota (CF319a), respectively. CF319a-positive cells were subtracted from EUB-I to avoid overlap. Positive signals of substrate-stained cells were compared with the results of microbial diversity analysis to define the potentially selfish bacteria in karst lakes.

In some samples, cumulative positive cell counts reached only 25%–50% of the total bacterial abundance, even with included Cyanobacteria cells or autofluorescence cells (Figure S5), likely due to FISH detection limits of small fraction size and low-ribosome-content Actinobacteriota,[25] which comprised up to 40% of the community.

In Kozjak, remaining bacteria (EUB-I) dominated in winter, with Bacteroidota, Planctomycetota, and Verrucomicrobiota present in smaller amounts (Figure S5A). In spring, Verrucomicrobiota had the highest average abundance, and in summer, Planctomycetota was dominant.

In Crniševo, Planctomycetota and Verrucomicrobiota generally had higher cell abundances (Figures S5B and S5C). Bacteroidota were more abundant in spring pullulan and xylan incubations (Figure S5B) and in most summer incubations except for laminarin (Figure S5C). Verrucomicrobiota were dominant in spring and summer (Figures S5B and S5C), while remaining bacteria (EUB-I) dominated in winter (Figure S5C).

Identification of selfish organisms using FISH and sequencing

We taxonomically identified the selfishly active organisms using a combination of FLAPS and FISH staining. Using probes EUB-I, EUB-II, EUB-III, and CF319a, we characterized a total of 82% of selfish bacteria across all lakes, seasons, and polysaccharides. Furthermore, we compared our FLAPS-FISH results, which yield a phylum-level taxonomic identification, with our community analysis by 16S rRNA gene amplicon sequencing to identify the specific bacterial genera that might be selfishly active in lakes.

In Kozjak, all targeted bacterial groups (Bacteroidota, Planctomycetota, and Verrucomicrobiota) accounted for 70% of FLAPS uptake, with selfish activity varying by season and incubation time (Figure 6A). Several FLAPS-stained cells remained unidentified, suggesting that other bacterial phyla also exhibit selfish behavior in the lakes. In winter, all three phyla showed selfish pullulan uptake, while Bacteroidota showed pullulan uptake in all seasons during the first 6 days (Figure 6A). In the xylan incubation, fewer selfish cells were identified, with only Verrucomicrobiota showing high selfish activity at day 0 in summer.

The absolute abundance calculated from total cell counts and 16S rRNA gene amplicon sequencing data (see STAR Methods for details) showed an increase in the cell count of members of the phylum Bacteroidota during summer and winter incubations (Figure 6C), although they were only sometimes selfishly active (Figure 6A). During summer, the phyla Planctomycetota and Verrucomicrobiota showed an increase in absolute abundance throughout the incubation time (Figure 6C). The class Gammaproteobacteria had an increased absolute abundance during winter and a decreased one during spring (Figure 6C). A positive correlation between calculated abundances of Bacteroidota and FISH counts in Kozjak was confirmed with Spearman's rank correlation coefficient, while Verrucomicrobiota and FISH counts had a negative correlation (Figure S6A).

Our microbial diversity analysis speculates that several genera are selfish organisms in Lake Kozjak. Pullulan was likely taken selfishly by *Candidatus Aquirestis* and *Emticicia* in spring, *Dinghuibacter* and *Sediminibacterium* in summer, *Pseudarcicella* in winter, and *Candidatus Methylopumilus* and *Methylotenera* in summer and winter. Meanwhile, xylan was selfishly utilized by *IMCC26134* in summer, *Chthoniobacter* in spring and winter, *LD29* in winter, and *Acinetobacter* and *Limnohabitans* in summer and winter. *Pirellula* took up both polysaccharides selfishly in winter (Figure S7A).

In Crniševo, Verrucomicrobiota showed selfish uptake of all polysaccharides (Figures 6B and 7A). Surprisingly, there was no selfishly active Bacteroidota in any of the incubations. The remaining bacteria (EUB-I) and Planctomycetota showed a little selfish uptake of pullulan and xylan across the seasons and high selfish activity for fucoidan in the summer (Figure 7A). In total, bacteria mentioned above accounted for 78% of the total selfish bacteria in Crniševo.

The calculated absolute abundance showed that Verrucomicrobiota were most abundant (Figures 6D and 7B), corresponding to their selfish activity. Planctomycetota had a higher abundance in summer in all incubations and were not present in the other seasons. Furthermore, Gammaproteobacteria had a low abundance in the summer but a higher abundance in the spring and winter incubations of pullulan and xylan (Figures 6B and 6D). A positive correlation between the calculated abundances of Verrucomicrobiota and Planctomycetota with FISH counts in Crniševo was confirmed with Spearman's rank correlation coefficient, while Gammaproteobacteria had a negative correlation (Figure S6B).

