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ORIGINAL ARTICLE

Links between bacterial production, amino-acid utilization and community composition in productive lakes

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Influence of distribution and abundance of bacterial taxa on ecosystem function are poorly understood for natural microbial communities. We related 16S rRNA-based terminal restriction fragment length polymorphism to bacterial production and arginine uptake kinetics to test if functional features of bacterioplankton in four lakes could be predicted from community composition. Maximum arginine uptake rate (arginine V_{max}) ranged from 10% to 100% of bacterial production. Owing to high growth efficiencies on arginine (63–77%), the bacterial community could potentially saturate its carbon demand using this single organic substrate, for example, during sudden surges of free amino acids. However, due to low *in situ* concentrations of arginine in these lakes ($<0.9\,\mu g\,l^{-1}$), actual uptake rates at ambient concentrations rarely exceeded 10% of V_{max}. Bacterial production and arginine V_{max} could be predicted from a subset of bacterial ribotypes, tentatively affiliated with several bacterial divisions (*Cyanobacteria, Actinobacteria, Bacteroidetes* and *Proteobacteria*). Multivariate statistical analysis indicates that there were both highly important and less important ribotypes for the prediction of bacterial production and arginine V_{max}. These populations were either negatively or positively related to the respective functional feature, indicating contrasting ecological roles. Our study provides a statistically robust demonstration that, apart from environmental conditions, patterns in bacterial community composition can also be used to predict lake ecosystem function.
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Introduction

One challenge in microbial ecology is to identify environmental factors that shape ecosystem functions and bacterial community composition. It is known that multiple factors, like interspecific interactions (for example, predation and competition) as well as chemical and physical constraints, affect the function of natural bacterial communities (see, for example, Fuhrman, 1999). An important general function is the utilization of specific dissolved organic matter (DOM) in freshwater environments. Natural DOM contains a variable fraction of carbohydrates and various forms of free and combined amino acids (for example, proteins and peptides; Jørgensen and Søndergaard, 1984; Tranvik and Jørgensen, 1995). These and other structurally defined biopolymers mainly originate from recent production by planktonic and sessile organisms such as phytoplankton, plants and bacteria (Zygmuntowa, 1981; Bertilsson and Jones, 2003), and are rapidly being hydrolyzed to low-molecular weight compounds and efficiently depleted by heterotrophic bacterial uptake (Tranvik and Jørgensen, 1995; Kirchman, 2003). Changes in concentrations of monomers such as dissolved free amino acids (DFAA) seem to be coupled to phytoplankton growth and the simultaneous stimulation of zooplankton and heterotrophic bacterial activity (Eppley *et al.*, 1978). The compounds being released likely create new niches that are rapidly being filled by various microbial heterotrophs. This has been demonstrated by marked shifts in bacterial community composition following phytoplankton blooms (van Hannen *et al.*, 1999; Riemann *et al.*, 2000). Several other studies have also shown that availability of resources have the potential to regulate

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bacterial community composition (Pinhassi *et al.*, 1999; van Hannen *et al.*, 1999; Crump *et al.*, 2003; Eiler *et al.*, 2003; Newton *et al.*, 2006).

Much less is known about the role that community composition plays in regulating ecosystem function (Gray and Head, 2001). Although it is well established that different bacteria exhibit different functional features and hence contribute to combined ecosystem function in different ways, most current models assume that ecosystem function is a direct result of prevailing environmental conditions. It has often been argued that environmental conditions are more important in determining community function than bacterial community composition (see, for example, Fernandéz *et al.*, 1999; Findlay *et al.*, 2003; Langenheder *et al.*, 2005). This uncoupling of function from bacterial community composition is proposed to be caused by a high degree of functional redundancy within the bacterial community (Groffman and Bohlen, 1999). However, recent laboratory experiments suggest that the composition of bacterial communities and the presence of specific populations can affect general community functions (for example, ability to degrade organic matter: Eiler *et al.*, 2003; Bell *et al.*, 2005; Szabó *et al.*, 2007).

Here, we test if variation in some common and quantitatively significant microbial functions could be predicted from variation in bacterial community composition. The functions were bacterial biomass production and kinetics of amino-acid utilization with arginine as a model substance. Uptake of arginine by bacterial populations has previously been used as an indicator of microbial activity in soils and has been shown to correlate with respiration of the microbial community (Alef and Kleiner, 1986; Bonde *et al.*, 2001).

These functional parameters were analyzed in four productive lakes during 2002 and 2003, paralleled by terminal-restriction fragment length polymorphism (T-RFLP) analysis of amplified 16S rRNA genes to assess variation in bacterial community composition. We show that amino-acid utilization can be a significant source of nutrients and energy for heterotrophic bacteria in these lakes and that bacterial community composition can explain both spatial and seasonal variation in bacterial production and maximum arginine uptake rates. Using phylogenetic analyses from previously published 16S rRNA clone libraries from these lakes, we were able to putatively identify some populations that were positively or negatively related to these community functions.

Materials and methods

Sample collection

Four Swedish lakes (Ekoln, Erken, Limmaren and Vallentunasjön) were sampled during the summer seasons of 2002 and 2003 (Table 1). These high-productive lakes are all located in the region of Uppland in central Sweden (for details, see Eiler and Bertilsson, 2004). Lake Ekoln is the northernmost basin of Lake Mälaren, the third largest lake in Sweden. The drainage area is characterized by a high proportion of agricultural land and urban areas which results in a high-nutrient load to the system and a hydraulic retention time of 1.2 years. Lake Erken is a moderately eutrophic lake and usually has a stable thermal stratification during summer. Lake Limmaren does not develop summer stratification and has an exchange of nutrients between the sediment and the photic zone (Karlsson-Elfgren, 2003). The drainage areas of Erken and Limmaren are smaller than Ekoln and are mostly dominated by coniferous forests. Consequently, their hydraulic retention times are longer (7.4 and 5.8 years, respectively). Lake Vallentunasjön is a shallow lake, previously used as a recipient of sewage from the surrounding urban areas and the sediment still acts as a significant source of nutrients to the lake (Brunberg and Boström, 1992). The hydraulic retention time of this lake is 2 years.

