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Experimental manipulations of microbial food web interactions in a humic lake: shifting biological drivers of bacterial community structure

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Summary

A previous multiyear study observed correlations between bacterioplankton community composition (BCC) and abundance and the dynamics of phytoplankton populations and bacterivorous grazers in a humic lake. These observations generated hypotheses about the importance of trophic interactions (both top-down and bottom-up) for structuring bacterial communities in this lake, which were tested using two multifactorial food web manipulation experiments that separately manipulated the intensity of grazing and the composition of the phytoplankton community. Our results, combined with field observations, suggest that a hierarchy of drivers structures bacterial communities in this lake. While other studies have noted links between aggregate measures of phytoplankton and bacterioplankton communities, we demonstrate here correlations between succession of phytoplankton assemblages and BCC as assessed by automated ribosomal intergenic spacer analysis (ARISA). We used a novel approach linking community ARISA data to phylogenetic assignments from sequence analysis of 16S rRNA gene clone libraries to examine the responses of specific bacterial phylotypes to the experimental manipulations. The synchronous dynamics of these populations suggests that primary producers may mediate BCC and diversity through labile organic matter production, which evolves in quality and quantity during phytoplankton succession. Superimposed on this resource-mediated control of BCC are brief periods of intense bacterivory that impact bacterial abundance and composition.

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Introduction

Aquatic bacteria interact with multiple ecological factors that have the potential to influence the bacterial community species composition. Bacterioplankton community composition (BCC) determines the suite of biogeochemical functions available in pelagic ecosystems; BCC also influences the availability of bacteria as a food source for the microbial food web (Pernthaler *et al*., 1996; 2004). Through their role in nutrient recycling and organic matter decomposition, bacteria have the potential to impact higher trophic levels (Azam *et al*., 1983). In turn, both food web interactions and resource availability are known to influence aquatic bacterial abundance, size distribution and activity (Pernthaler *et al*., 1996; Fisher *et al*., 2000; Jürgens and Jeppesen, 2000; Jürgens and Sala, 2000; Langenheder and Jürgens, 2001). Because individual bacterial populations differ in their response to shifts in resource availability, this factor will necessarily influence BCC (van Hannen *et al*., 1999a; Fisher *et al*., 2000; Crump *et al*., 2003). Thus, there is a need to understand the key drivers structuring bacterial communities in order to improve our understanding of freshwater food webs and ecosystem function.

Grazing by bacterivorous protists is a significant factor impacting bacterial mortality in aquatic ecosystems (Jürgens and Sala, 2000; Langenheder and Jürgens, 2001). Nanoflagellate grazers appear to selectively prey on the most active bacteria, or those in a specific size range (Pernthaler *et al*., 1996). Some taxa can adapt to grazing pressure by forming filaments or aggregates that exceed the upper size limit for ingestion (Hahn and Höfle, 2001). Such morphological shifts may represent either population-specific phenotypic plasticity or selection for populations already possessing a grazing-resistant morphology (Pernthaler *et al*., 1996; Hahn and Höfle, 2001).

Dissolved organic matter (DOM) source and composition can also affect BCC. Organic carbon fixed photosynthetically within the lake (autochthonous DOM) is an important resource for pelagic bacterial populations (Cole,

1982), and previous studies have noted strong correlations between aggregate measures (biomass, abundance and production) of bacterioplankton and phytoplankton communities (Azam *et al*., 1983; White *et al*., 1991; Pinhassi and Hagström, 2000). Terrestrially derived (allochthonous) organic carbon from the surrounding landscape is also an important component of available DOM. Crump and colleagues (2003) demonstrated that seasonal variation in DOM source (allochthonous vs. autochthonous) influenced BCC. The different DOM sources likely represented organic substrates of varying composition and lability; concurrent seasonal changes in BCC may reflect bacterial populations best suited to utilize particular substrates.

As phytoplankton communities undergo seasonal succession, labile DOM concentration and quality also change (Kirchman *et al*., 1991). While measures of phytoplankton abundance or productivity have been correlated to whole-community measures for the bacterial community in both natural and experimental systems (Cole, 1982; White *et al*., 1991), few studies have examined correlation between phytoplankton and bacterioplankton populations at the species level in freshwater ecosystems. However, van Hannen demonstrated that organic matter derived from different phytoplankton species is utilized by distinct bacterial groups in an experimental system (van Hannen *et al*., 1999a). This phenomenon was also recently observed in marine systems (Pinhassi *et al*., 2004).

We recently examined a number of chemical, physical and biological factors potentially impacting BCC in Crystal Bog Lake. On an annual time scale, physical factors such as water temperature or mixing events appear to be important in determining BCC (Yannarell *et al*., 2003). The microbial community was most variable during the summer months (June–August), where physical changes were less well correlated with changes in BCC. Seasonal patterns in BCC were most strongly correlated to changes in the grazer and phytoplankton community over three consecutive years (Kent *et al*., 2004). Our observations generated hypotheses relating seasonal changes in BCC to the dynamics of other planktonic populations. Specifically, we propose that summer bacterial community dynamics in this lake are determined by a hierarchy of food web interactions: resource quality and availability (bottom-up factors mediated by phytoplankton succession) are consistently acting to structure BCC, while the impact of top-down interactions (bacterivory) is superimposed upon these drivers in early summer. In the current study, two multifactorial experiments were conducted to test these hypotheses. The early summer experiment was carried out during the annual peak in abundance of heterotrophic and mixotrophic grazing populations. The late summer experiment took place while the phytoplankton community experienced an intense bloom of a single dinoflagellate species.

