The following supplement accompanies the article

Interspecific interactions drive chitin and cellulose degradation by aquatic microorganisms

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Supplement.

Summary: Brief description of the model strains, of the applied CARD-FISH methodology and of DOC/TOC standard measurements. Analysis of competitive interactions between bacterial strains. Protist abundances and predation impact on the bacterial strains and communities. Morphological distribution of bacteria in the different treatments.

Ecology and physiology of the microbial model strains

Our simplified resident community comprised four bacterial species characterized by differing ecology, physiology and grazing resistance. *Flectobacillus* was selected as a member of our resident community because of its ability to avoid protozoan grazing by developing long filaments (Corno & Juergens 2006). In nature it is widely distributed in European freshwaters (Newton et al. 2011) where its abundance is usually strongly limited by competition. The Gram-positive actinobacteria strain *A. agilis* occurs occasionally in freshwaters and is limited by its restricted substrate uptake abilities (Hervàs et al. 2009). In experimental systems, it appears to be well protected against grazing because of its cell wall properties and its peculiar aggregation behaviour (Corno et al. 2013). In terrestrial systems, it has been widely studied as a plant pathogen, but its ecological role in freshwater ecosystems is little known. In contrast, *Brevundimonas* spp. (Alphaproteobacteria) are common in freshwaters where they can grow even in low carbon conditions (Newton et al. 2011). Abundance of *Brevundimonas* sp. strain GC044 is controlled by predation in co-cultures with flagellates. The fast-growing *A. hydrophila* (Gammaproteobacteria) is widespread and abundant in freshwaters (Jagmann et al. 2010). While generally considered unable to actively develop anti-predation strategies, it is potentially motile and can also switch to a facultative anaerobe metabolism. The selected *Burkholderia* strain can be considered a potentially good competitor in our systems, as the presence of several peroxidase genes in the genomes of closely related strains of *Burkholderia* suggests an ability to use complex OM substrates. The mixotrophic flagellate *Poterioochromonas* sp. strain DS, chosen as protist predator in our experiment, is easy to cultivate and can attach to surfaces such as aggregates and bacterial microcolonies (Rothhaupt 1997). Being efficient interception-feeders, many members of the family *Ochromonadaceae* (e.g. *Poterioochromonas*) can be considered well-suited model organisms to study the impact of predation by mixotrophic flagellates on bacteria (Rothhaupt 1997). These organisms can also alter substrate chemistry by releasing a complex mixture of secondary metabolites during grazing (Blom et al. 2010), including chemical cues that can affect bacterial growth and morphology (Corno et al. 2013).

CARD FISH Protocol and DOC/TOC Determination

15 ml samples for CARD FISH counts were concentrated on 0.2 µm polycarbonate filters (type GTTP, diameter 47 mm, Millipore). Filters were air-dried and stored at -20°C until further processing. CARD-FISH was performed according to Sekar et al. (2003) with the following modifications: Filters were fixed in 50% ethanol for one hour and subsequently embedded in agarose gel solution and dried. Afterwards, filters were consecutively dehydrated with 80% and 96% ethanol for 5 min each before cells were permeabilized first in lysozyme solution and then in achromopeptidase solution. The following horseradish peroxidase-labelled probes were used to determine the relative proportions of specific bacterial populations: ALF968 for *Brevundimonas* sp., Alphaproteobacteria (Glöckner et al. 1998), BET42a for *Burkholderia* sp., Betaproteobacteria (Glöckner et al. 1998) and GAM42a for *A. hydrophila*, Gammaproteobacteria (Manz et al. 1992) both mixed with the corresponding competitor probe, CF319a for *Flectobacillus* sp., Cytophaga-Flavobacteria (Manz et al. 1996), and HGC69a for *A. agilis*, Actinobacteria (Roller et al. 1994). Subsequently, bacteria were stained with DAPI (final concentration 1 µg l^{-1}) and the relative proportions of hybridized cells were determined by epifluorescence microscopy (AxioPlan, Zeiss, Germany) equipped with filter set 62 HE (Zeiss). Imaging was performed using a HD camera (DP72, Olympus) and the image analysis software Image Pro-Plus. At least 500 cells were counted per sample. In all samples, the overall CARD-FISH detection rate was 96-100 % of the total bacterial number (mean 98.3±4.6 %).