All four lakes experience seasonal cyanobacterial blooms. Blooms in Lake Ekoln usually feature non-nitrogen-fixing *Microcystis aeruginosa* as the dominant component, while the shallow lake Limmaren and Vallentunasjön foster blooms composed of more complex cyanobacterial assemblages of *Anabaena flos aquae, Aphanizomenon* sp. and *Microcystis* sp. Cyanobacterial blooms in Lake Erken are usually characterized by persistent blooms of *Gloeotrichia echinulata* often followed by a late summer bloom of *Microcystis.*

For each of the eight sampling occasions in the four lakes, single surface water samples (0–0.5 m) were collected using acid rinsed and autoclaved polycarbonate bottles. Water temperature was measured on site with a digital thermometer. Samples were transported to the laboratory at *in situ* temperature and processed within 4 h of sampling.

*Chemical analyses, chlorophyll-*a *and bacterial abundance*

Water samples for analysis of dissolved organic carbon (DOC) were first filtered through pre-rinsed 0.2 μm Supor membranes (Gelman, Pall Norden AB Lund, Sweden). Subsamples were acidified by hydrochloric acid added to a final concentration of 0.02 M, and subsequently purged with CO_2-free air for 6 min to remove inorganic carbon. DOC was analyzed by IR detection of CO_2 formed following high-temperature catalytic oxidation on a Shimadzu TOC-5000 instrument (Eiler *et al.*, 2003).

DFAA were analyzed by high-performance liquid chromatography (HPLC) and detected as fluorescent primary amines after derivatization with *o*-phthaldialdehyde (OPA) according to Lindroth and Mopper (1979) with modifications according to Jørgensen *et al.* (1993). For the derivatization, a

Table 1 Abiotic and biotic characteristics of water samples analyzed for bacterial community composition, bacterial production and amino-acid utilization in four Swedish lakes

Lake	*Date*	*Abiotic and biotic characteristics*								
		Temp (°C)	*Chl*-a ($\mu g\,l^{-1}$)	*DOC* ($mg\,l^{-1}$)	*Arg* ($\mu g\,l^{-1}$)	*DFAA* ($\mu g\,l^{-1}$)	*BN* ($10^6\,l^{-1}$)	*Arg-pept.* ($\mu g\ C\,l^{-1}\,h^{-1}$)	V_{amb} ($\mu g\ C\,l^{-1}\,h^{-1}$)	% of BCD[a]
Ekoln	12/8/2002	23.2	249	13.0	0.19	8.69	47.2	77 (4)	0.108	1.06
	22/5/2003	13.8	50.9	14.6	0.35	7.60	5.1	17 (3)	ND	ND
	5/6/2003	18.0	26.5	12.9	0.26	8.91	11.6	28 (3)	0.019	0.58
	30/6/2003	17.1	5.5	11.9	0.13	3.69	6.4	18 (2)	0.007	0.67
	17/7/2003	22.0	23.6	11.5	0.41	5.76	10.8	12 (3)	0.020	2.00
	14/8/2003	20.5	39.4	11.3	0.91	5.15	7.0	11 (1)	0.075	2.03
	11/9/2003	15.7	39.8	11.1	0.30	7.31	6.6	12 (3)	0.019	0.86
	16/10/2003	9.4	23.2	10.8	0.07	1.94	5.2	ND	0.003	0.67
Erken	8/8/2002	21.8	39.1	10.1	0.42	16.50	14.5	107 (3)	0.013	0.71
	21/5/2003	9.5	2.1	9.8	0.10	4.33	9.8	8 (0)	0.001	0.11
	10/6/2003	15.9	4.2	9.8	0.12	4.65	8.6	6 (2)	0.013	2.09
	2/7/2003	17.6	2.0	10.1	0.09	4.40	9.6	7 (2)	0.003	0.30
	18/7/2003	21.0	48.7	10.0	0.25	16.12	4.9	9 (1)	0.012	0.38
	13/8/2003	20.2	26.4	10.3	0.14	3.29	5.7	10 (2)	0.011	0.24
	15/9/2003	15.7	11.1	10.1	0.06	1.91	5.0	ND	0.006	0.14
	7/10/2003	12.3	39.3	9.8	0.11	6.13	2.4	ND	0.002	0.45
Limmaren	14/8/2002	22.7	99.7	10.8	0.82	2.56	68.7	43 (5)	0.098	2.29
	21/5/2003	12.0	11.1	10.5	0.18	8.59	17.4	11 (0)	0.019	1.08
	10/6/2003	17.1	23.5	10.6	0.13	6.45	16.1	16 (2)	0.016	0.94
	2/7/2003	18.3	59.2	11.0	0.66	6.89	22.8	16 (2)	0.106	2.50
	18/7/2003	23.5	141	11.0	0.17	12.53	7.9	31 (14)	0.060	0.51
	13/8/2003	20.3	60.6	10.7	0.15	5.85	8.8	23 (2)	0.038	0.38
	15/9/2003	15.8	55.9	10.90	0.14	10.73	6.4	ND	0.022	0.61
	7/10/2003	11.0	26.1	10.9	0.19	3.17	6.4	ND	0.035	1.22
Vallentunasjön	15/8/2002	23.5	225	11.1	0.09	3.75	109	30 (2)	0.025	0.13
	22/5/2003	14.7	40.9	12.0	0.17	5.42	17.8	32 (2)	ND	ND
	5/6/2003	17.9	42.0	11.5	0.15	5.97	47.6	16 (1)	0.067	0.71
	30/6/2003	17.4	40.4	11.0	0.12	5.88	57.9	33 (3)	0.031	0.82
	17/7/2003	23.6	56.2	11.1	0.25	6.98	45.0	27 (3)	0.122	1.19
	14/8/2003	19.2	59.1	10.1	ND	ND	17.6	17 (2)	ND	ND
	11/9/2003	15.8	101	10.4	0.22	6.60	19.0	13 (2)	0.132	0.83
	16/10/2003	5.4	80.0	9.9	0.34	14.20	10.8	ND	0.137	6.69

Abbreviations: Arg, arginine concentration; Arg-pept, arginine-aminopeptidase activity; BN, bacterial abundance; Chl-*a*, chlorophyll-*a*; DOC, dissolved organic carbon; ND, not determined; % of BCD, % of bacterial carbon demand accounted for by arginine uptake; Temp, temperature; DFAA, dissolved free amino acid; V_{amb}, arginine uptake rate at ambient concentration.
For arginine-aminopeptidase activity, s.e. derived from linear regressions are given in parentheses.
[a]V_{amb} corrected for yield (growth efficiency on arginine; 63–77%) in percentage of bacterial production.

20 μl water sample was mixed with 4 μl OPA solution in a Waters Alliance 2695 HPLC module and was allowed to react for 1 min before injection and separation on a 3.9×150 mm Nova-Pak C18 steel column (Waters Corporation, Milford, MA, USA). After the separation, the derivatized primary amines were quantified on a Waters 470 fluorescence detector. The detection limit for the different amino acids was generally 0.2 nM. The analytical conditions employed enabled detection and quantification of all protein amino acids except proline and cysteine.