Based on our microbial diversity analysis, we speculate that pullulan and xylan were selfishly taken up by *LD29* and *Prosthecobacter* in summer, *Luteolibacter* and *Acinetobacter* in spring and winter, and *Limnohabitans* in winter or spring (Figure S7B). During summer, all polysaccharides were likely selfishly taken up by *Luteolibacter* and *Limnohabitans* (Figure S7C). *Pirellula* was selfishly active for all polysaccharides (Figures S6B and S6C), while *Blastopirellula* was active during summer (Figure S6C).

DISCUSSION

In this study, we reveal the temporal dynamics of selfish polysaccharide utilization in two karst lakes with different trophic

Figure 6. Comparison of FISH results, FLAPS-specific staining, and absolute abundance of singled out phyla microbial diversity analysis in pullulan and xylan incubations

(A and B) Absolute cell counts of substrate-stained cells (%) enumerated by FISH in pullulan and xylan incubations in different seasons in Kozjak (A) and Crniševo (B). Group-specific FISH probes EUB-I, EUB-II, EUB-III, and CF319a target remaining bacteria (EUB-I), Planctomycetota, Verrucomicrobiota, and Bacteroidota. Probes are color coded, and unidentified selfish cells are colored gray and labeled as uncovered FLAPSs.

(C and D) Absolute abundance of phyla Bacteroidota, Planctomycetota, and Verrucomicrobiota and class Gammaproteobacteria in pullulan and xylan incubations in Kozjak (C) and Crniševo (D) calculated from normalization of 16S sequencing results and total cell counts.

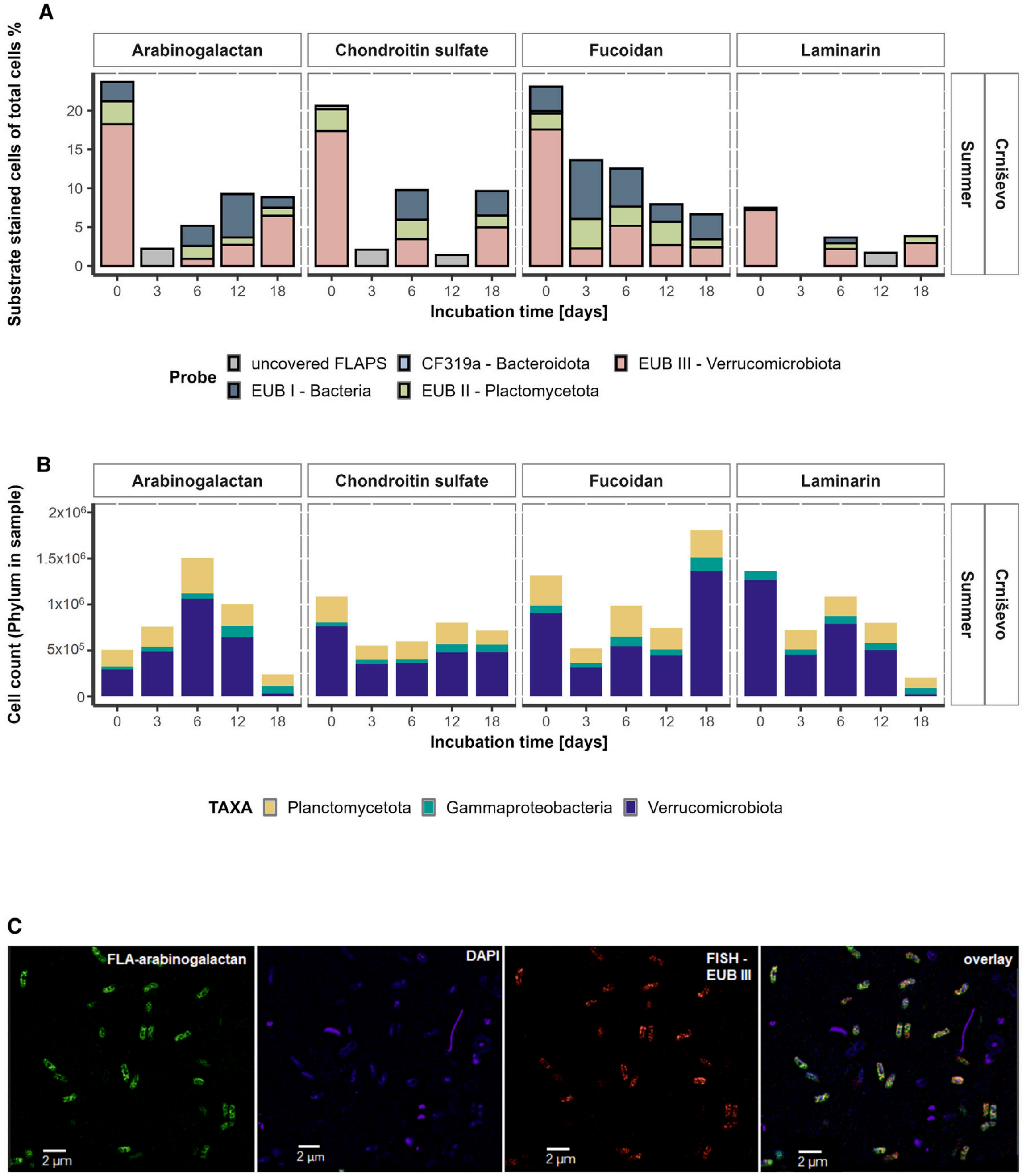

Figure 7. Comparison of FISH results, FLAPS-specific staining, and absolute abundance of singled out phyla from microbial diversity analysis in summer in Crniševo, with shown selfish bacteria in Lake Crniševo