Results

Planktonic population dynamics

Bacterioplankton community composition and phytoplankton community composition (PCC) in this humic lake are quite variable over the summer months. Non-metric multidimensional scaling (MDS) ordinations based on standardized automated ribosomal intergenic spacer analysis (ARISA) profiles (Fig. 1A) or phytoplankton abundance (Fig. 1B) illustrate bacterial and phytoplankton community succession, respectively, in 2002. The sample dates form temporally distinct clusters in Fig. 1A. Sample dates in both Fig. 1A and B are coded by the dominant species in the phytoplankton assemblage present on each date in order to visualize the correlation between BCC (Fig. 1A) and phytoplankton succession (Fig. 1B). The pattern of sample dates in each plot was compared using the Spearman rank correlation (ρ) between the similarity matrix elements used to generate these plots. The rank correlation (ρ) of 0.851 between the bacterial and algal similarity matrices indicates good agreement between the patterns in each ordination (Fig. 1). Sample dates affiliated with the early summer cluster (triangles) correspond to the peak in mixotrophic flagellates and heterotrophic nanoflagellates (HNF) (Kent *et al*., 2004). Total bacterioplankton abundance was low, while the abundance and proportion of grazing-resistant filaments also peaked during this time (Kent *et al*., 2004). The grazer manipulation experiment was conducted during this period. *Peridinium limbatum* dominated the phytoplankton community on the sample dates shown as diamonds (Fig. 1 and Kent *et al*., 2004). The intense bloom of this dinoflagellate accounted for more than 80% of the primary producer biomass in the lake on these days. The phytoplankton manipulation experiment took place during this period. Bacterioplankton community composition was significantly different in each of these groups of sample dates in Fig. 1A [analysis of similarity (ANOSIM) $R = 0.881$, $P < 0.001$].

Microbial food web manipulations

Grazer manipulation experiment. The grazer manipulation experiment was conducted to examine the importance of flagellate grazing for determining early summer BCC. The <1 m size fraction was virtually free of flagellate grazers ($0.08 \pm 0.03 \times 10^3$ HNF per millilitre, mixotrophic flagellates were completely removed) while all other treatments initially had ambient levels of flagellates ($5.2 \pm 0.2 \times 10^3$ HNF per millilitre, $1.8 \pm 0.4 \times 10^3$ mixotrophs per millilitre). Large-bodied cladoceran removal from

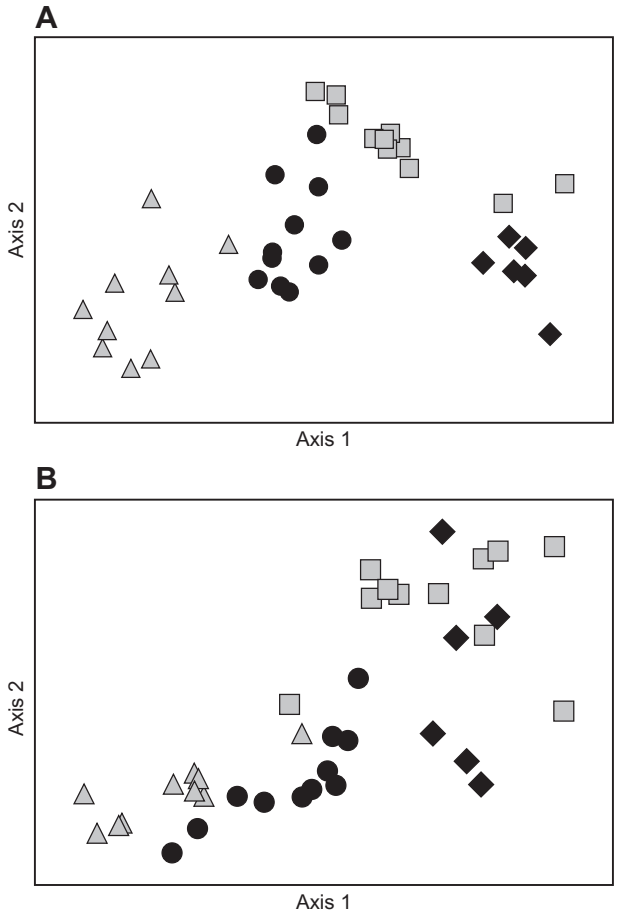

Fig. 1. A. Non-metric multidimensional scaling plot showing the succession of bacterial communities in 2002 in Crystal Bog Lake. Bacterioplankton community composition was assessed by ARISA, and analysed in PRIMER using normalized fluorescence for the similarity analysis. This ordination has a stress of 0.07.
B. Non-metric multidimensional scaling plot showing the succession of phytoplankton communities in 2002 in Crystal Bog Lake. Stress is 0.08 for this plot. Bray-Curtis similarity indices were calculated between all pairs of samples based on abundance of phytoplankton species. Spearman rank correlation $\rho = 0.851$ for comparison of the similarity matrices underlying A and B. For both plots, samples were coded based on the dominant phytoplankton species present on each date. Triangles (▲) represent sample dates from 20 May to 3 June when the bacterial community was experiencing intense grazing pressure from heterotrophic and mixotrophic flagellates. Circles (●) represent sample dates between 4 June and 19 June when the phytoplankton community was dominated by a *Cryptomonas* species. Sample dates shown as squares (■) include 21 June through 12 July, and also 14 August and 28 September. The phytoplankton community on these dates was dominated by two dinoflagellate species: *Gymnodinium fuscum* and *Peridinium limbatum.* Diamonds (◆) represent sample dates between 18 July and 21 August (excluding 14 August), when an intense bloom of *P. limbatum* characterized the phytoplankton community.

all treatments resulted in enhanced flagellate grazing in treatments with the <10 and <243 m size fractions compared with the lake (ambient) control. This caused a dramatic increase in grazing-resistant filamentous bacteria in all 'grazer-enhanced' treatments after incubation for 7 days (Fig. 2). The selection for populations able to assume a grazing-resistant morphology was reflected in the assessment of BCC by ARISA (Fig. 3A). The different grazing intensities resulted in distinct bacterial communities (ANOSIM $R = 1.0$, $P < 0.001$).

Comparison of the two grazer-enhanced treatments suggests that differences in phytoplankton biomass or community composition did not affect BCC in this experiment. The size fractionation resulted in significantly different phytoplankton populations in mesocosms receiving water from the < 10 and < 243 m size fractions (ANOSIM $R = 1.0$, $P = 0.002$ for a pairwise comparison of phytoplankton communities between these two treatments) (Fig. 3B). Despite the difference in primary producer populations between these size fractions, no significant difference in BCC was noted between the two grazer-enhanced treatments (ANOSIM $R = 0.233$, $P = 0.08$ for a pairwise comparison of BCC between the < 10 and < 243 m size fractions) (Fig. 3A). Furthermore, no significant differences in BCC were detected between treatments with and without N and P additions within each size fraction ($P = 0.1$).

