Strong coupling between natural Planctomycetes and changes in the quality of dissolved organic matter in freshwater samples

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Abstract
Dilution-regrowth experiments coupled to fluorescence in situ hybridization were conducted with samples from two humic reservoirs in order to examine how inorganic nutrients (N, P) affect free-living bacterioplankton phylogenetic groups and subsequently the quality of dissolved organic matter (DOM). The experiments were complemented by analyses of the empirical relationships between the targeted bacteria, nutrients, DOM and grazers. The ratio of absorbance of waters ($A_{250}$ nm:/$A_{365}$ nm), which has been found to increase with the proportion of small molecules in the DOM pool in other humic waters, was used as an index of DOM quality. When nutrient stimulated bacteria, both the responses of bacterial groups (in the absence of grazers) and the ratio $A_{250}$ nm:/$A_{365}$ nm were generally different between treatments (+N, +P, +NP), suggesting that in nutrient-poor systems, differences in the type of inorganic nutrient supply will ultimately cause differences in DOM quality. The ratio $A_{250}$ nm:/$A_{365}$ nm peaked in the +N treatments where members of the Planctomycetes (PLA) were the most stimulated group, and across treatments, PLA best explained (positive relationship) variations in this ratio. Consistent with this, the in situ data showed that the removal of the negative effects of flagellates on PLA yielded the highest $R^2$ in attempts to use bacterial groups to explain variations in $A_{250}$ nm:/$A_{365}$ nm. These findings provide lines of evidence, not previously demonstrated in natural waters, that Planctomycetes may be an important factor changing the DOM quality, particularly in nutrient-poor systems when supplied with inorganic N.

Introduction
It is well known that bacterioplankton total abundance and production are influenced by inorganic nutrients such as phosphorus (P) and/or nitrogen (N) in many aquatic systems (e.g. Toolan et al., 1991; Rivkin & Anderson, 1997). In nutrient-poor waters in particular, bacterial utilization of dissolved organic matter (DOM) seems to be strongly dependent on inorganic nutrient availability (Tranvik, 1988; Skoog et al., 1999). It is now well established that the bacterioplankton community is phylogenetically very diverse (see Giovannoni & Rappé, 2000; Zwart et al., 2002). One of the current challenges in aquatic microbial ecology is to understand the function of the different members of this community and how these functions affect the functioning of the ecosystem. DOM decomposition is one of the key processes in the functioning of aquatic systems (Tranvik, 1988; Wetzel et al., 1995; Del Giorgio et al., 1997). Several studies have attempted to identify the bacterioplankton groups or lineages that are involved in this decomposition (e.g. Cottrell & Kirchman, 2000; Kisand et al., 2002; Kisand & Wikner, 2003; Eiler et al., 2003). However, these studies do not often take into account the fact that most aquatic systems receive pulses of inorganic nutrients, and that bacterial groups or lineages may respond differently to inorganic nutrient additions (e.g. Fisher et al., 2000; Pinhasi & Berman, 2003). Hence, the effects that the inorganic nutrient availability or type may have on the links between DOM and bacterioplankton phylogenetic groups are poorly known. The present study experimentally examined these links following inorganic nutrient additions. The experiments were complemented by analyses of the empirical relationships between these variables in ambient waters. Given that strong interactions often occur between resources, bacteria and grazers (Pace & Cole, 1994), it was expected that the bacterial groups playing a major role in the degradation of DOM in this context (i.e. following amendments) would be more strongly related to changes in DOM.
quality if they are not influenced by limiting factors such as protists, which are the main grazers of bacteria. Bacterial activity and the abiotic variables that may change with it (e.g. oxygen concentration), or influence it indirectly through effects on DOM (e.g. light), were also considered. The study sites were two boreal humic reservoirs chosen to provide contrasting abiotic characteristics. One was old and the other was newly flooded. Newly flooded reservoirs are expected to have higher amounts of nutrients and readily degradable organic matter than old reservoirs (St Louis et al., 2000).

Materials and methods

Study sites and sampling

The two reservoirs under study, Sainte Marguerite 3 (SM3) and Manicouagan 5 (M5), are situated in the northern part of the Canadian Shield in the province of Quebec (50°–52°N, 66°–70°W). They are used for hydroelectric power production. SM3 was flooded in 2000, whereas M5 was flooded in 1967. SM3 is a canyon-shaped reservoir that has a surface area of 253 km², a length of 140 km and a width that varies from a few hundred meters to 10 km. Maximum and mean depths are 350 and 62 m, respectively for M5, compared with 180 and 49 m, respectively, for SM3. Finally, the average water residence time is longer in M5 (6.4 ± 0.9 years) than in SM3 (2.4 ± 1.1 years).