Chlorophyll-a was determined from absorbance at 665 nm with correction for suspended solids at 750 nm (Jespersen and Christoffersen, 1987). Volumes of 30–200 ml of water were collected on duplicate Whatman GF/F glass fiber filters by gentle vacuum filtration (<10 kPa). The filters, all visibly green from pigments, were stored in the dark at −80°C before extraction in 96% ethanol for 6 h. The extracts were filtered through a 0.2 μm Supor filter to remove cell debris before absorbance measurements in 1-cm quartz-glass cuvettes.

Samples for bacterial abundance were preserved by adding borax-buffered formaldehyde to a final concentration of 2% (v/v). Samples were stored at 4°C in the dark before analysis. Samples were stained with 4′-6-diamidino-2-phenylindole (DAPI) and counted by epifluorescence microscopy as described previously (Bertilsson and Tranvik, 1998). Duplicate filters were analyzed for each sample and at least 250 cells or 10 fields of view were counted for each filter.

Bacterial production and arginine uptake kinetics

Bacterial production was estimated from ^{14}C-leucine incorporation into proteins as described previously

(Eiler *et al.*, 2006). For each water sample, four 1.7 ml subsamples were spiked with ^{14}C-leucine (specific activity of 306 mCi mmol^{-1}; product CFB67, Amersham Pharmacia Biotech, Uppsala, Sweden) to a final concentration of 100 nM and subsequently incubated for 1 h. The incubation was stopped by adding trichloroacetic acid to 5% final concentration. To one of the subsamples, trichloroacetic acid was added before ^{14}C-tracer additions for use as abiotic controls. Protein precipitates were pelletized by 10 min centrifugation at 14 600 *g*. The supernatants were discarded and pellets were sequentially washed in 5% trichloroacetic acid and 80% ethanol. Optiphase Hisafe 2 liquid scintillation cocktail (1 ml; Perkin Elmer Life and Analytical Sciences, Waltham, MA, USA) was added to each sample and the ^{14}C was determined by liquid scintillation in a Packard TriCarb 2100 TR instrument (Perkin Elmer Life and Analytical Sciences). The signal quenching and counting efficiency was determined in parallel counts of external reference ^{14}C samples. Bacterial carbon production was estimated using the conversion factors of Simon and Azam (1989).

As a model for analyzing the ability of the bacterial community to take up and process amino acids, a kinetic ^{14}C-tracer approach was used to study bacterial utilization of the basic amino acid arginine. It has previously been shown that free arginine is efficiently taken up by freshwater microorganisms at rates corresponding to other free amino acids (Burnison and Morita, 1974). For each sample, uniformly labeled ^{14}C-arginine (specific activity 300 mCi mmol^{-1}; product CFB63, Amersham Pharmacia Biotech) was added to sterile polypropylene screw-cap vials containing aliquots (1.7 ml) of the lake water. The ^{14}C-labeled substrate was added at concentrations of 1, 5, 10, 20, 40 and 80 μg l^{-1}, with two replicates and a formaldehyde-killed control (2% v/v) for each concentration. All vials were incubated on a rotary shaker for 60 min in darkness at *in situ* temperature. Incubations were terminated by adding formaldehyde to 2% final concentration and the samples were immediately processed to separate bacterial biomass from the liquid medium. Particulate matters, including microorganisms, were captured by gentle vacuum filtration ($<$10 kPa) through 0.2 μm membrane filters (Supor 25 mm diameter). Filters were washed with 2 ml of filter-sterilized lake water to minimize absorption of arginine to the filter and the exterior of cells. Processed filters were placed cell-side facing up in 20 ml scintillation vials. Scintillation cocktail (10 ml) was added to each sample for analysis of ^{14}C by liquid scintillation as described above.

The maximum arginine uptake rate (V_{max}) was derived from a modified Lineweaver–Burke plot (Wright, 1978), with the reciprocal slope of a linear regression of the concentration of added arginine as the independent variable and arginine turnover time (T_t) as the dependent variable (Bertilsson and Tranvik, 1998). The regression curve intercept with the dependent axis represents the arginine turnover time at ambient concentration (T_{amb}). Arginine uptake rates at ambient concentration (V_{amb}) can then be obtained by dividing the measured arginine *in situ* concentration with T_{amb}. The s.e. for V_{max}, T_t and T_{amb} was estimated as described previously (Bertilsson and Tranvik, 1998).

In a separate set of experiments performed for the first sampling occasion in each lake, the bacterial respiration of utilized ^{14}C-arginine into biomass and CO_2 (that is, bacterial growth efficiency) was assessed. Similar incubations as above were carried out in larger volumes (5 ml water in 20 ml acid washed scintillation vials) and for a total incubation time of 4 h. For each lake, four replicates were incubated in this way along with two formaldehyde-killed controls. Particles, including bacteria, were retained on membrane filters as outlined above but filtrates were also collected for analysis of ^{14}C-arginine remaining in the water after the incubation. To strip water of $^{14}CO_2$, samples were acidified by adding HCl to a final concentration of 0.02 M followed by vigorous shaking before leaving the vials open overnight in a fumehood. One milliliter of the remaining CO_2-free liquid was mixed with 10 ml liquid scintillation cocktail and the ^{14}C signal was measured as described above. The bacterial growth efficiency was estimated as the fraction of the utilized ^{14}C-arginine partitioning into biomass during the incubation with correction for abiotic controls.

Aminopeptidase activity

Proteolytic activity (arginine-aminopeptidase and leucine-aminopeptidase) was assessed for each water sample. The fluorogenic-peptide analogs L-arginine, 7-amido-4-methylcoumarin hydrochloride (Sigma Aldrich Sweden AB, Stockholm, Sweden) and L-leucine, 7-amido-4-methylcoumarin hydrochloride were first individually dissolved in 5 mM bicarbonate buffer (pH 8). Individual wells in sterile, low-absorption, 96-well polystyrene plates (Nunc A/S, Roskilde, Denmark) were filled with 200 μl aliquots of water and each substrate analog was separately added to nine replicate wells at a final concentration of 40 μM. Plates were incubated in the dark at *in situ* temperature. The enzymatic hydrolysis of the substrate and the coupled release of highly fluorescent 7-amino-4-methylcoumarin (AMC) was measured hourly over a period of 8–16 h depending on the degree of activity. AMC fluorescence was assessed using a MicroWell plate reader connected to a FluroMax-2 spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ, USA) set at 380 nm excitation and 485 nm emission. Potential aminopeptidase hydrolysis rates were calculated from linear regressions of AMC accumulation over time, where fluorescence was converted to AMC

concentration by using a standard curve based on triplicate AMC standards in water samples (0, 5, 10, 25, 50 and 100 μM) on the same plate.