Dynamics of specific phylotypes (inferred from ARISA profiles) suggest that specific taxa responded to changes in grazing pressure (Fig. 4). In particular, ARISA peaks assigned to the Beta III and Beta IV clades, the CB_Be2 phylotype affiliated with the *Betaproteobacteria,* and to the Soil II-III clade of the *Actinobacteria* (Table 1) had a higher relative signal intensity in treatments containing bacterivorous grazers (the grazer-enhanced treatments and the lake) (Fig. 4). Similar, but less dramatic, differences were also observed for peaks assigned to several phylotypes associated with *Bacteroidetes* (CB_Ba1) and *Gammaproteobacteria* (CB_Ga4, CB_Ga5 and CB_Ga6) (Table 1).

The normalized signal intensity of several peaks assigned to groups within the *Alphaproteobacteria* (Alpha IV clade), *Betaproteobacteria* (Beta I clade and CB_Be1 phylotype) and *Bacteroidetes* (CB_Ba4 phylotype) increased significantly in the grazer-free treatments (Fig. 4 and Table 1).

Phytoplankton manipulation experiment. The phytoplankton manipulation experiment was conducted in late summer when grazing pressure was much lower (Kent *et al*., 2004). The proportion of filamentous bacteria was low (compared with early summer) throughout the mesocosm and lake samples (Fig. 5), reflecting the reduced grazing pressure at this time. An intense bloom of the large dinoflagellate *P. limbatum* characterized the phytoplankton community in the lake during this period. Size fractionation of the planktonic communities (< 243 m, < 10 m and < 1 m) resulted in phytoplankton assemblages that differed in species composition, diversity and biovolume

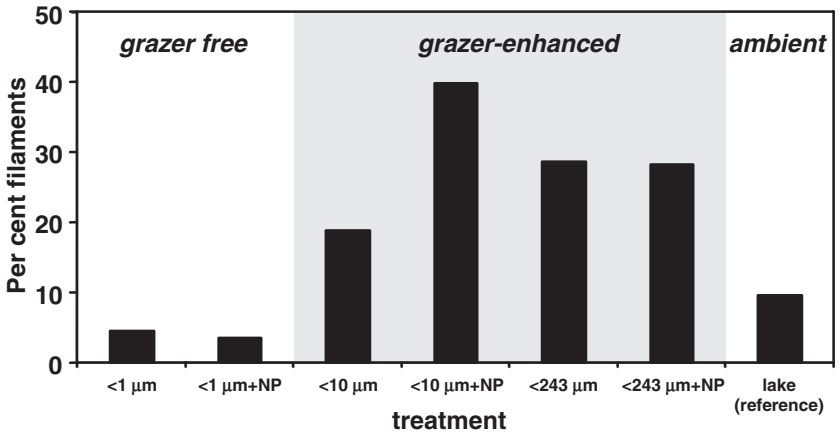

Fig. 2. Per cent of bacterial population with filamentous morphology 7 days after the density of flagellate grazers was manipulated, during the first mesocosm experiment.

(Table 2). The phytoplankton community in the treatments receiving 243 m of filtered water did not differ significantly from the phytoplankton communities in the lake initially (pairwise $R = 0$, $P = 0.39$). After incubation for 7 days, however, the lake community had higher densities of *Cryptomonas, Mallomonas* and all dinoflagellate species, particularly *P. limbatum*. Both the reference communities in the lake and the < 243 m mesocosm had significantly higher algal biovolume than the other treatments, and also higher phytoplankton diversity (Table 2).

Distinct bacterial assemblages formed following incubation with different phytoplankton communities (Fig. 6A). The relationship between patterns in BCC and PCC that was observed in the lake (Fig. 1) is also apparent in the manipulated communities ($\rho = 0.827$) (Fig. 6B).

Automated ribosomal intergenic spacer analysis peaks assigned to specific clades showed differential responses to the manipulated phytoplankton communities (Fig. 7 and Table 1). Peaks assigned to the Alpha IV clade of the *Alphaproteobacteria* and to *Bacteroidetes* phylotype CB_Ba3 had significantly higher normalized fluorescence in the <1 m fraction where phytoplankton abundance and diversity was reduced. Peaks assigned to the *Betaproteobacteria* Beta II clade, *Actinobacteria* acI-B clade and *Gammaproteobacteria* CB_Ga1 phylotype showed significantly higher normalized signal strength in the treatments with higher phytoplankton biovolume, along with peaks assigned to *Firmicutes* phylotype CB_Fi1, *Deltaproteobacteria phylotype* CB_De1 and *Bacteroidetes* clade CFI.

Table 1. Automated ribosomal intergenic spacer analysis (ARISA) fragment lengths from Crystal Bog Lake assigned to specific phylotypes (Newton *et al*., 2006).

Phylotypes	ARISA fragment length (bp)
Actinobacteria	
acI-B (AY792223)[a]	545, 556, 581, 594, 600, 611
Soil II_III (AY792232)	615, 633, 675
Alphaproteobacteria	
Alpha I (AY792286)	891, 950
Alpha IV (AY792290)	898
Betaproteobacteria	
Beta I (AY792257)	958
Beta II (AY792239)	797
Beta III (AY792259)	1066
Beta IV (AY792265)	741, 828
CB_Be1 (AY792253)	619, 646 648, 873
CB_Be2 (AY792246)	565, 755, 767
Bacteriodetes	
CB_Ba1 (AY792303)	495, 626
CB_Ba2 (DQ093402)	821
CFI (AY792297)	749, 780, 806, 817
Firmicutes	
CB_Fi1 (AY792314)	586
Gammaproteobacteria	
CB_Ga1 (AY792266)	492, 510, 516, 664, 732, 752, 824
CB_Ga3 (AY792278)	689
CB_Ga4 (AY792280)	715
CB_Ga5 (AY792281)	771
CB_Ga6 (AY792282)	763
Deltaproteobacteria	
CB_De1 (AY792293)	684

Automated ribosomal intergenic spacer analysis fragment lengths and phylogenetic assignments were derived from analysis of clone libraries derived from partial *rrn* operons (near full-length 16S rRNA plus the intergenic transcribed spacer) amplified from aquatic bacterial communities. Only peaks which could be unambiguously assigned to a single clade have been included. Clade definitions are described elsewhere (Glockner *et al*., 2000; Zwart *et al*., 2002; Warnecke *et al*., 2004; Newton *et al*., 2006).

a. GenBank accession number for a representative sequence assigned to this clade as defined in Newton and colleagues, 2006).