Samples for the present study were collected in June (mid–spring, ~2 weeks after snow melt) and July–August (summer) 2002, at 11 stations in the two reservoirs, using a sea plane. The stations were located in the longitudinal axis in SM3, and round the water ring in M5. In the latter reservoir, the number assigned to each station corresponds to a given hour in a clock and indicates its location (e.g. 1200 for 12:00 h). Prior to sampling at each station, oxygen concentration, temperature and light profiles were determined in the water column using a multiparameter probe (YSI 6600, for oxygen and temperature) and a Li-1400 apparatus equipped with a submersible probe (Li-193 SA, for light). The average light amount (I_m) in the euphotic zone or in the epilimnion (both named here Z_i) was calculated later using the equation I_m = I_0(1−exp[−kZ_i])/kZ_i (Stefan et al., 1976), where I_0 is the irradiance measured a few centimeters below the water surface, and k the light attenuation coefficient determined from regressions of depth with light intensities in the water column.

At each station, samples were collected every 0.5 m in the epilimnion or the euphotic zone (when there was no thermal stratification), pooled, placed in cans that isolated them from external temperature, and transported with ice to the field laboratory where they were processed upon arrival (see below), using acid-washed, extensively rinsed and autoclaved material.

Dilution culture experimental set-up

Three dilution experiments, which consisted of inoculating natural bacterioplankton assemblages (inoculum) into 0.2-μm-filtered lake water (diluent), were performed to examine bacterial growth with and without nutrients. A dilution ratio of 1:4 (inoculum/diluent, instead of the commonly used ratio of 1:9) was used in order to avoid large reduction in the complexity of the bacterial assemblages. The inoculum contained free-living bacterioplankton and was obtained by filtering natural lake water through 0.8-μm filters (filamentous forms were not found in the samples), which eliminate protists and other eukaryotes. The diluent was obtained by gently (< 50 mmHg) filtering lake water through precombusted Whatman GF/F glass-fiber filters, and then through a 0.2-μm GTTP filter. The filters were changed regularly in order to minimize clogging, cell bursting and enrichment of filtrates. All the dilutions were prepared in triplicate 1-L dark brown bottles. The treatments were either unamended (control, no nutrient solution added) or enriched with nitrogen (N, as NH_3NO_3) and phosphorus (P, as K_2PO_4), alone or in combination (i.e. N+P = +NP). Because no nutrient data from these reservoirs were available prior to this study, N and P were added (50 and 6.5 μM, respectively) to yield, in the +NP treatments, a ratio of added nutrients close to the average N:P in natural bacterioplankton, i.e. 9–10 (Fagerbakke et al., 1996). Experiment 1 (Exp1) was conducted over 96 h with samples from M5, while experiment 2 (Exp2) was performed over 88 h with samples from SM3. Experiment 3 (Exp3), in which free-living bacteria from M5 were grown in a diluent from SM3, lasted only 48 h because the sampling period was shortened owing to poor weather. For Exp3, an additional control was prepared, in which the diluent was from M5. Note that, except when bacteria from M5 were grown in diluent from SM3, the inoculum and the diluent always came from the same sample and sampling station. The diluted samples were gently mixed before and after nutrient addition, sampled immediately after enrichments for initial conditions (T_0) and then incubated in the field laboratory at 16 °C in darkness until the end of the experiment. Temperatures around 16 °C are common in summer in surface waters of reservoirs in these areas of the Canadian Shield (information from Hydro-Quebec; see also Tadonléké et al., 2005). Sub-samples were taken, in general, every 24 h. Total bacterial abundance and production in the experimental bottles were determined at all time points, whereas bacterial phylogenetic groups and the absorbance of water were examined at T_0 and T_48. Preliminary tests, which consisted of measuring
absorbance at $T_0$ and $T_{48}$ in diluents with and without nutrients and incubated without bacteria in the same conditions as the experimental bottles, showed that neither the absorbances of interest (listed below) nor their ratios were significantly different between treatments or sampling times (data not shown). It was concluded that added nutrients alone did not affect the absorbance of waters.