Community composition

For DNA extraction, between 1×10^9 and 2×10^{10} bacterial cells were collected on 0.2 μm membrane filters (Supor; Gelman) by vacuum filtration (<30 kPa). Filters were immediately frozen and stored at −80°C until analysis. Screw cap tubes (2 ml) containing frozen filters were mixed with 0.5 g of precombusted zirconium beads (0.1 mm diameter) and 600 μl lysis buffer (5.25 M guanidine-HCl, 1.5% Triton X-100, 2.5 mM Tris, 0.25 mM ethylenediaminetetraacetic acid (EDTA), pH 7.5). Total nucleic acids were extracted by bead beating at 5000 r.p.m. for 3×45 s in a Mini-Bead beater (Biospec Products, Bartlesville, OK, USA). Filter debris and beads were pelletized by centrifugation at 12 000 *g* for 1 min after which 400 μl of the crude lysates (the supernatants) were mixed with 200 μl of isopropanol before purification on SNAP columns as recommended by the manufacturer (Invitrogen, Carlsbad, CA, USA). This procedure resulted in high-quality genomic DNA with an average size of ~20 kb as revealed by electrophoretic separation on a 1% agarose gel followed by ethidium bromide staining and detection by UV transillumination. This procedure has been used successfully to extract DNA from purified *Bacillus subtilis* endospores as well as a wide range of aquatic isolates affiliated with *Cyanobacteria*, α-, β-, γ-*Proteobacteria, Bacteroidetes* and *Firmicutes* (data not shown), suggesting that few bacteria escape detection. All nucleic acid extracts also contained ribosomal RNA but the concentration of high-molecular-weight DNA was always more than fivefold higher than ribosomal RNA. The concentration of DNA in the final extracts was quantified by agarose gel electrophoresis and comparison with a low DNA mass ladder (Invitrogen).

T-RFLP of bacterial 16S rDNA genes was used to assess bacterial community composition in the studied lakes. Bacterial 16S rDNA was amplified from mixed genomic samples using PCR with bacterial primer 27 forward, modified to match also the *Planctomycetales* (5′-AGRGTTTGATCMTGGCTCAG-3′; Vergin *et al*., 1998) and universal primer 519 reverse (5′-GWATTACCGCGGCKGCTG-3′; Lane *et al*., 1985). The forward primer was labeled with hexachlorofluoroscein at the 5′-end (MWG Biotech AG, Ebersberg, Germany) to enable fluorescent detection of T-RFs. For each sample, four replicate PCRs of 20 μl were amplified in a Stratagene Robocycler with initial denaturation at 94°C for 3 min followed by 25 cycles of 1 min at 94°C, 1 min annealing at 55°C and 3 min primer extension at 72°C and a final extension at 72°C for 7 min. Each tube contained 1–4 ng of target DNA, PCR buffer (10 mM Tris-HCl, pH 9, 50 mM KCl, 0.1% Triton X-100 and 2 mM $MgCl_2$), 100 nM of each primer, 200 μM of each dNTP and 0.25 U Taq DNA Polymerase (Invitrogen). Pseudo-terminal fragments were eliminated by digesting single-stranded DNA generated in the amplification with mung bean nuclease (Egert and Friedrich, 2003). PCR products from single samples were then pooled and purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany). For each sample, duplicates containing approximately 50 ng of PCR product were separately digested with the four-cutter endonuclease *Hha*I for 16 h. Fluorescently labeled T-RFs were separated by size and quantified in an ABI 3700 96-capillary sequencer running in GeneScan mode (Applied Biosystems, Foster City, CA, USA). T-RFLP electropherograms were analyzed with GenScanView 4 (CRIBI group, http://grup.cribi.unipd.it) using 1% of the total peak area as a lower threshold for positive T-RFs.

Multivariate statistics

Only peaks detected in the both analytical restriction digest replicates were included in the binary data of presence or absence of individual T-RFs. To determine the community similarities between the 32 samples, the binary data were used to calculate a similarity matrix (D_{xy}) according to the Sørensen equation: $D_{xy} = 2N_{xy}/(N_x + N_y)$, where N_x and N_y represent the number of operational taxonomic units (OTUs) either in sample *x* or *y*, respectively, and N_{xy} is the number of OTUs present in both samples. Semi-strong hybrid nonmetric multidimensional scaling (NMDS) was performed in Statistica (Statsoft, Tulsa, OK, USA) to reduce the multidimensional similarity matrix to two dimensions (Eiler *et al*., 2003). A Mantel test was used to correlate the dissimilarity matrix of bacterial community composition with a dissimilarity matrix of biotic parameters; bacterial production, chlorophyll-*a*, arginine-peptidase activity and arginine V_{max}.

In parallel, the average concentrations of each T-RF in the different samples were used as variables in a multivariate analysis to link function and composition of the bacterial communities. Partial least square projection to latent structures (PLS) with autoscaling was performed in Simca-P + version 11.0 (Umetrics AB, Umeå, Sweden; Wold *et al*., 2001). The heterotrophic response variables, bacterial production and arginine V_{max} were log-transformed before analysis. Bacterial ribotypes (T-RFs) with little importance for the model were, five at the time, iteratively excluded from the analysis according to the VIP plot until the goodness of prediction (Q^2) reached a maximum. PLS has some advantages to other multivariate statistical methods, such as multiple linear regression, since it can analyze data with strongly collinear (correlated), noisy and numerous *X* variables (T-RFs). PLS also performs well on matrices with few observations and many variables (Wold *et al*., 2001).

Results

Dissolved organic carbon concentration ranged from 9.8 to 14.6 mg C l^{-1} in the 32 samples from the four lakes (Table 1). In addition to the limited variation in DOC within and among each of the four lakes, there were no significant correlations between this parameter and either chlorophyll-*a*, bacterial biomass, bacterial production, peptidase activity or arginine V_{max} ($r^2 < 0.07$, $P > 0.05$).

Chlorophyll-*a* on the other hand, was much more variable and ranged from 2 to 250 mg l^{-1} among the 32 samples. Even within a single lake (Lake Ekoln), chlorophyll-*a* varied almost 50-fold (Table 1). The chlorophyll-*a* content (assumed to be a proxy for phytoplankton-derived autochthonous organic carbon input) was positively correlated with bacterial production ($r^2 = 0.40$, $P < 0.0001$) and there was also a weak but significant positive correlation between chlorophyll-*a* and arginine V_{max} ($r^2 = 0.17$, $P < 0.05$).