(A) Absolute cell counts of substrate-stained cells (%) enumerated by FISH in incubations with different polysaccharides in summer in Crniševo. Group-specific FISH probes EUB-I, EUB-II, EUB-III, and CF319a target remaining bacteria (EUB-I), Planctomycetota, Verrucomicrobiota, and Bacteroidota. Probes are color coded, and unidentified selfish cells are colored gray and labeled as uncovered FLAPSs.

(B) Absolute abundance of phyla Planctomycetota and Verrucomicrobiota and class Gammaproteobacteria in summer Crniševo calculated from normalization of 16S sequencing results and total cell counts.

(C) SR-SIM of cells stained by FLA-arabinogalactan, DAPI, and FISH EUB-III probe and an overlay of all signals in Lake Crniševo, summer, day 0. White scale bar: 2 µm.

statuses. We show, for the first time, that selfish organisms are active in freshwater ecosystems, quantifying their rate of activity and identifying them taxonomically (Figure 7C).

Seasonal dynamics of autochthonous and allochthonous polysaccharides' selfish uptake in two lakes

Lake trophic status affects microbial enzyme activity[26] as DOM degradation releases nutrients.[8,9] DOM origin and composition differ between oligotrophic and mesotrophic lakes, shaping microbial communities with distinct enzymatic capabilities. In our study, selfish organisms formed a small community fraction but processed six diverse polysaccharides, showing peak selfish activity in winter in the oligotrophic lake and in summer in the mesotrophic lake (Figures 3A and 3B).

Oligotrophic lake Kozjak,[27] influenced by a continental climate, has a low DOC and primary production rates,[28] with carbon-rich DOM leaching from surrounding coniferous soil.[29,30] Winter increases in DOC (Figure 1B) and nitrate (Table S1) suggest substantial allochthonous, likely terrestrial, DOM input.[22] High selfish activity, common in nutrient-limited environments like oligotrophic marine gyres and the deep sea,[15,31,32] was observed in Kozjak during winter (Figure 3A), probably triggered by complex DOM from soil runoff during early spring bloom, when bacteria react to new organic inputs.[20] Increased allochthonous input in winter leads to higher selfish activity for xylan (Figure 3A), a plant cell polysaccharide,[33] similar to observations in the northern Adriatic.[34]

In contrast, the highest selfish activity was observed in mesotrophic Lake Crniševo during summer (Figure 3B). Mild winters maintain stable temperatures year round (Figure 1B) and elevated nutrient levels (Table 1), characteristic of mesotrophic lakes[27] with higher microbial diversity, polysaccharide concentrations, and enzymatic activity.[21] High DOC levels year round suggest mixed autochthonous and allochthonous DOM sources, with summer O_2 peaks (Figure 1B) indicating increased phytoplankton activity[28] and autochthonous DOM influence.[22] This summer phytoplankton bloom provides abundant DOM, driving higher selfish activity.[20] Seawater inflow through porous karst[22] also affects microbial communities, evident in higher selfish activity for fucoidan and laminarin (Figure 3B), which are produced by phytoplankton and macroalgae.[35] High external hydrolysis rates for these polysaccharides have been documented in oligotrophic lakes[21] along with selfish uptake of laminarin in marine environments.[11,14,17,20,32] Higher selfish activity for these polysaccharides was expected; however, bacteria may instead utilize them through alternative mechanisms. Further research is needed to fully understand bacterial polysaccharide degradation in freshwater ecosystems. For instance, the abundance of Bacteroidota suggests that they respond to available polysaccharides in the ecosystem, yet the lack of selfish behavior may indicate they utilize FLAPSs through alternative mechanisms, warranting further research to clarify these pathways and interactions.

Bacteria showed the highest selfish activity for pullulan in both lakes, despite its low selfish uptake in surface marine environments[20,32] and external degradation in eutrophic, but not oligotrophic, lakes.[21] Pullulan, a simple polysaccharide produced by fungi as an extracellular coating agent,[36] is found in diverse environments, including fresh water, soil forests, and plant leaf surfaces.[37] Increased pullulan uptake occurred during periods of higher allochthonous DOM input in lakes (Figures 3A and 3B), reflecting previous analysis of pullulan processing in the deep ocean[32] where fungi comprise a significant fraction of bathypelagic marine snow particles.[38] This trend is also observed in terrestrial-influenced environments, such as the highly river-influenced north Adriatic.[34]

Predictions of selfishly active bacteria in freshwater lakes

The composition of the heterotrophic microbial community differed with lake trophic status and season. Higher trophic lakes, such as mesotrophic Crniševo, have a higher diversity.[39] During periods between two phytoplankton blooms, when nutrients peak, the community richness increases,[40] but diversity decreases, indicating the presence of only specific bacteria[41] (Table S2).