Discussion

Humic lakes as model systems for examining microbial food web interactions

The importance of top-down versus bottom-up factors on BCC, abundance and morphology was examined in Crystal Bog lake, a humic lake in northern Wisconsin. This lake, and other humic systems in northern temperate regions generally lack planktivorous fishes. This simplified food web readily lends itself to examination of trophic interactions among planktonic populations without the

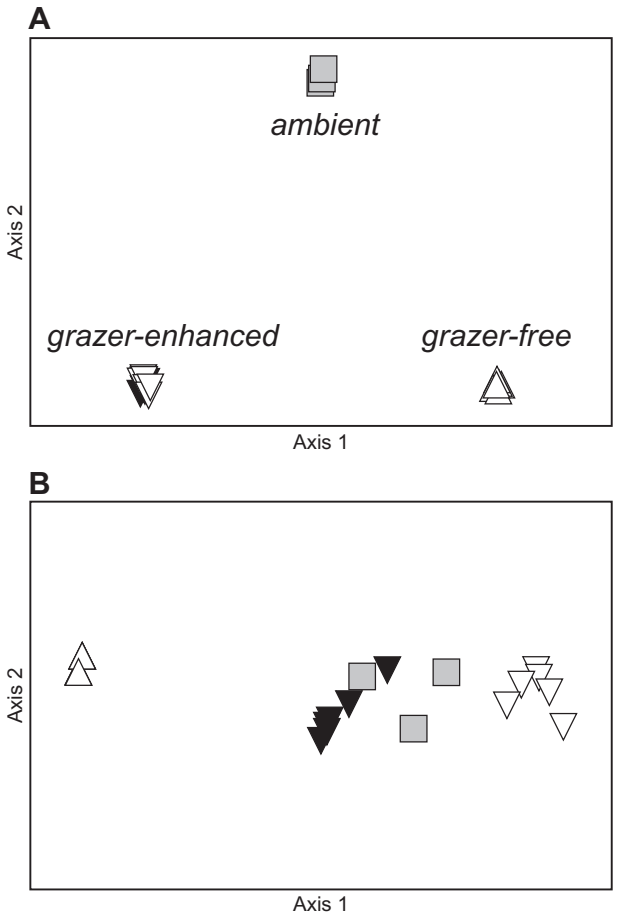

Fig. 3. Non-metric multidimensional scaling plot of BCC (A) and PCC (B) 7 days after manipulation of flagellate grazing pressure. Each point in the plot represents the community composition determined by ARISA profiles generated from individual lake or mesocosm treatments (A) or microscopic counts (B) in the grazer manipulation experiment. Similarity in community composition was determined using Bray-Curtis similarity index in PRIMER. Distance between points represents dissimilarity in community composition; samples that have similar communities plot close together. The samples are labelled with the grazer manipulation treatment in A (△ – grazer-free samples; ▽ – grazer-enhanced samples; ■ – lake samples); within the grazer-enhanced treatments, communities in the < 10 m size fraction are indicated in black (▼) and those from the < 243 m size fraction are indicated in white (▽). Phytoplankton communities from corresponding samples are coded similarly in B. Samples with and without N and P additions are included for each size fraction. The stress for each ordination is 0.01, indicating that the two-dimensional representation of this multidimensional ordination is not unduly distorted. Analysis of similarity (ANOSIM) between treatment groups indicates that bacterial communities (A) had much greater similarity to each other within treatments compared with between treatments ($R = 1.0$, $P < 0.001$).

complication of higher trophic level influence. Crystal Bog lake experiences a succession of dinoflagellate blooms each summer, culminating in a particularly intense annual *P. limbatum* bloom in August, as do many other humic lakes in this region (Graham *et al*., 2004; Kent *et al*., 2004). This succession likely affects the concentration and

Table 2. Phytoplankton community composition in mesocosms following size fractionation (initial) and incubation for 7 days (final) during the phytoplankton manipulation experiment.

Phytoplankton population	Phytoplankton abundance (cells l^{-1}) in each size fraction							
	< 1 m		< 10 m		< 243 m		Lake	
	initial	final	initial	final	initial	final	initial	final
Cryptomonas	$2.0 \pm 2.0 \times 10^2$	$1.4 \pm 0.4 \times 10^3$	$1.7 \pm 0.1 \times 10^5$	$1.1 \pm 0.4 \times 10^5$	$2.5 \pm 0.2 \times 10^5$	$1.3 \pm 0.4 \times 10^5$	$1.9 \pm 0.7 \times 10^5$	$5.9 \pm 1.9 \times 10^5$
Dinobryon	n.d.	$4.9 \pm 0.0 \times 10^4$	$5.3 \pm 2.0 \times 10^3$	$1.3 \pm 0.2 \times 10^4$	$3.9 \pm 0.6 \times 10^4$	$1.3 \pm 0.3 \times 10^4$	$4.8 \pm 2.3 \times 10^4$	$1.1 \pm 0.2 \times 10^4$
G. fuscum	n.d.	n.d.	$2.9 \pm 1.0 \times 10^3$	$2.2 \pm 0.5 \times 10^3$	$9.4 \pm 2.4 \times 10^3$	$2.0 \pm 0.4 \times 10^3$	$7.7 \pm 2.5 \times 10^3$	$3.3 \pm 0.1 \times 10^4$
P. cinctum	$2.0 \pm 2.0 \times 10^2$	n.d.	$2.6 \pm 0.8 \times 10^3$	$1.5 \pm 0.5 \times 10^3$	$8.6 \pm 1.0 \times 10^3$	$5.1 \pm 1.6 \times 10^3$	$6.9 \pm 2.3 \times 10^3$	$1.5 \pm 1.3 \times 10^4$
P. limbatum	n.d.	$1.7 \pm 1.7 \times 10^2$	$3.2 \pm 0.6 \times 10^3$	$1.7 \pm 0.6 \times 10^3$	$9.0 \pm 1.7 \times 10^3$	$7.9 \pm 1.4 \times 10^3$	$9.8 \pm 1.4 \times 10^2$	$1.1 \pm 1.0 \times 10^4$
Mallomonas	n.d.	n.d.	$1.7 \pm 0.2 \times 10^4$	$1.7 \pm 1.7 \times 10^2$	$4.5 \pm 0.5 \times 10^4$	$2.0 \pm 2.0 \times 10^2$	$2.8 \pm 1.1 \times 10^4$	$2.8 \pm 1.4 \times 10^4$
Total biovolume ($m^3 l^{-1}$)	$1.8 \pm 1.7 \times 10^7$	$6.2 \pm 4.8 \times 10^7$	$1.7 \pm 0.2 \times 10^9$	$9.7 \pm 2.1 \times 10^9$	$4.4 \pm 0.6 \times 10^9$	$3.1 \pm 0.5 \times 10^9$	$4.3 \pm 0.5 \times 10^9$	$7.3 \pm 3.6 \times 10^9$

Populations were averaged for six replicate mesocosms from each size fraction, and three replicates for the lake samples. Range indicates standard error.
n.d., not detected.