**Chemical analyses**

Subsamples for dissolved nutrient, dissolved organic carbon (DOC) and DOM absorbance analyses were gently (< 50 mmHg) filtered through 0.2-μm filters. Nutrients were analyzed by common colorimetric methods using a Traccs autoanalyzer (Bran and Luebbe Analyzing Technologies Inc). DOC concentrations were determined by the high-temperature catalytic method, using a Shimadzu TOC-5000A analyzer. The absorbance of DOM (A) in the samples was measured at different wavelengths commonly used in attempts to characterize DOM in waters (250, 280, 340, 350, 365 and 440 nm), using a fluorescence spectrometer (F 2000, Hitachi). Different indexes of DOM quality commonly used in the literature were tested ($A_{250 \text{ nm}}/A_{365 \text{ nm}}, A_{350 \text{ nm}}/\text{DOC}, \text{C:N}, \text{C:P}$). Among them, the ratio $A_{250 \text{ nm}}/A_{365 \text{ nm}}$ gave the best statistical relationships with bacteria and light, two of the factors that may change DOM quality. This ratio was therefore chosen as the index of DOM quality. Using a chromatographic method and samples from humic lakes, De Haan (1972) found that this ratio increases as the proportion of small molecules increases in the DOM pool.

**Biological analyses**

Subsamples for microbial community analyses were preserved using formaldehyde (final concentration 2%) for bacteria, glutaraldehyde (final concentration 1%) for heterotrophic nanoflagellates (HNF) and acidic Lugol's solution for ciliates. HNF were counted under an epifluorescence microscope, after staining preserved samples with DAPI (4’6 diamidino-2-phenylindole, 3 mg L$^{-1}$ final concentration) and filtration through 0.8-μm polycarbonate filters. Ciliates were counted under an inverted microscope with phase contrast, after sedimentation of subsamples for 48–60 h. Cell sizes were measured for these protists. All the experimental samples were checked for flagellates, whereas for ciliates, because a relatively high volume of subsample is required for counts, only samples collected after dilution and at the end of the experiments were examined. No ciliate was found whereas a few flagellates were found in some samples but only after $T_{48}$. The abundance of bacteria was determined using both microscopy (for the bacterial phylogenetic groups) and flow cytometry (to distinguish bacteria with high nucleic acid content from those with low nucleic acid content). For counts by microscopy, preserved bacteria were stained with DAPI and collected on 0.2-μm polycarbonate filters. For the flow cytometry analyses, bacteria were stained with the nucleic acid dye SYBR Green II. Bacterial production was estimated from $^3H$-leucine (40 nM) incorporation by bacteria (Kirchman, 1993). The methods used for these analyses are the same as those described in detail elsewhere (Tadonléké et al., 2005).

After 8 h in fixative at 4 °C, subsamples for the analysis of bacterial phylogenetic groups were filtered through 0.2-μm polycarbonate filters, which were then stored at −20 °C and analyzed within 3 months. This analysis was done using the standard fluorescence in situ hybridization (FISH) method. Several probes were used to detect prokaryotes, among which were HGC69a (for *Actinobacteria*), ARCH915 (for *Archaea*) and CF319a (for members of the *Cytophaga*-Flavobacterium cluster). Later (i.e. after all the analyses had been performed), I found literature showing that (1) the probe ARCH915 may bind to some bacteria (Pernthaler et al., 2002a), and (2) that enzyme treatment is needed for a better analysis of *Actinobacteria* with the probe HGC69a (Sekar et al., 2003). Based on this, it was suspected that the results obtained with these two probes here might have been affected. On the other hand, none of these three prokaryote groups shows significant relationships with DOM [see Table 1 for CF319a and note that this probe has been found to miss several *Cytophaga*-Flavobacteria (Weller et al., 2000)]. The present results thus focused on bacterial groups that showed links with the index of DOM quality, namely the Betaproteobacteria, targeted by the probe BET42a (Manz et al., 1992), the Gammaproteobacteria, targeted by the probe GAM42a (Manz et al., 1992) and members of the phylum Planctomycetes, targeted by the probe PLA886 (Neef et al., 1998). For BET42a and GAM42a, competitor oligonucleotides were used to increase the specificity of probes during hybridization (Manz et al., 1992). The probe NON338 (which generally bound < 1% of DAPI-stained bacteria in this study) was used as a negative control. The probes were labeled (by the manufacturer) with the fluorophore Cy3 (Indocarbocyanine). For BET42a and GAM42a, the hybridization solution contained 2 ng of probe μL$^{-1}$, 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% sodium dodecyl sulfate (SDS) and formamide. The composition of the hybridization solution was the same for PLA886, except that the pH was 8.0. The formamide concentration was 35% for BET42a, GAM42a and PLA886. The wash solution contained 20 mM Tris-HCl (pH 7.4 for BET42a and GAM42a and pH 8 for PLA886), 10 mM EDTA (for BET42a and GAM42a), 0.01% SDS and NaCl at a concentration appropriate for the probe (Manz et al., 1992; Neef et al., 1998). Each filter (diameter 25 mm) containing the collected bacterioplankton was cut into several small sections. Each section received a 4-μL droplet of the hybridization solution.
Table 1. Abiotic and biotic variables at sampled stations during the investigation