Total (TOC) as well as dissolved organic carbon (DOC) were determined by the method of Wetzel & Likens (1991). Samples for DOC analysis were filtered through 0.2 □m Nuclepore Track-Etch polycarbonate membranes (Whatman), which were pre-rinsed with double distilled water. Filtered (DOC) or non-filtered (TOC) samples were acidified with 1% (v/v) of 2 mol l^{-1} HCl, and TOC/DOC was determined as the non-purgeable organic carbon by an organic carbon analyzer (multi N/C 3100, Analytic Jena AG).

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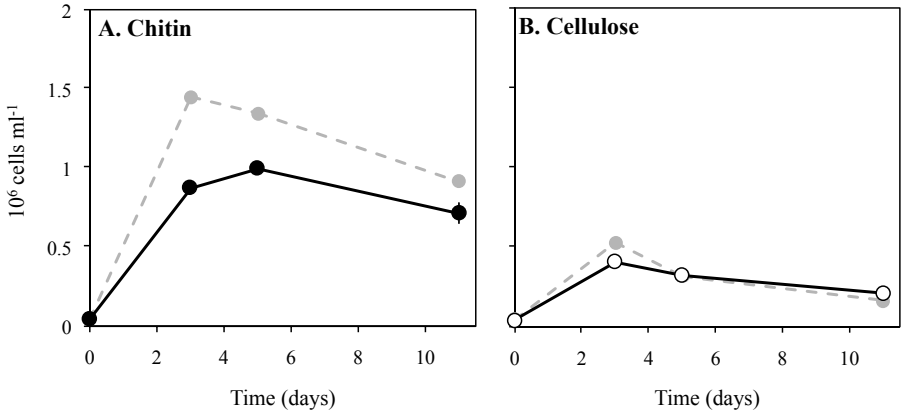
Figure S1: Competitive interactions in mixed cultures. Points show average (±s.e., *n*=3) total population densities in mixed cultures in the Control treatment (dark lines, black circles for chitin; dark lines, white circles for cellulose) compared to predictions from a null model of no competition (dashed lines, grey circles) in (A) chitin and (B) cellulose cultures.



Following Foster & Bell (2012), the null model of no competition among species predicts that each performs in a mixed community exactly as if it were growing in pure culture, so that total population density in a mixed community equals the sum of observed pure culture densities. Competitive interactions result in an observed density lower than the null prediction, and cooperative interactions result in an observed density greater than the null prediction (Mitri & Foster 2013). Total population densities in every mixed culture with chitin were lower than those predicted by the null model, indicating competitive interactions. By contrast, there was no significant evidence of competitive interactions in cellulose cultures: mixed-culture densities were lower than null predictions only after 3 days, but this was not statistically significant after sequential Bonferroni correction to account for multiple testing (P=0.04 at □=0.016).