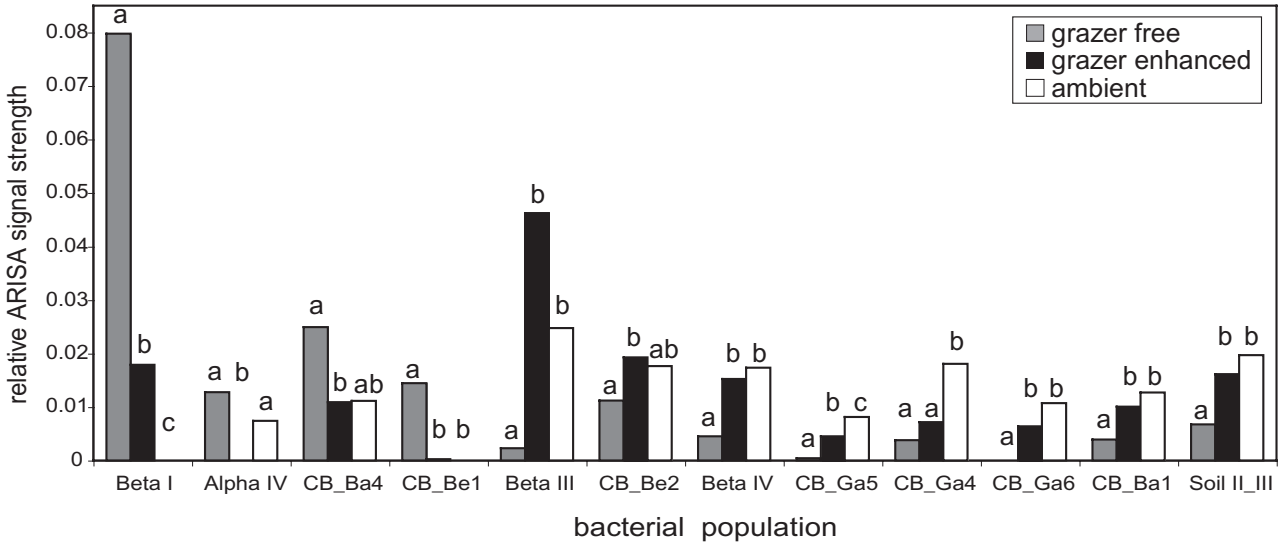

Fig. 4. Mean normalized fluorescence of ARISA peaks assigned to the indicated clades is shown for each treatment following 7 days' incubation with different grazing pressures (treatment groups are described in Table 3). Letters indicate significant differences between mean values following pairwise comparisons with Bonferroni corrections ($\alpha = 0.05/3$).

biochemical composition of labile DOM available to bacterioplankton populations (van Hannen *et al*., 1999a; Pinhassi *et al*., 2004). Though this lake is consistently high in dissolved organic carbon (average 10 mg l^{-1}), a large fraction of this is presumed to be comprised of recalcitrant high-molecular-weight humic compounds (Sachse *et al*., 2001; Burkert *et al*., 2003). Other researchers have demonstrated in both mesocosm and field studies that organic matter of differing quality will select for different bacterioplankton populations (van Hannen *et al*., 1999a; Arrieta and Herndl, 2002; Burkert *et al*., 2003; Crump *et al*., 2003; Pinhassi *et al*., 2004). The intense bloom of a single phytoplankton species each August may limit the diversity of readily available DOM, and enrich for the bacterioplankton populations that specialize in using this resource. The synchronous dynamics of bacterial and phytoplankton populations (Fig. 1) are consistent with the hypothesis that resource quality or availability may be an important factor in determining BCC in this lake.

Part of the observed phytoplankton community succession in Crystal Bog lake includes annual blooms of mixotrophic flagellates each year in early summer (Graham *et al*., 2004; Kent *et al*., 2004). This bloom coincides with the peak in HNF grazers each year, and bacterial communities are subjected to intense grazing pressure for the bloom duration. Previous studies in model systems have shown that bacterivory can influence not only bacterial abundance, but also the genotypic and phenotypic composition of the community (Pernthaler *et al*., 1997; 2001; 2004; Jürgens *et al*., 1999; van Hannen *et al*., 1999b; Hahn and Höfle, 2001; Langenheder and Jürgens, 2001). Dramatic shifts in bacterial abundance, bacterial commu-

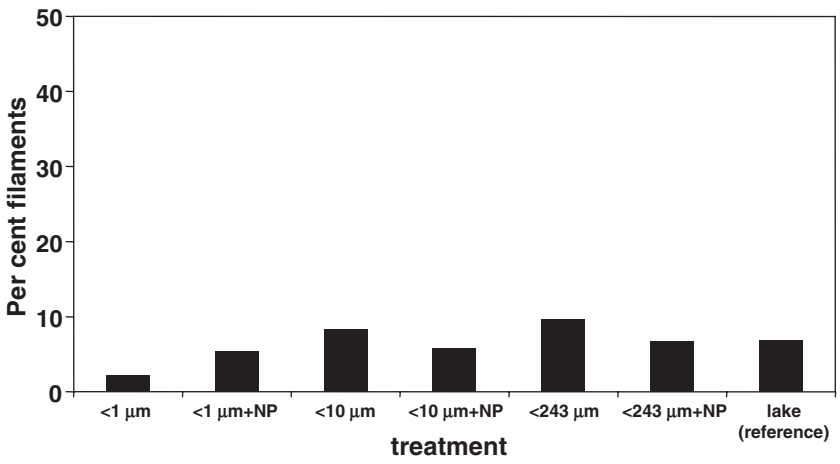

Fig. 5. Per cent of bacterial population with filamentous morphology 7 days after the PCC was manipulated, during the second mesocosm experiment. This figure uses the same scale as Fig. 2 in order to illustrate the difference in relative abundance of filamentous bacteria between the two experiments.