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Station</th>
<th>Im (μm² m⁻² s⁻¹)</th>
<th>Dissolved O₂ (mg L⁻¹)</th>
<th>DIN (μM)</th>
<th>DIP (mg L⁻¹)</th>
<th>A₃₅₀ nm:DOC</th>
<th>A₃₅₀ nm: DOC</th>
<th>GAM (x 10⁵ cells mL⁻¹)</th>
<th>BETA</th>
<th>PLA</th>
<th>CF</th>
<th>% HNA</th>
<th>BP (pmol Leu L⁻¹ h⁻¹)</th>
<th>HNFA (cells mL⁻¹)</th>
<th>% HNF &lt; 5 μm</th>
<th>% HNF</th>
<th>CilA</th>
<th>Mean (SM3)</th>
<th>Mean (M5)</th>
<th>P (Wilcoxon test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 Jun</td>
<td>SM3-5048 (178)</td>
<td>19.100</td>
<td>6.88</td>
<td>0.85</td>
<td>20.45</td>
<td>0.65</td>
<td>4.26</td>
<td>0.51</td>
<td>0.85</td>
<td>0.31</td>
<td>0.23</td>
<td>30</td>
<td>2.63</td>
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<td>91</td>
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<td>SM3-5107 (169)</td>
<td>14.72</td>
<td>4.34</td>
<td>0.49</td>
<td>8.45</td>
<td>1.57</td>
<td>3.00</td>
<td>0.82</td>
<td>0.96</td>
<td>0.40</td>
<td>0.62</td>
<td>68</td>
<td>6.21</td>
<td>1968</td>
<td>90</td>
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<td>SM3-5057 (78)</td>
<td>26.93</td>
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<td>6.13</td>
<td>0.50</td>
<td>3.34</td>
<td>4.24</td>
<td>3.11</td>
<td>0.91</td>
<td>0.28</td>
<td>0.48</td>
<td>0.54</td>
<td>91</td>
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<td>911</td>
<td>93</td>
<td>7159</td>
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<td>23 Jul</td>
<td>SM3-5048 (178)</td>
<td>19.27</td>
<td>6.08</td>
<td>0.57</td>
<td>6.81</td>
<td>1.92</td>
<td>3.18</td>
<td>1.73</td>
<td>2.07</td>
<td>2.52</td>
<td>0.17</td>
<td>66</td>
<td>22.58</td>
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<td>28 Jul</td>
<td>SM3-5146 (13)</td>
<td>64.91</td>
<td>1.75</td>
<td>0.31</td>
<td>9.44</td>
<td>1.27</td>
<td>3.13</td>
<td>0.68</td>
<td>1.67</td>
<td>0.51</td>
<td>0.43</td>
<td>65</td>
<td>11.18</td>
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<tr>
<td>28 Jul</td>
<td>SM3-5121 (74)</td>
<td>175.2</td>
<td>0.63</td>
<td>0.25</td>
<td>7.17</td>
<td>1.92</td>
<td>3.41</td>
<td>2.49</td>
<td>0.74</td>
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<td>2.92</td>
<td>68</td>
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<td>3 Aug</td>
<td>SM3-5124 (31)</td>
<td>14.84</td>
<td>14.58</td>
<td>0.26</td>
<td>6.39</td>
<td>1.91</td>
<td>3.42</td>
<td>2.70</td>
<td>3.13</td>
<td>1.08</td>
<td>0.65</td>
<td>60</td>
<td>11.97</td>
<td>722</td>
<td>92</td>
<td>3721</td>
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<tr>
<td>23 Jun</td>
<td>M5-1200 (55)</td>
<td>91.4</td>
<td>6.55</td>
<td>0.27</td>
<td>7.64</td>
<td>1.48</td>
<td>2.91</td>
<td>0.57</td>
<td>0.31</td>
<td>0.31</td>
<td>0.26</td>
<td>66</td>
<td>0.29</td>
<td>718</td>
<td>96</td>
<td>2808</td>
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<tr>
<td>28 Jun</td>
<td>M5-0800 (114)</td>
<td>94.1</td>
<td>6.07</td>
<td>0.35</td>
<td>6.39</td>
<td>1.72</td>
<td>2.74</td>
<td>0.23</td>
<td>0.23</td>
<td>0.17</td>
<td>0.31</td>
<td>58</td>
<td>3.65</td>
<td>1048</td>
<td>96</td>
<td>1322</td>
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<tr>
<td>2 Jul</td>