Comparing FISH results, FLAPS-specific staining, and 16S sequencing allowed us identify selfishly active bacteria in karst lakes. Several genera from the phyla Bacteroidota, Planctomycetota, and Verrucomicrobiota and the class Gammaproteobacteria were identified as selfishly active in fresh water (Figures 6, 7, and S7), with variations observed according to lake, season, and polysaccharide type.

Bacteroidota, particularly the genus *Flavobacterium*, depend on DOC input and showed increased abundance and selfish activity in Kozjak, likely due to high external DOC loading.[42,43] Similar to their role in marine and gut ecosystems, where Bacteroidota degrade complex DOM and selfishly take up laminarin,[20] they perform similar function in lakes, showing seasonal selfish uptake of pullulan (Figures 6A and 6C). Thus, genera *Candidatus Aquirestis* and *Dinghuibacter* were active during periods of high DOC from algal blooms or external inputs.[44] *Emticicia* exhibited selfish activity after the spring phytoplankton bloom, whereas *Pseudarcicella*, typically linked to phytoplankton blooms, was active beforehand (Figure S7A). Additionally, *Sediminibacterium*, known for carbon uptake in soil surrounding freshwater rivers,[45] likely possesses enzymes for pullulan degradation (Figure S7A), a polysaccharide most commonly associated with the fungus *Aureobasidium pullulans*.[46]

During summer, Planctomycetota increased in abundance (Figure S4) due to phytoplankton blooms, which provide a nutrient source.[47] Previously identified as selfishly active in marine environment,[11,15,20,32] many Planctomycetota possess sulfatase genes and are involved in the decomposition of high-molecular-weight dissolved organic carbon (HMW-DOC) molecules, such as sulfated polysaccharides from algae,[48] and thus are more abundant in Crniševo. The genus *Pirellula* is abundant and selfishly active in both marine[49] and freshwater habitats[50] (Figures 6 and 7), while members of *Blastopirellula*, adapted to saltwater habitats,[50] showed selfish activity during seawater intrusion[22] (Figures S7B and S7C).

The composition of the Verrucomicrobiota community is primarily driven by DOC input, leading to higher abundances during elevated DOC levels (Figure S4) and variations in the dominance of genera and selfish activity between the two lakes, attributed to extensive carbohydrate utilization genes.[51] For instance, genus

Luteolibacter, associated with land plant rhizospheres,[52] was prevalent in allochthonous-influenced Kozjak and demonstrated selfish activity[53] for all polysaccharides in both lakes, along with the polysaccharide-degrading genus *LD29*.[54] Genus *LD29*, capable of utilizing phytoplankton-produced carbon polysaccharides, played a significant role in the selfish uptake of autochthonous polysaccharides.[55] In the autochthonous-influenced Crniševo, *Prosthecobacter*, associated with planktonic algae,[56] dominated and was overall selfishly active there (Figures 5B, S7B, and S7C), thanks to its diverse gene repertoire for degrading complex carbohydrates.[50] Additionally, genera *Chthoniobacter* and *IMCC26134* were selfishly active (Figure S7A), reflecting their roles in soil microbiome and carbohydrate degradation.[57,58]

Proteobacteria, characteristic of oligotrophic lakes[43] and karst waters,[59] dominated the initial communities in both lakes (Figure 4A). Alphaproteobacteria became abundant in phytoplankton blooms, especially genus *Sphingorhabdus* (Figure 5), which are known for metabolizing organics.[60] Gammaproteobacteria were initially abundant after phytoplankton bloom (Figure S4), with genera *Candidatus Methylopumilus* and *Acinetobacter* dominating (Figure 5), both associated with biodegradation.[61] In our study, we observed a large proportion of positive polysaccharide staining with the EUB-I probe, which we assume to be bacteria from the class Gammaproteobacteria, known for selfish uptake in marine environments,[17] particularly genera *Acinetobacter* and *Limnohabitans* (Figure S7). Genus *Acinetobacter* is known for its ability to utilize polysaccharides in fresh water,[62] while genus *Limnohabitans*, a phytoplankton colonizer, actively degrades algal exudates, proteins, and polysaccharides,[63] especially autochthonous (Figure S7). A negative correlation between FISH counts and the calculated absolute abundance of Gammaproteobacteria and Verrucomicrobiota was observed. This could have several explanations: one possibility is that cells from growing members of these phylogenetic groups are not well covered by the FISH probe used but are detected by the primers used for amplification and sequencing of the 16S rRNA gene. This could lead to an increase in their calculated absolute abundance, while FISH counts remain unchanged. Alternatively, if cells of a specific subpopulation are not active, then they might not be effectively detected by FISH due to a low ribosome count in the cell, despite being amplified and detected in 16S rRNA gene sequencing. On the other hand, it is also possible that cells from a specific group are covered by the FISH probe but not by the primers used for 16S rRNA gene amplification, which could result in an increase in FISH counts while the abundance based on amplicon sequencing remains unchanged or even decreases. These differences in quantification methods—such as varying coverage by probes or primers, as well as physiological differences in bacterial groups regarding the number of ribosomes—could explain the observed negative correlations.[64] Unfortunately, differences in primer vs. probe coverage cannot be evaluated for these specific groups, as both FISH probes target regions (the Gamma 42a Probe even targets the 23S rRNA) outside of the amplified and sequenced V4 16S rRNA gene region.