As expected, there was a positive correlation between bacterial production and arginine V_{max} ($r^2 = 0.66$, $P < 0.0001$). Arginine V_{max} varied between 10% and 100% of bacterial biomass production (Figure 1). Over the study period, arginine V_{amb} (Table 1) ranged from 0.3% to 21% of V_{max} with no significant correlations to any of the studied biotic or abiotic lakewater characteristics ($r^2 < 0.06$, $P > 0.05$), except arginine concentration *in situ* (Table 1; $r^2 = 0.53$, $P < 0.0001$). This latter correlation is expected since arginine V_{amb} is estimated from arginine turnover time at ambient concentration and the *in situ* concentration of this amino acid.

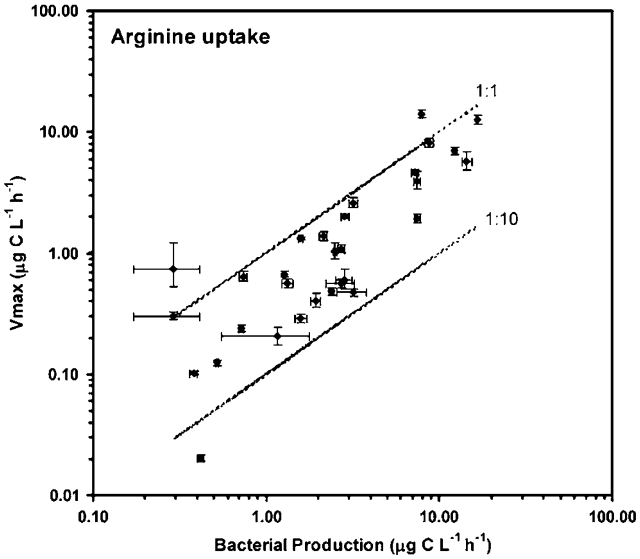

Figure 1 Log – log plot of bacterial biomass production and maximum arginine uptake rate (V_{max}) in seasonal samples from the four studied Swedish lakes. Data points represent averages with error bars marking the estimated s.d. (bacterial production; $n = 3$) or the s.e. for V_{max} derived from the linear regressions. Dotted lines mark break points where estimated V_{max} is 10% or 100% of bacterial production.

Measured arginine concentrations were always low, ranging from 0.06 to 0.91 µg C l^{-1} (0.8–12.6 nM). The *in situ* concentration of dissolved free amino acids (DFAA) was also low, ranging from 1.9 to 16.5 µg C l^{-1} (50–440 nM) with a variable fraction of the DFAAs accounted for by arginine (1.3–32%; Table 1). The bacterial growth efficiency on arginine was always high, ranging from 63% in the least productive lake (Erken) to 73%, 75% and 77% in lake Ekoln, Limmaren and Vallentunasjön, respectively.

Arginine- and leucine-peptidase activity was strongly positively correlated ($r^2 = 0.65$, $P < 0.0001$), indicating overlapping substrate specificity of the exoenzymes mediating these processes, or some other link between the two hydrolytic processes (for example related to enzyme expression or organisms involved). Potential arginine-peptidase activity was always higher than arginine V_{max} but the difference between these two measures of functional community potential was highly variable (Table 1).

The average community similarities within each lake, determined from the Sørensen similarity matrix of eight samples, showed moderate variation as it ranged from 0.48 to 0.53 within each of the four lakes. In all four lakes, bacterial community composition was dynamic as revealed by the NMDS analysis (see Supplementary Figure S1). Changes in community structure are given in detail in Figure 2, showing the distribution of T-RFs in each lake and sampling occasion. A total of 93 different T-RFs could be detected, whereof 27 were observed only in a single sample (not included in Figure 2). Most T-RFs were observed at low frequency among samples, and only one T-RF (293 bp) was detected in all 32 samples.

A Mantel test revealed weak but significant correlations between binary data of bacterial community similarity, and bacterial production ($r = 0.093$; $P < 0.05$) and arginine V_{max} ($r = 0.114$; $P < 0.05$), but not to arginine-peptidase activity ($r = -0.06$; $P > 0.05$). A significant correlation was also observed between chlorophyll-*a* and bacterial community similarity ($r = 0.128$, $P < 0.01$).

In parallel, PLS was used to determine how variances of bacterial production and arginine V_{max} are associated with the intensity of detected T-RFs (*X* variables). The PLS models exhibited high goodness of fit (R^2) and goodness of prediction (Q^2; Table 2). Also the residuals, obtained from regression models using chlorophyll-*a* as independent variable and either bacterial production or arginine V_{max} as *Y* variables, could be predicted from bacterial community composition (variance in the intensity of detected T-RFs; Table 2). Permutation tests with randomized *Y* variables had mostly negative effects on Q^2 for the four models. Many of the T-RFs had a strong influence on all or most of these PLS models (for example, T-RFs; 139, 204, 468, 473, 499, 513 and 519 bp; Figure 3), whereas many more appeared to be important for predicting either