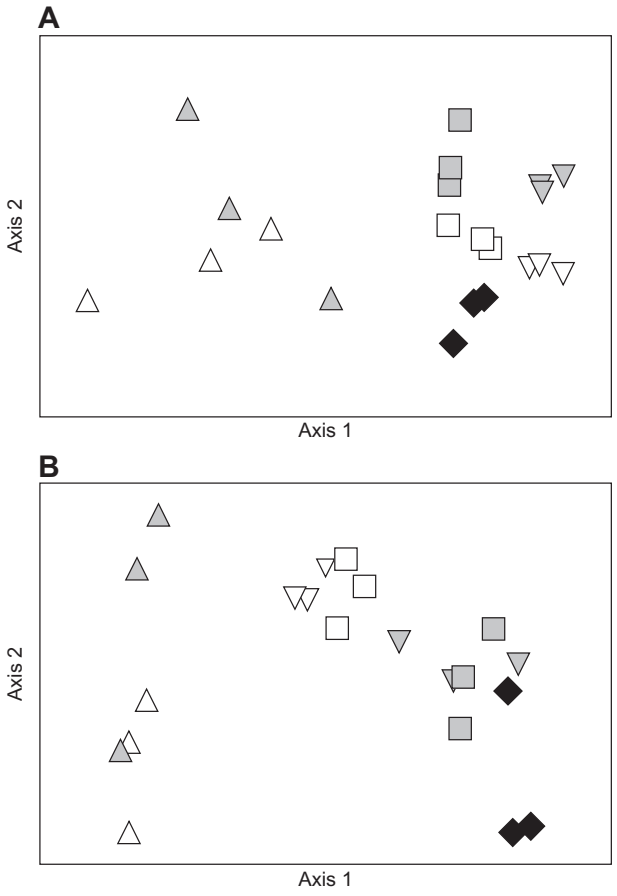

Fig. 6. Comparison of mesocosm BCC (A) with PCC (B) yields similar MDS plots at the conclusion of the phytoplankton manipulation experiment. The symbols correspond to the different size fractions: (△ – <1 m; ▽– < 10 m; □– < 243 m; ◆– lake). Samples with (grey) and without (white) N and P additions are included for each size fraction. Phytoplankton communities from corresponding samples are coded similarly in B. The stress for the BCC ordination is 0.09, the phytoplankton community ordination has a stress of 0.05. Analysis of similarity (ANOSIM) between treatment groups indicates that bacterial communities (A) had much greater similarity to each other within treatments compared with between treatments ($R = 0.711$, $P < 0.001$). Phytoplankton community composition differences among treatments are described in Table 2. Phytoplankton community composition also differed significantly between treatment groups (ANOSIM $R = 0.733$, $P < 0.001$). Spearman rank correlation $\rho = 0.827$ for comparison of the similarity matrices underlying the ordinations shown in A and B.

nity richness and composition (assessed by ARISA), as well as an increase in the proportion of grazing resistant filaments are observed annually in Crystal Bog lake, concurrent with this increase in nanoflagellate grazers (Kent *et al*., 2004; Newton *et al*., 2006).

In order to test our hypotheses about the importance of both top-down and resource-mediated controls for BCC, the food web structure was manipulated at different points during microbial community succession. The temporal separation of intense grazing pressure (early summer) and the potentially limited resource diversity represented by low phytoplankton diversity (late summer) allowed us to separately examine the importance of each factor for structuring bacterial communities in this lake.

Effect of food web interactions on BCC

Top-down factors. Studies examining the influence of flagellate grazers on the phenotypic composition of bacterial communities have reported conflicting results on the correlation between the formation of filamentous bacterial morphology and the presence of bacterivorous protists (Jürgens *et al*., 1999; Jürgens and Sala, 2000; Langenheder and Jürgens, 2001; Pernthaler *et al*., 2004; Wu *et al*., 2004). In the current study, enhanced bacterivory increased the proportion of filamentous bacteria, regardless of resource-related factors that were also manipulated (Fig. 2). In addition, flagellate removal resulted in fewer filamentous bacteria at the conclusion of the experiment. The strong differences in BCC related to changes in grazing pressure lead us to conclude that bacterivory is the most important factor structuring the bacterial communities in early summer in this lake.

Resource-mediated factors. The phytoplankton manipulation experiment was conducted to test the hypothesis that the annual late summer *P. limbatum* bloom impacts BCC. This intense bloom presumably provides the bacterial community with abundant autochthonous DOM resources (van Hannen *et al*., 1999a; Arrieta and Herndl, 2002; Pinhassi *et al*., 2004). However, the limited phytoplankton community diversity during this time (Graham *et al*., 2004) may influence DOM resource diversity and lead to enrichment of bacterial populations best suited to take advantage of particular substrates. Analytical methods for DOM characterization were not available for this study; however, because labile autochthonous DOM may be metabolized by bacterioplankton very soon after it is released by phytoplankton (Kritzberg *et al*., 2004), such measures likely would not have allowed us to accurately calculate the flux of autochothonous carbon between these two compartments of the food web. Instead, we propose that PCC provides a useful proxy for evaluating diversity and abundance of labile organic carbon resources available to aquatic bacteria. By reducing or eliminating *P. limbatum* abundance through size fractionation, the community composition of primary producers available in each experimental treatment was altered (Fig. 6). The strong relationship between BCC and PCC in the mesocosm experiment mirrors the synchronous dynamics of these two food web compartments that were observed in the lake over the ice-free season in 2002 (Fig. 1), and supports our hypothesis regarding the relationship between BCC and the primary producer community.