Conclusion

The study on polysaccharide utilization carried out in two lakes revealed the presence of a selfish uptake and utilization mechanism in freshwater ecosystems. The selfish activity varied depending on the trophic status of the lakes, with higher trophic lakes exhibiting greater microbial diversity and increased selfish uptake. A temporal influence on microbial communities and selfish activity within the lakes was visible, with selfish uptake being the most widespread among polysaccharides during phytoplankton blooms (Crniševo) or intense terrestrial influence (Kozjak). Bacteria from phyla Bacteroidota, Planctomycetota, and Verrucomicrobiota and class Gammaproteobacteria were found to be associated with selfish activity. These bacteria exhibited varying degrees of selfish uptake across different lakes and seasons, suggesting a complex interplay between microbial community structure and selfish behavior. Overall, this study emphasizes the importance of considering selfish mechanisms in nutrient cycling in freshwater ecosystems. However, our results also show that many bacteria responded to added polysaccharides without exhibiting the selfish mechanism. This suggests that certain bacteria may rely on alternative, extracellular mechanisms for polysaccharide degradation, opening new avenues for our future research.

Furthermore, genomic analysis of the genera we have identified here would be invaluable in understanding their potential for selfish activities. While the SusCD system is a known transporter associated with selfish behavior, it remains unclear which other transporters in freshwater bacteria might contribute to this phenomenon. Investigating these systems could lead to the discovery of novel transport mechanisms that differ from those in marine or gut environments and expand our understanding of bacterial polysaccharide utilization in lakes.

Limitations of the study

Although our study provides novel insights into selfish polysaccharide utilization in freshwater lakes, a few limitations should be noted. The use of 16S rRNA gene sequencing and FISH limited taxonomic resolution, potentially overlooking some taxa involved in selfish activity. Incubation experiments with FLAPSs simplified natural DOM conditions and may not fully capture *in situ* microbial dynamics. Additionally, the focus on six model polysaccharides, while informative, may not represent the full diversity of DOM in freshwater systems. Future studies integrating multi-omics approaches and broader environmental sampling could further elucidate the mechanisms and ecological relevance of selfish uptake.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Greta Reintjes (reintjes@uni-bremen.de).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Bacterial 16S rRNA gene sequences were archived as Illumina-generated libraries at the EBI-EMBL ENA database under the accession number EBI-EMBL: PRJEB74624; https://www.ebi.ac.uk/ena/browser/view/PRJEB45634.
- All original code has been deposited at GitHub and is publicly available at https://github.com/Andrea2710-afk/Selfishbacteria as of the date of publication.

- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

A.C. thanks Dr. Rudolf Amann and Dr. Bernhard M. Fuchs for their hospitality at the Max Planck Institute for Marine Microbiology in Bremen. The authors would also like to thank Dr. Lorena Selak, Dr. Maja Mitrović, and Ivana Stanić for their help with the incubations. We thank Dr. Tamara Marković for DOC measurements. A.C. was partially supported by DAAD Research Grants - Short-Term Grants (57681230) and FEMS Research and Training Grant (ID 2765). G.R. is funded by the German Research Foundation (DFG) through project no. 496342779. This research was partially supported under the project by the Croatian Science Foundation through grant HRZZ IP-2020-02-9021.

AUTHOR CONTRIBUTIONS

Conceptualization, A.C., S.O., and G.R.; data curation, A.C., P.P., S.O., and G.R.; formal analysis, A.C. and P.P.; investigation, A.C.; visualization, A.C.; writing – original draft, A.C.; writing – review & editing, P.P., S.O., and G.R.; methodology, P.P., S.O., and G.R.; funding acquisition, S.O. and G.R.; project administration, S.O.; resources, S.O. and G.R.; supervision, S.O. and G.R.; validation, G.R. All of the authors read and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2025.115415.

Received: September 4, 2024
Revised: November 22, 2024
Accepted: February 18, 2025
Published: April 10, 2025