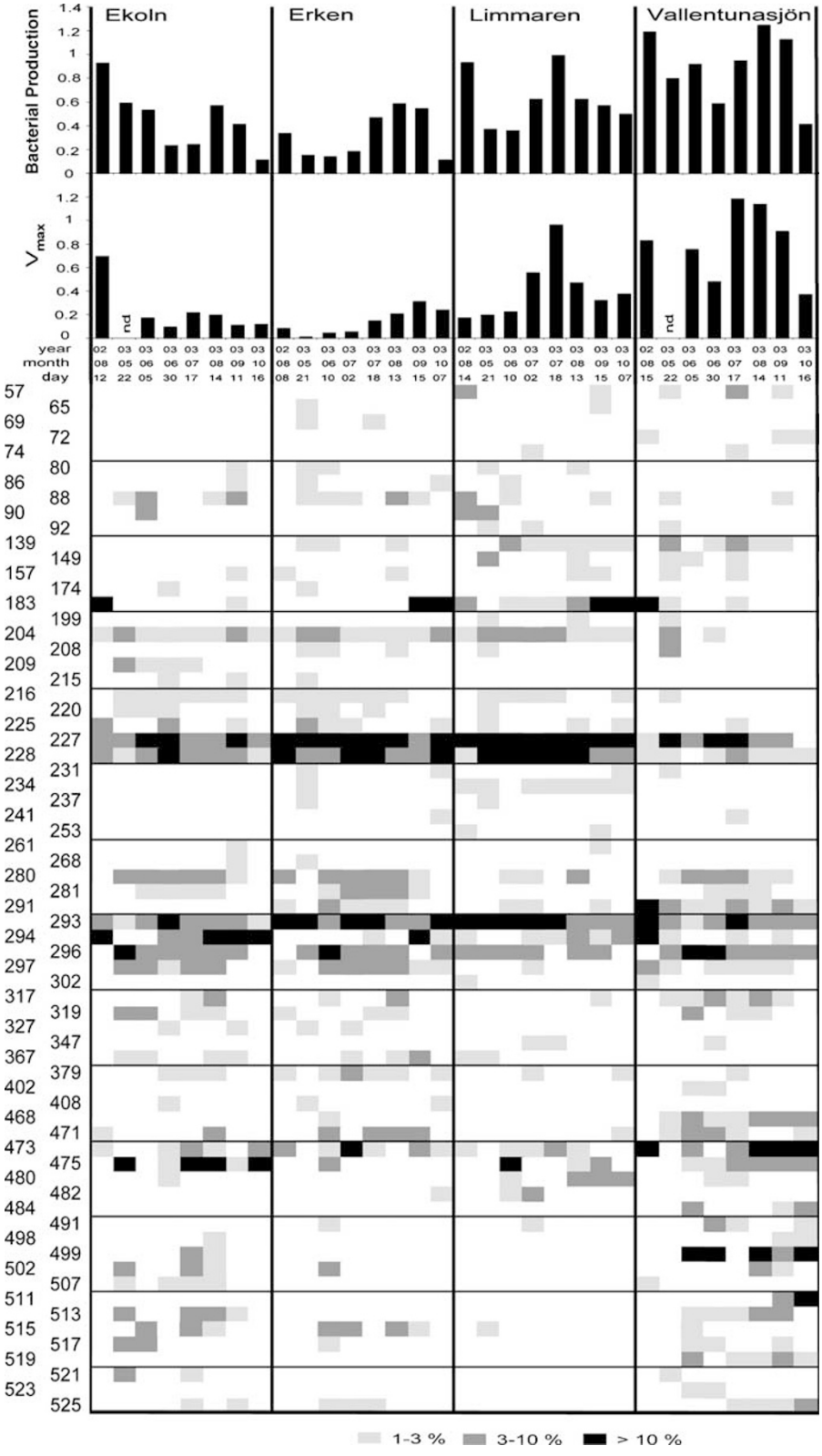

Figure 2 Measured bacterial production (μg C l^{-1} h^{-1}) and maximum arginine uptake rate (arginine V_{max}; μg C l^{-1} h^{-1}) for each lake sample (date and location) in the four Swedish lakes (top panels). The lower part of the graph shows, for each sample, the presence/absence of T-RFs detected in more than 1 of the 32 samples. The size (bp) of each detected T-RF is given to the left. The presence of a black or gray cell indicates that the T-RF was present on that sample date with the degree of shading marking its relative abundance in percentage of total T-RF peak area (<3%, 3–10%, >10%).

Table 2 Model statistics of PLS

Response variables	R^2	Q^2	*No. of samples*	*No. of T-RFs*	N
Bacterial production	0.85	0.53	32	32	3
Residual bacterial production vs chlorophyll-*a*	0.79	0.54	32	22	2
Arginine V_{max}	0.84	0.69	30	37	2
Residual arginine V_{max} vs chlorophyll-*a*	0.81	0.49	31	22	2

Abbreviations: *N*, number of PLS components; PLS, partial least square projection to latent structures; Q^2, goodness of prediction; R^2, goodness of fit; arginine V_{max}, maximum arginine uptake rate.
Each model is based on a number of terminal restriction fragments (no. of T-RFs) that describe the response variable measured for each sample (no. of samples). Additional information is provided in Supplementary Figure S2.

production, arginine V_{max}, or the residuals after normalizing these heterotrophic functions for chlorophyll-*a* levels (Figure 3).

Discussion

Proteins and peptides represent a major component of all living cells and have also been identified as major substrates for heterotrophic microorganisms in fresh waters (Kirchman *et al.*, 1985; Rosenstock and Simon, 1993; Jørgensen *et al.*, 1993; Tranvik and Jørgensen, 1995). A recent review by Kirchman (2003) concludes that amino acids typically sustain more than 20% of the bacterial carbon demand in a wide range of habitats (lakes, estuarine and marine), but the variation between and within these habitats can be large (from 6% to all of the carbon demand for lake bacterioplankton). An even greater variability in the fraction of bacterial production accounted for by total amino-acid uptake was observed in Lake Constance (Rosenstock and Simon, 1993). This variability may simply be an effect of the concentration of labile substrates (amino acids) presented to the organisms, but it may also reflect the ability of unique bacterial communities to utilize labile amino-acid substrates at high rates, or at low concentrations or in a specific polymer form (as proteins and peptides).

In the present study, arginine was used as a model to probe bacterial amino-acid utilization. Arginine makes up a rather constant proportion of proteins in eukaryote and bacterial planktonic cells, typically 3–6% (Lourenço *et al.*, 1998). Utilization of this single amino acid accounted for 0.1–7% of estimated bacterial carbon demand in our study (Table 1). The uptake rates at ambient concentration were usually less than 10% of arginine V_{max}. This is most likely linked to the very low-ambient arginine concentrations in these lakes ($<0.9\,\mu g\,l^{-1}$; Table 1). Arginine V_{max} sometimes exceeded bacterial production (Figure 1). Hence, considering the high-estimated bacterial growth efficiency on arginine in the studied lakes (63–77%), bacterial communities could potentially saturate their carbon demand by using a single organic substrate, at least during sudden episodes of high-arginine inputs. In surface waters, such inputs are not necessarily visible at larger scales (milliter to liter), but may instead occur at the microliter scale, for example, during lysis or predation of individual cells. Hence, there may be a need for studies at the single-cell level if we are to fully understand bacterial strategies for substrate acquisition in aquatic ecosystems.