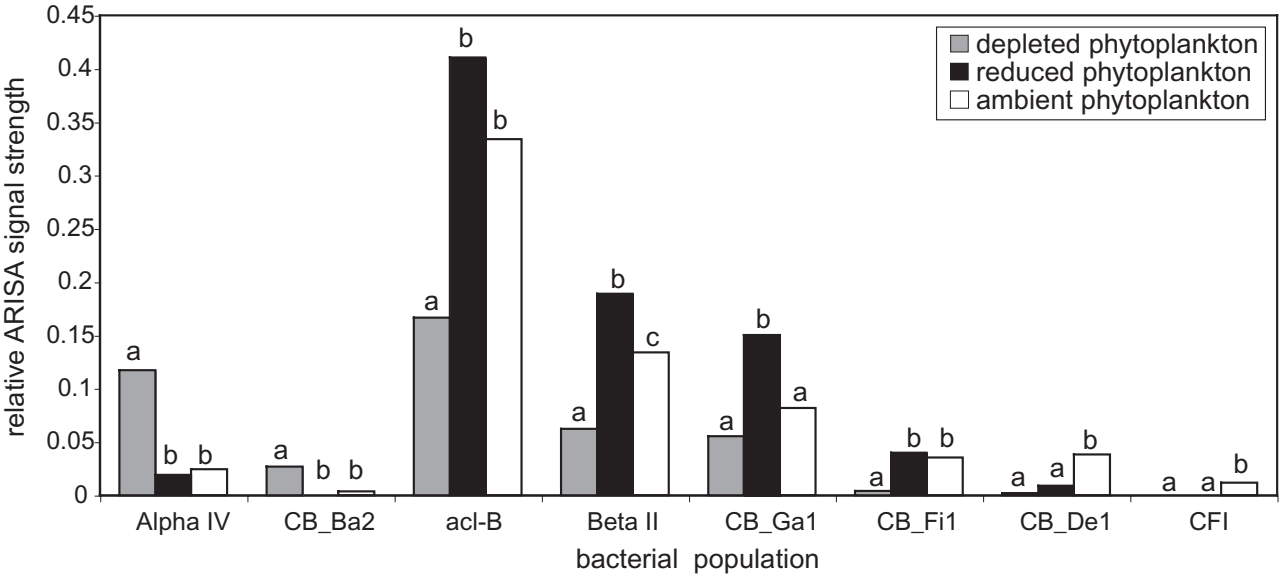

Fig. 7. Mean normalized fluorescence of ARISA peaks assigned to the indicated clades is shown for each treatment group described in Table 3 7 days after manipulation of the phytoplankton community diversity and biomass. Letters indicate significant differences between mean values following pairwise comparisons with Bonferroni corrections ($\alpha = 0.05/3$).

Population-level response to food web dynamics

Changes in ARISA peak intensity have been found to be well correlated with the dynamics of the populations represented by specific peaks (Brown *et al*., 2005), thus we are able to make general comparative observations regarding the response of bacterial populations to experimental manipulations based on ARISA profiles. Intensity of ARISA peaks assigned to several *Betaproteobacteria* clades increased in the grazer-enhanced treatments, or was greatly reduced in the grazer-free treatments, suggesting that grazing pressure may select for these taxa. The response to increased grazing was most dramatic for the Beta III clade of *Betaproteobacteria*. A dramatic increase in the relative signal intensity associated with this clade was also observed in the lake during this time (Newton *et al*., 2006), leading us to speculate that the grazing-resistant (filamentous) populations observed in this lake might be *Betaproteobacteria*. Previous studies have demonstrated that cells affiliated with the *Betaproteobacteria* form a significant portion of the filamentous bacteria in some freshwater ecosystems (Langenheder and Jürgens, 2001), though several other phylogenetic groups are also able to assume a grazing-resistant morphology (Jürgens *et al*., 1999; Hahn and Höfle, 2001; Boenigk *et al*., 2004; Pernthaler *et al*., 2004). Additional experiments using whole-cell detection methods such as fluorescent *in situ* hybridization are required to conclusively determine the phylogenetic affiliation of the filamentous populations in this lake.

Phytoplankton community manipulation also resulted in differential responses from specific bacterial populations. Because availability of autochthonous DOM is related to primary producer abundance (Kirchman *et al*., 1991; Crump *et al*., 2003), we presume that labile substrate was more limiting in mesocosms with smaller phytoplankton populations. The bacterial populations represented by the ARISA peaks that increase in normalized signal strength in these treatments may represent populations that are more successful in resource-limited situations, or 'K-strategists'. Automated ribosomal intergenic spacer analysis peaks showing the opposite trend may represent bacterial populations enriched by the substrate provided by the *Peridinium* bloom. As this experiment was carried out during the period when the peak in total primary producer biovolume for the ice-free season was observed, a more general interpretation would be that these populations may represent 'r-strategists' responding to the temporary abundance of autochthonous DOM resources. The enrichment of Beta II *Betaproteobacteria* from humic lake bacterial communities following organic carbon addition has also been observed in other studies (Burkert *et al*., 2003). Further investigation into the ecophysiology of individual freshwater taxa is required to thoroughly test these hypotheses.

Conclusion

Previous multiyear observations of planktonic population dynamics in this humic lake generated hypotheses about

Table 3. Planktonic population manipulations in two mesocosm experiments.

Size fraction	Grazer-manipulation experiment	Phytoplankton removal experiment
< 1 m	grazer-free	depleted phytoplankton levels
< 10 m	grazer-enhanced	reduced phytoplankton levels
< 243 m	grazer-enhanced	ambient (high) phytoplankton levels
Lake (control)	ambient grazer levels	ambient (high) phytoplankton levels

the interactions among these populations and the impact such interactions have on BCC (Kent *et al*., 2004). The present study demonstrated that the 'top-down' interactions between the heterotrophic and mixotrophic flagellates and the bacterial populations are important for structuring BCC in this lake in the early summer. Intense bacterivory may temporarily eclipse the effects of resource-mediated controls on BCC, a hypothesis supported by the synchronous dynamics of bacterioplankton and phytoplankton populations throughout the rest of the ice-free season (Fig. 1). We propose that these trophic interactions represent a hierarchy of factors impacting BCC. As microbial communities underpin ecosystem function in aquatic environments, it is critical to understand the factors that influence these communities.