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Fluoresceinamine	Sigma-Aldrich; isomer II	CAS No.: 51649-83-3
Arabinogalactan	Sigma-Aldrich	CAS No.: 9036-66-2
Chondroitin sulfate	Sigma-Aldrich	CAS No.: 9082-07-9
Fucoidan	Sigma-Aldrich	CAS No.:9072-19-9
Laminarin	Sigma-Aldrich	CAS No.: 9008-22-4
Pullulan	Sigma-Aldrich	CAS No.: 9057-02-7
Xylan	Sigma-Aldrich	CAS No.:9014-63-5
Formaldehyde	Emprove Evolve	CAS No.: 50-00-0
H_3PO_4	Sial	CAS No.: 7664-38-2
4′,6-diamidin-2-phenylindol (DAPI)	Sigma-Aldrich	CAS No.:28718-90-3
Citifluor	Citifluor, Electron Microscopy Science	Cat No.: 17970-100
VectaShield	Vector laboratories	H-1000-10
NaCl	Carl Roth	CAS No. 7647-14-5
Tris-HCl	Cayman Chemical	600200
Sodium dodecyl sulfate	Sigma-Aldrich	CAS No.: 151-21-3
Maleic acid	Sigma-Aldrich	CAS No.: 110-16-7
EDTA	Carl Roth	CAS No. 6381-92-6
Formamide	Sigma-Aldrich	CAS No.: 75-12-7
Critical commercial assays		
DNeasy PowerWater kit	Qiagene	14900-100-NF
innuPREP PCRpure Kit	Analytik Jena	845-KS-5010250
Deposited data		
16S rRNA sequencing data	ENA (EBI-EMBL)	PRJEB74624; https://www.ebi.ac.uk/ena/browser/view/PRJEB45634
Analysis code	GitHub	https://github.com/Andrea2710-afk/Selfishbacteria
Oligonucleotides		
EUB338-I	Amann et al.[65]	S-D-Bact-0338-a-A-18
EUB338-II	Daims et al.[66]	S-*-BactP-0338-a-A-18
EUB338-III	Daims et al.[66]	S-*-BactV-0338-a-A-18
CF319a	Manz et al.[67]	pB-42
Primer: 515F Parada	Parada et al.[24]	N/A
Primer: 806R Apprill	Apprill et al.[68]	N/A
Software and algorithms		
BBDuk	Bushnell[69]	N/A
demultiplex	Laros JFJ, github.com/jfjlaros/demultiplex	N/A
DADA2	Callahan et al.[70]	N/A
SILVA database	https://www.ncbi.nlm.nih.gov/pubmed/23193283[71]	N/A
SINA	https://www.ncbi.nlm.nih.gov/pubmed/22556368[72]	N/A
phyloseq	McMurdie and Holmes[73]	N/A
vegan	Oksanen et al.[74]	N/A
dplyr	Wickham et al.[75]	N/A
ggplot2	Wickham[76]	N/A
BBTools	Bushnell[69]	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
demultiplex	Laros JFJ, github.com/jfjlaros/demultiplex	N/A
ZEN2011 software	Carl Zeiss	N/A
ACMETOOL software	https://www.mpi-bremen.de/automated-microscopy.html#section19794	N/A

METHOD DETAILS

Sampling locations

Two natural karst lakes were sampled in Croatia: Kozjak and Crniševo (Figure 1A). Lake Kozjak is the largest and deepest of the Plitvice Lakes, a tufa-forming freshwater network ecosystem consisting of streams and 16 cascading lakes interconnected with waterfalls. The Plitvice Lakes are in western Croatia's Dinaric Karst region, characterized by a continental climate featuring cold winters and dry summers. Lake Crniševo is the deepest lake from a group of cryptodepression lakes (Baćina Lakes) located in southern Mediterranean Croatia, characterized a Mediterranean climate with hot, dry summers and mild, wet winters. Lake Crniševo has a partial inflow of seawater through the porous karst.[22] Water samples were collected in spring, summer 2022 and winter 2023 at 10 m depth using Ruttner water sampler 3L (KC Denmark).

Physicochemical measurements

A multisensor probe (EXO3, YSI, USA) was used to measure dissolved oxygen (O_2), temperature, pH, conductivity, and salinity *in situ*. Concentrations of major cations (Ca^{2+}, Mg^{2+}, and Na^{+}) and anions (Cl^{-}, SO_4^{2}, NO_3^{-}) in filtered water samples were measured on an ion chromatography system Dionex ICS-1100 (Sunnyvale, CA, USA). DOC concentrations in filtered water samples were quantified using the HACH QBD1200 analyser. Prior to analysis, dissolved inorganic carbon (DIC) was first removed (i.e., converted to CO_2 and outgassed) by adding H_3PO_4. DOC analyses were performed in the Hydrochemical Laboratory of the Croatian Geological Survey.

Fluorescently labeled polysaccharide incubations

At each sampling point, triplicates of 1 L water samples were collected in sterile, acid-rinsed bottles. These samples were incubated in the dark at *in situ* temperature for up to 18 days with one of six fluorescently labeled polysaccharides (FLAPS): arabinogalactan, chondroitin sulfate, fucoidan, laminarin, pullulan, and xylan. Each polysaccharide was added at a concentration of 3.5 μmol monomer-equivalent. The selected FLAPS and their concentration were deliberately chosen for the experiment to enable a meaningful comparison between freshwater and marine environments. Additionally, a control sample consisting of freshwater without the addition of any polysaccharide was also incubated under the same conditions. Subsamples were taken from each incubation at 0, 3, 6, 12, and 18 days for microbial community composition analysis. This analysis was done by 16S rRNA gene amplicon sequencing, selfish activity quantification, and fluorescence *in situ* hybridization (FISH). For DNA-based microbial diversity analysis, 50 mL of water was filtered onto a 0.22-μm-pore size PC filter (Whatman Nuclepore Track-Etch membrane; diameter, 47 mm) and immediately stored at −20°C until DNA extraction. Subsample taken at day 0 was filtered within 2 h of adding FLAPS to lake sampled water.