The measured arginine V_{max} in the lakes of $0.1–10\,\mu g\ C\,l^{-1}\,h^{-1}$ ($1–140\,nmol\,l^{-1}\,h^{-1}$) were more variable than rates previously measured for arginine in both lake water ($0.02–0.07\,\mu g\ C\,l^{-1}\,h^{-1}$; Burnison and Morita, 1974) and in estuarine water ($0.13–0.17\,\mu g\ C\,l^{-1}\,h^{-1}$; Decho *et al.*, 1998), but may reflect that seasonal changes were not examined in these studies. Another divergence was the large (typically 10- to 100-fold) difference between V_{max} and actual uptake rates (V_{amb}) in the Swedish lakes, relative to amino-acid uptake kinetics in some other lakes. Thus, in three Danish lakes, V_{amb} of free amino acids (not including arginine) were found to make up 30–87% of the V_{max} (Jørgensen and Søndergaard, 1984). Possibly, as discussed above, microorganisms in the productive Swedish lakes could be adapted to sporadic and local bursts of organic matter with a high content of amino acids, for example inputs linked to blooms of cyanobacteria. In the present study, we did not test for possible multiphasic uptake kinetics at high substrate concentrations (Azam and Hodson, 1981; Logan and Fleury, 1993). Such information could give a more complete understanding of the arginine-processing features of the microbial community, but would require incubations with a wider range of arginine concentrations than the 5–460 nm used in our study.

The low occurrence of arginine among free amino acids in the four lakes (arginine on average made up 3% of all free amino acids) agrees with amino-acid profiles of other lakes (Jørgensen, 1987; Simon and Rosenstock, 1992). In general, arginine is not a major amino acid in proteins of aquatic organisms (constitutes 3–8% of protein amino acids; compilation from several sources), but arginine may still be an important amino acid to bacteria owing to its high C/N ratio of 3. During degradation of arginine, microorganisms are supplied with excess nitrogen and this nitrogen can subsequently be used for

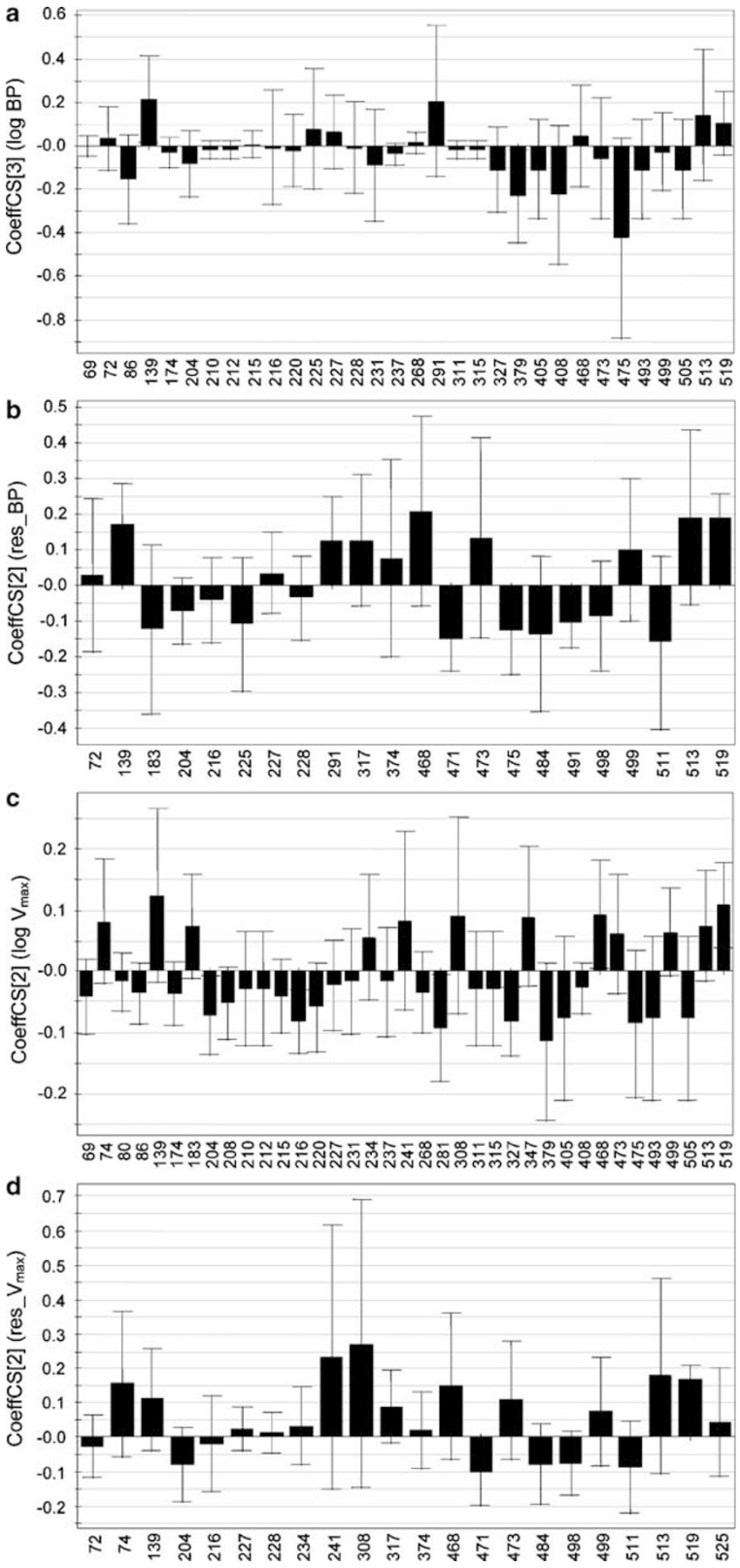

Figure 3 Plot of regression coefficients of T-RFs of PLS models using (**a**) log bacterial production (BP), (**b**) residual_BP as predicted from chlorophyll-*a*, (**c**) log maximum arginine uptake rate (V_{max}) and (**d**) residual_V_{max} as predicted from chlorophyll-*a* as the response variable, respectively. Positive columns mean that the variables are positively correlated to the response variable. Negative columns are variables in which high values diminish the response. Error bars denote 95% confidence intervals derived from jackknifing.

incorporation of other, low-nitrogen organic compounds into for example amino acids.

Like in previous studies, we could show that aminopeptidase activity and uptake of the resulting DFAA seem to be uncoupled (Karner and Herndl, 1992; Smith *et al*., 1992; Middelboe *et al*., 1995). This may suggest that peptidases expressed by the community have the potential to saturate bacterial uptake systems in the presence of high amounts of peptides and proteins susceptible to enzyme attack. One explanation for this apparent uncoupling could be variation in the composition of the heterotrophic bacterial community and in the ability of different populations in the community to mediate peptidase activity and DFAA uptake (Cottrell and Kirchman, 2000). Thus, variation in the composition of bacterial communities could explain some variability in amino-acid uptake observed in natural environments.