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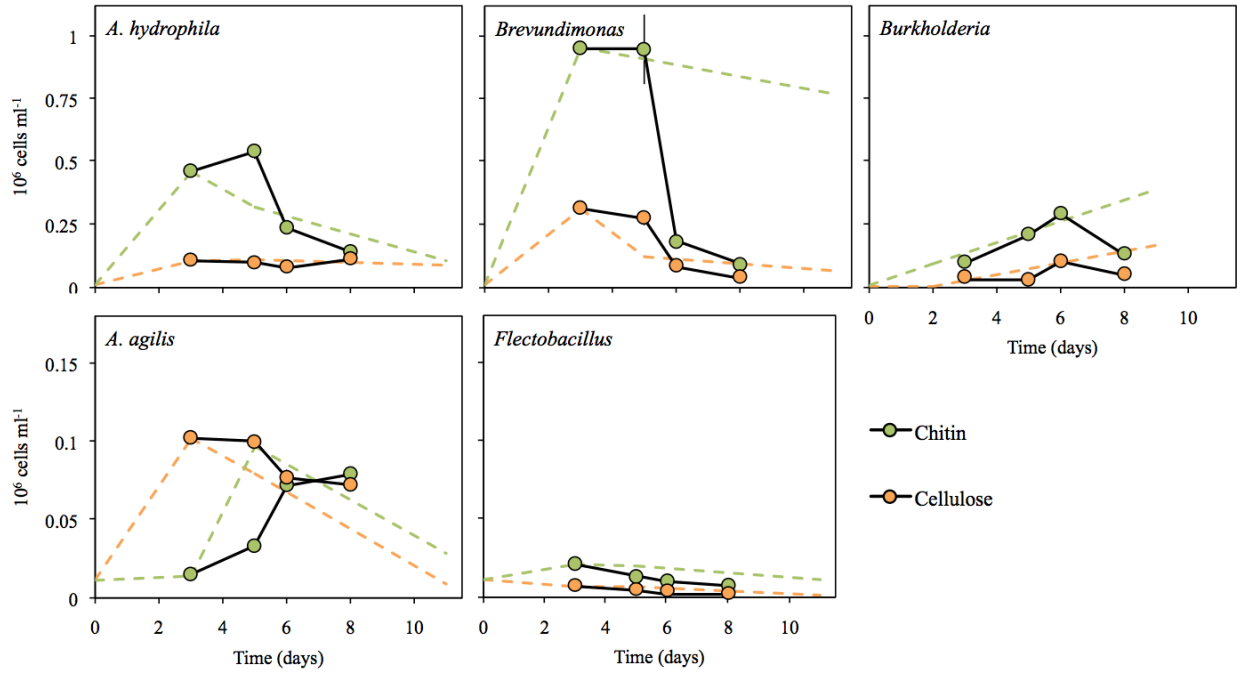
Figure S2. Effect of protist on individual bacterial species. Each panel shows growth of a single species in cultures with the protist in either chitin or cellulose. Circles show means ± s.e. (n=3). Dashed lines show the average density in pure cultures of the same species without the protist (see main text and Figure 1). Note the y-axis scale is smaller in the lower panels.



The protist did not significantly alter the maximum population density reached by any bacterial species in either cellulose or chitin (t-tests for each species in chitin or cellulose: $P>$□ after sequential Bonferroni correction) except for *Burkholderia* in chitin, where it was lower in protist cultures (P=0.004). Note that densities in Protist+ and Protist- treatments were collected at different time points. By contrast, in mixed communities containing all four bacterial species, the protist increased total population size by up to 19-fold in cellulose and five-fold in chitin (see main text; Fig. 2).

Figure S3. Protist growth with and without bacteria. Each point shows the average (± s.e.) number of protist cells in cultures without (A) and with (B-C) bacteria from three replicates.

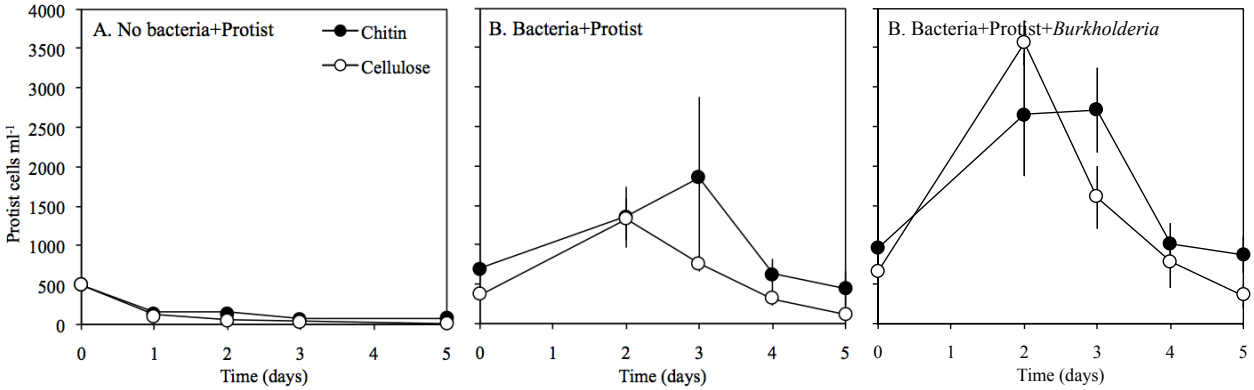


Figure S4. Morphotype distribution for each species in each treatment. Distribution of principal morphotypes (single cells, microcolonies, and aggregates) for each bacterial species on chitin and cellulose at the end of the experiment (day 11). Values are means of six replicates (three for *Burkholderia*). *Flectobacillus* has been outcompeted in Cellulose treatments before day 11.