Additionally, negative controls were also incubated under the same conditions. Negative controls included autoclaved water samples incubated with one of six FLAPS. Microscopic images confirmed non-existence of cells in samples and no degraded FLAPS. (Figure S8).

Microbial diversity analysis by 16S rRNA gene amplicon sequencing

Total genomic DNA was extracted with the DNeasy PowerWater kit (Qiagen, Inc., Valencia, CA, USA) following the manufacturer's protocol. The hypervariable V4 region of the prokaryotic 16S rRNA gene was amplified by PCR using primer pair 515F Parada (5′-GTG YCA GCM GCC GCG GTA A-3′)[24] and 806R Apprill (5′-GGA CTA CNV GGG TWT CTA AT-3′),[68] modified with two 16 bp head sequences, which allowed for sample barcoding in a second PCR step at the Joint Microbiome Facility of the of the Medical University of Vienna and the University of Vienna, under project IDs JMF-2212-13 and JMF-2304-03. Samples were amplified, barcoded, purified, normalized PCR amplicons were multiplexed concentrated with the innuPREP PCRpure Kit (Analytik Jena) and sequenced on an Illumina MiSeq System (v3 chemistry, 2 × 300 bp).[77]

Individual amplicon pools were extracted from the raw sequencing data using the FASTQ workflow in BaseSpace (Illumina) with default parameters, allowing one mismatch for the 6-nucleotide (nt) library indexes. The input data were filtered for PhiX contamination with BBDuk (BBTools).[69] Further demultiplexing of each amplicon pool library into single amplicon libraries was performed with the Python package demultiplex (Laros JFJ, github.com/jfjlaros/demultiplex), allowing one mismatch for barcodes and two mismatches for linkers and primer sequences, respectively. FASTQ reads were trimmed at 150/220 nt with allowed expected error 2. Amplicon sequence variants (ASVs) were inferred using the DADA2 R package version 1.14.1[70] with R version 3.6.1[78] applying the recommended workflow[78] in pooled mode using all amplicon libraries per sequencing run. Finally, taxonomy was assigned to

ASVs based on SILVA database SSU Ref NR 99 release 138.1 (https://www.ncbi.nlm.nih.gov/pubmed/23193283)[71] using SINA version 1.6.1 classifier (https://www.ncbi.nlm.nih.gov/pubmed/22556368).[72]

Super-resolution imaging of selfish polysaccharide uptake

The FLAPS uptake was visualized on a Zeiss ELYRA PS.1 (Carl Zeiss) using 561, 488, and 405 nm lasers and BP 573–613, BP 502–538, and BP 420–480+LP 750 optical filters. z stack images were taken with a Plan-Apochromat 63 ×/1.4 Oil objective and processed with ZEN2011 software (Carl Zeiss).

QUANTIFICATION AND STATISTICAL ANALYSIS

Selfish activity quantification and FISH by microscopy

Filtered and fixed water samples were counterstained using 4′,6-diamidin-2-phenylindol (DAPI) and mounted with a Citifluor/VectaShield (4:1) solution. Polysaccharide-stained cells were visualized and enumerated using a fully automated microscope imaging system on a Zeiss AxioImager.Z2 microscope stand (Carl Zeiss Micro Imaging GmbH, Göttingen, Germany) equipped with a cooled charged-coupled-device (CCD) camera (AxioCam MRm + Colibri LED light source, Carl Zeiss), three light-emitting diodes (UV-emitting LED, 365 ± 4.5 nm for DAPI; blue emitting LED, 470 ± 14 nm for FLAPS488; red-emitting LED, 590 ± 17.5 nm for the tyramide Alexa 594, FISH) combined with the HE-62 multifilter module consisted of a triple emission filter (425/50 nm,527/54 nm, LP 615 nm, including a triple beam splitter of 395/495/610, CarlZeiss). Images were taken automatically of a minimum of 30 fields of view per filter using a 63X magnification oil immersion plan apochromatic objective with a numerical aperture of 1.4 (Carol Zeiss) at selected wavelengths (DAPI, FLAPS, FISH).[79] Fixed exposure times were used for imaging ranging from 30 to 100 ms for DAPI, 40 and 150 ms for FLAPS, and 200 to 250 ms for FISH. Cell quantification was performed using the ACMETOOL software (https://www.mpi-bremen.de/automated-microscopy.html#section19794).[11] Briefly, a positive signal in DAPI and either FISH or FLAPS images with a minimum overlap of 30%, as well as a minimum signal background ratio of 1, were required for positive identification as a FISH or FLAPS-stained cell. An overlap of all three signals indicated an FLAPS-stained cell identified by a specific FISH probe.