Correlation analysis of the seasonal data from the four lakes revealed weak or insignificant correlations between selected environmental variables that are likely to influence DOM processing, for example, DOC, bacterial abundance, chlorophyll-*a* and functional characteristics relevant for DOM dynamics, such as bacterial production and arginine V_{max}.

Both bacterial production and arginine V_{max} could be predicted from T-RFLP patterns, suggesting a link between function and community composition. This does not necessarily mean that there is a direct causal link since we cannot rule out a situation where shifts in composition and function are a result of covariation driven by environmental factors. It is also clear that PCR and T-RFLP have some well-known limitations (see, for example, Loisel *et al*., 2006 and references following it), but this technique represents a high-throughput approach to compare the composition of complex natural bacterial communities from many samples.

To provide further indications of a possible link between function and composition, two additional PLS analyses were performed, that is residuals from regression models in which chlorophyll-*a* concentrations were used to explain bacterial production and arginine V_{max} were predicted from T-RF abundances (Table 2). The high Q^2 (>0.5) in these models suggests that the relationship between environmental conditions (chlorophyll-*a*) and community function may be influenced by bacterial community composition. This pattern could, for example, be the result of population interactions (as suggested by Bell *et al*., 2005), feedbacks between populations and their environment, as well as a time lag for the bacterial community to adjust to fluctuating environmental conditions.

Previous studies on bacteria have rarely studied relationships between biogeochemical functions and bacterial community composition in natural environments. However, results from controlled laboratory experiments suggest weak or insignificant coupling between microbial community composition and metabolic function (Franklin and Mills, 2001; Findlay *et al*., 2003). Fernandéz *et al*. (1999) showed that natural bacterial communities can maintain a stable ecosystem function over time despite changes in composition. Similarly, Langenheder *et al*. (2006) observed high functional similarity (that is, exoenzyme activity and monomer processing) in bacterioplankton communities emerging from different inocula but incubated under identical conditions. These findings imply that coupling between function and bacterial community composition is not necessarily tight and that bacterial communities may exhibit considerable functional redundancy. These observations also suggest that the composition of bacterial communities have little influence on the metabolic features of the community and that such ecosystem-level function is a direct consequence of the abiotic and biotic environment.

In natural environments, physicochemical parameters, predation and resource availability are free to fluctuate concurrently over time and space resulting in a greater total variability than under controlled conditions. Previous studies have shown that the bacterial community composition strongly depends on the physicochemical conditions of the environment (Lindström, 2000; Yannarell and Triplett, 2004; Lindström *et al*., 2006b), interspecific interactions (Pernthaler *et al*., 2004; Schwalbach *et al*., 2005; Kent *et al*., 2006), available resources (Pinhassi *et al*., 1999; van Hannen *et al*., 1999; Crump *et al*., 2003; Newton *et al*., 2006) and dispersal (Curtis and Sloan, 2004; Langenheder *et al*., 2006; Lindström *et al*., 2006a). However, in none of these studies were there any attempts to predict bacterial distribution patterns from nonbacterial parameters. In a recent study, discriminate function and multiple regression analysis were used to predict successfully seasonal changes in marine bacterial community composition over seasons (Fuhrman *et al*., 2006). This study indicates the existence of specific niches of individual populations within planktonic bacterial communities. If this is a general feature, one consequence might be that functional responses to environmental change may vary depending on the bacterial community composition on hand (Langenheder *et al*., 2006; Szabó *et al*., 2007). However, more experimental studies are certainly needed to verify if the rivet theory, which states that every niche is filled by a single species, applies to microbial communities (Ehrlich and Ehrlich, 1981).

On the basis of PLS coefficients and the VIP plots (data not shown), it was possible to identify the T-RFs, which were most important for predicting the studied indicators of community function (bacterial production and arginine V_{max}), although verifying experiments are necessary to directly link particular functions to individual populations. Some of the T-RFs observed in our community analyses could be

tentatively affiliated with bacterial groups by comparing the T-RF lengths to *in silico* digests of 16S rRNA sequences in clone library data from the same set of lakes (Eiler and Bertilsson, 2004). In this way, many of the T-RFs, strongly influencing the PLS models, could be putatively linked to specific phylogenetic groups. For example, T-RF 519 bp likely represents an *Actinobacteria* population that was positively related to both bacterial production and V_{max}. In contrast, T-RF 204 bp likely represents a *Bacteroidetes* population that was negatively correlated to both bacterial production and arginine V_{max}. Several of the cyanobacterial T-RFs (291 and 473 bp) also had a strong positive relationship to bacterial production and arginine V_{max}, while other cyanobacterial T-RFs had a negative relationship to either bacterial production (T-RF 475 bp) or residual variance in bacterial production after chlorophyll normalization (T-RF 471 bp). Altogether, the results suggest that several bacterial populations like *Cyanobacteria* as well as members of diverse phylogenetic groups, including *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* covaried with bacterial production and arginine-processing ability of the community. Previous work suggests that the ability to assimilate ^{3}H-leucine differs among major phylogenetic groups. For example, in freshwater samples from the Delaware Estuary, *β-Proteobacteria* dominated the assimilation of leucine and Cytophaga-like bacteria (*Bacteriodetes*) comprised the second most important group (Cottrell and Kirchman, 2003). It has also been shown that different bacterial lineages develop on DOM of different quality and concentration (Kisand *et al*., 2002; Eiler *et al*., 2003; Kent *et al*., 2006).

Even so, it was not possible to affiliate all T-RFs with bacterial taxa, since many T-RFs either included multiple phylogenetic groups or were not detected in the existing clone libraries. Also, several of the putatively identified bacterial groups had little influence on the PLS models, for example the cyanobacterial T-RFs 293, 294, 296 and 297 bp. Other putatively identified T-RFs with minor influence on the PLS models were T-RFs 57 bp (*Planctomycetes*, LiUU-9–218), 216 bp (*β-Proteobacteria*, LiUU-11–179.2), 220 bp (*β-Proteobacteria*, LiUU-5–340), 225 bp (*Actinobacteria*, acI) and 327 bp (*Bacteroidetes*, LiUU-9–28) (Figure 3).

Our analyses did not demonstrate that these T-RFs were the only ones in the community performing these functions. Yet, as the studied functional response variables are biogeochemically significant processes in the lakes, we propose that the subset of ribotypes (represented by T-RFs) being identified as strong predictors may represent functionally important groups in productive freshwater environments. To conclude, the high predictability and significance of the PLS models indicate a link between bacterial community composition and ecosystem function, but further studies are needed to elucidate the underlying mechanisms.

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