Experimental procedures

Study sites and sample collection

Crystal Bog Lake is a shallow humic lake located in northern Wisconsin, USA (89 36′22.5″W, 46 00′26.8″N). This lake has a surface area of 0.5 ha, a maximum depth of 2.5 m, and is surrounded by an extensive *Sphagnum* mat. Sample collection procedures were described previously (Kent *et al*., 2004). Physical and chemical limnological characteristics of the lake water during the study period are available on the North Temperate Lakes Long-term Ecological Research website: http://lter.limnology.wisc.edu

Mesocosm experiments

Lake water for the 7-day grazer manipulation experiment was collected on May 24, 2002 during a peak in flagellate populations (Kent *et al*., 2004). The phytoplankton manipulation experiment began on August 9, 2002 during the peak of an intense dinoflagellate bloom (Kent *et al*., 2004). For each experiment, manipulated planktonic communities were assembled in 10-l low-density polyethylene, square carboys (I-Chem). Water 150 l for these mesocosms was passed through a 243- m mesh. This filtration served to remove only the largest of the macrozooplankton (mostly the largest *Daphnia*), which have been shown to have disproportionately large impacts on microbial food webs in such container experiments (Jürgens and Jeppesen, 2000). The complexity of the microbial food web following this filtration step was similar to that in the lake, especially during the phytoplankton manipulation experiment, during which no large zooplankton populations were present (Kent *et al*., 2004). From this water, 40 l was filtered through a 10- m mesh to remove remaining zooplankton and large phytoplankton populations. Another 40-l aliquot was filtered through a 1- m in-line filter capsule (Whatman Polycap) to remove flagellate and smaller phytoplankton populations. Population manipulations are summarized in Table 3. Filtered water (6.2 l) from each size fraction (< 243 m, <10 m and <1 m) was dispensed to six replicate mesocosms. In order to eliminate the effects of nutrient limitation in these experiments, three replicates for each size fraction received inorganic N and P additions. For the grazer manipulation experiment, the final concentrations of soluble reactive P (SRP) and NH_4^+ were 5.8 ± 0.1 g l^{-1} SRP and 375 ± 24 g l^{-1} NH_4^+ (approximately twice the ambient N and P concentration). For the phytoplankton manipulation experiment, the final nutrient concentrations were 6.1 ± 2.3 g l^{-1} SRP and 267 ± 55 g l^{-1} NH_4^+ (a 3.6- and 1.6-fold increase over the ambient N and P concentration respectively). The mesocosms were attached to a floating frame and incubated *in situ*. Aliquots of 1.5 l were removed from each mesocosm on day 3 and day 7 after mixing the container contents. Previous studies suggested that BCC changes on a time scale of a few days in this system (Fisher *et al*., 2000; Kent *et al*., 2004). Water from each aliquot was passed through a 70- m mesh to recover zooplankton populations which were subsequently preserved in 80% ethanol. Bacterial, flagellate and phytoplankton samples were preserved for analysis as described previously (Kent *et al*., 2004).

Planktonic community composition

Abundance of bacteria, phytoplankton, zooplankton and HNF. To determine bacterial abundance, cells were stained with 4′, 6′-diamidino-2-phenylindole (DAPI) and counted on black 0.2- m PCTE filters using epifluorescence microscopy (Porter and Feig, 1980), as described previously (Kent *et al*., 2004). In addition to total abundance, filamentous bacteria were counted. Filamentous bacteria are defined here as elongated single cells > 5 m in length; colonies of cells arranged end-to-end were not observed in these samples. Bacteria thus classified are presumed to be less accessible to flagellate grazing (Pernthaler *et al*., 1996).

Phytoplankton biovolume and abundance and zooplankton populations were identified and enumerated as described previously (Kent *et al*., 2004). Heterotrophic nanoflagellates were visualized with DAPI and enumerated as described previously (Kent *et al*., 2004).

Bacterioplankton community analysis

Bacterioplankton community composition and diversity was assessed using ARISA (Fisher and Triplett, 1999), as

described previously (Kent *et al*., 2004; Yannarell and Triplett, 2005). To include the maximum number of peaks while excluding background fluorescence, a threshold of 100 fluorescence units was used. The signal strength (i.e. peak area) of each peak was normalized to account for run-to-run variations in signal detection by dividing the area of individual peaks by the total fluorescence (area) detected in each profile, expressing each peak as a proportion of the observed community (Rees *et al*., 2004; Yannarell and Triplett, 2005).

Bacterioplankton population analysis

Clone libraries and clone ARISA. To evaluate the impact of the food web manipulations on individual taxa, the normalized fluorescence of individual ARISA fragments was compared between treatments. Taxonomic assignments for individual ARISA peaks were determined using polymerase chain reaction-based clone libraries containing the 16S and intergenic transcribed spacer (ITS) region of *rrn* operons amplified from Crystal Bog bacterioplankton communities (Brown *et al*., 2005; Jacob *et al*., 2005; Newton *et al*., 2006). Details of phylogenetic assignments for microbial communities in this lake are described elsewhere (Newton *et al*., 2006).

Data analysis

Comparisons of community composition. For each ARISA dataset, the Bray-Curtis similarity coefficient ($S_{17} = 1 - D_{14}$ of Legendre and Legendre, 1998) was calculated to assess the degree of similarity between ARISA profiles obtained from different samples. A similarity matrix containing these comparisons was generated for all possible pairs of samples in each experiment. This matrix was used to generate non-MDS plots. This ordination results in a visual representation of BCC similarity between treatments (Yannarell *et al*., 2003; Rees *et al*., 2004).

Analysis of similarity, as described by Clarke and Green (Clarke and Green, 1988; Clarke, 1993), was used previously to distinguish bacterial communities in lake (Yannarell *et al*., 2003; Yannarell and Triplett, 2004) and sediment (Rees *et al*., 2004) samples and to determine the annual differences in BCC within a lake (Kent *et al*., 2004). In the current study, bacterial and phytoplankton communities were classified by treatment or grouped by sample date, and ANOSIM was used to test the hypothesis that communities from the same group were more similar to each other than to communities in different groups. ANOSIM generates a test statistic, *R*, with a value between −1 and 1. The magnitude of *R* indicates the degree of separation between groups of samples, with a score of 1 indicating complete separation and 0 indicating no separation. Monte-Carlo randomization of group labels was used to generate the null distribution of *R* in order to test the hypothesis that within-group similarities were higher than would be expected from random grouping of samples.

To determine the relationship between BCC and PCC, a non-parametric form of the Mantel test (Legendre and Legendre, 1998) was used. Matrices containing the similarity coefficients for pairwise comparisons of phytoplankton or BCC between each sample were compared using the Spearman rank correlation coefficient (ρ). The production of the similarity matrices, calculation of Spearman's ρ, and the ANOSIM described above were all performed using the software package PRIMER 5 for Windows v. 5.2.7 (PRIMER-E, 2001; routines SIMILARITY, RELATE and ANOSIM).

Population-level response to manipulations

The significance of changes in normalized ARISA peak fluorescence for each clade was tested by ANOVA between treatment groups defined in Table 3. Only ARISA peaks that could be assigned to a single clade (Table 1) were considered for the analysis of populations responding to the experimental manipulation. The Bonferroni correction for multiple testing was applied for significance tests between each treatment group.

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