FISH was carried out on filter sections with a selection of probes to quantify the abundance of specific phylogenetic groups. The hybridization buffer was composed of 900 mM NaCl, 20 mM Tris-HCl (pH 7.5), 0.02% sodium dodecyl sulfate, 10% dextran sulfate (wt/vol), and 1% (wt/vol) blocking reagent (Boehringer; Mannheim, Germany), with 35% formamide concentration for all used probes. All hybridizations were carried out at 46°C in a humidity chamber for 3 h, followed by a wash in a buffer containing 700 mM NaCl, 20 mM Tris/HCl (pH 8), 5 mM EDTA (pH 8), and 0.01% sodium dodecyl sulfate at 48°C. Four times Atto-594 labeled oligo-probes were used for detection of all bacteria. The domain specific probe EUB338-I was used for detection of bacteria in general,[65] and EUB388-II and EUB338-III for specifically targeting Planctomycetota and Verrucomicrobiota.[66] Probes EUB388-II and EUB 338-III have an overlap of converging,[66] resulting in certain bacteria being positive for both probes. Subsequently, probe CF19a was used for detecting Bacteroidota.[67] Detailed information about used probes are listed in Table S4.

Each image was recorded on the auto signal, and all bacteria that had a positive signal on the auto, DAPI and FLAPS channels were recognized as Cyanobacteria and excluded from the calculations for selfish uptake (Figure S1). Due to poor filter quality and lack of DAPI staining spring samples from lake Kozjak at day six from arabinogalactan, chondroitin sulfate, laminarin and pullulan incubation were not analyzed. As well as summer samples from lake Kozjak at day six from control incubation, arabinogalactan and laminarin incubations.

Statistical analysis

Statistical analyses were performed in the R environment (version 4.3.0)[80] using the packages phyloseq,[73] vegan,[74] dplyr[75] and ggplot2.[76]

The mean values for total cell counts and the proportion of the community showing selfish polysaccharide uptake were calculated using dplyr[75] package. Mean and standard deviation were calculated for each group, where the groups were defined by lake, sampling season and different polysaccharide incubation. Three filter replicates were done for each sample. The calculated mean values are represented in Figures 2 and 3.

Prior to statistical analysis, ASV classified as eukaryotes, mitochondria, or chloroplasts, as well as unassigned ASVs at the phylum level, singletons, and doubletons were removed from the dataset. Additionally, samples Kozjak, summer, chondroitin sulfate day 0 as well as Kozjak, winter, control day 6, chondroitin sulfate day 0, and laminarin days 6 and 12 were removed from the dataset due to low read numbers (<1000 reads). Filtering was performed with phyloseq[73] package. After filtering, the final dataset consisted of 205 samples.

Alpha diversity was computed by rarefaction of a subsampled dataset to the smallest library size (1024) and estimated as diversity according to the Shannon index[81] and richness, with a function to find the numbers of species, *specnumber*, in vegan[74] package. Alpha diversity analysis results are presented in Table S2. Beta diversity analysis was done using Bray-Curtis dissimilarity matrices of the total bacterial community of each sample and visualized in non-metric multi-dimensional scaling plots (NMDS) using phyloseq[73] package. Beta diversity results were visualized with ggplot2[76] package and presented in Figure 4B. The function *envfit* of the package vegan[74] was applied to the results of NMDS to visualize the correlations with environmental factors. Results were visualized with ggplot2[76] package and presented in Figure S3. Tests for significant differences in community composition between different lakes, sampling seasons, incubation time, and polysaccharides were performed by analysis of similarity (ANOSIM). Results are provided in the Results section and in Figure 4. Prior to analyses for bacteria involved in FLAPS selfish uptake, Archaea and Cyanobacteria were excluded. The taxonomic abundance of the initial community was studied at the phylum level including all taxa with relative abundance greater than 1%. The level used for this threshold was the phylum level to effectively capture the community composition. Results were visualized with ggplot2[76] package and are presented in Figure 4A. Next, we identified and selected genus-specific sequences with relevant changes compared to the initial community composition on genus level. The abundances of sequences were tracked through incubation time. Results were visualized with ggplot2[76] package and presented in Figure 5 and Figures S4 and S7. Finally, for comparison of FISH results with results of microbial diversity analysis absolute abundances of chosen phyla were calculated (Table S3). Absolute abundance was calculated based on formula:

$$\textit{Cell count} = \textit{Total cell count}\ (\textit{Sample } Y) \times \frac{\textit{Cumulative relative abundance}\ (\textit{Phylum } X \textit{ in sample } Y)}{\textit{Average } 16S\ \textit{rRNA gene copy number}\ (\textit{Phylum})}$$

(1) Total cell count represents cell counts counted during DAPI, FLAPS and FISH staining. Total cells number were counted with epifluorescence microscope and are shown in Figure 2.
(2) Cumulative relative abundance was calculated by summing up of all relative abundances of certain phylum in chosen sample.
(3) Average 16S rRNA gene copy number taken from Ibal et al.[82].

Results were visualized with ggplot2[76] and presented in Figures 6 and 7. Finally, Spearman's rank correlation was calculated between FISH results and calculated absolute abundances of chosen phyla, visualized with ggplot2[76] package and are presented in Figure S6.