<td>M5-1200 (55)</td>
<td>91.4</td>
<td>5.03</td>
<td>0.51</td>
<td>7.61</td>
<td>1.52</td>
<td>2.95</td>
<td>0.60</td>
<td>0.45</td>
<td>0.26</td>
<td>0.26</td>
<td>59</td>
<td>5.40</td>
<td>1080</td>
<td>97</td>
<td>9464</td>
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<tr>
<td>25 Jul</td>
<td>M5-1000 (109)</td>
<td>54.2</td>
<td>5.09</td>
<td>0.28</td>
<td>5.73</td>
<td>1.94</td>
<td>2.97</td>
<td>1.67</td>
<td>0.54</td>
<td>0.85</td>
<td>3.68</td>
<td>61</td>
<td>4.62</td>
<td>1207</td>
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<tr>
<td>1 Aug</td>
<td>M5-0800 (114)</td>
<td>98.3</td>
<td>9.38</td>
<td>0.28</td>
<td>8.34</td>
<td>1.34</td>
<td>2.91</td>
<td>0.54</td>
<td>0.11</td>
<td>0.10</td>
<td>0.34</td>
<td>61</td>
<td>2.93</td>
<td>1746</td>
<td>96</td>
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<tr>
<td>1 Aug</td>
<td>M5-0600 (49)</td>
<td>283.2</td>
<td>9.14</td>
<td>0.25</td>
<td>6.56</td>
<td>1.60</td>
<td>2.94</td>
<td>1.71</td>
<td>1.07</td>
<td>0.55</td>
<td>1.27</td>
<td>58</td>
<td>2.72</td>
<td>1746</td>
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<td>2 Aug</td>
<td>M5-0400 (194)</td>
<td>76.5</td>
<td>3.96</td>
<td>0.27</td>
<td>5.64</td>
<td>1.81</td>
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<td>0.40</td>
<td>0.51</td>
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<tr>
<td>Mean (SM3)</td>
<td>151.4</td>
<td>8.56</td>
<td>5.76</td>
<td>0.46</td>
<td>8.86</td>
<td>1.92</td>
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<td>1.41</td>
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<td>0.79</td>
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<tr>
<td>Mean (M5)</td>
<td>159.3</td>
<td>10.95</td>
<td>6.44</td>
<td>0.31</td>
<td>6.84</td>
<td>1.63</td>
<td>2.89</td>
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<td>96</td>
<td>3989</td>
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<tr>
<td>P (Wilcoxon test)</td>
<td>0.949</td>
<td>0.006</td>
<td>0.654</td>
<td>0.337</td>
<td>0.338</td>
<td>0.654</td>
<td>0.002</td>
<td>0.179</td>
<td>0.035</td>
<td>0.063</td>
<td>0.949</td>
<td>0.11</td>
<td>0.047</td>
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<td>0.047</td>
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</tbody>
</table>

The depth of the station at the sampling time in given in parentheses. Im is the average light in the euphotic zone or in the epilimnion. DIN is dissolved inorganic nitrogen. DIP is dissolved inorganic phosphorus. DOC is dissolved organic carbon. A₃₅₀ nm/A₃₆₅ nm is the ratio of absorbance of water at 350 and 365 nm. BETA, GAM, PLA and CF are the abundance (10⁵ cells mL⁻¹) of bacteria bound by the probes BET42a, GAM42a, PLA886 and CF319a, respectively. % HNA is the proportion of bacteria with high nucleic acid content. BP is bacterial production. HNFA is heterotrophic nanoflagellate abundance. % HNF < 5 μm is the proportion of HNF with size < 5 μm. CilA is ciliate abundance. The average proportion of CF (not given in the text) is 9.5% (1.3–45%) in situ, 8% (5–12%) in Exp1, 11% (9–25%) in Exp2 and 6% (2–19%) in Exp3.

*Calculated as the average of measurements at the different depths in the euphotic zone or in the epilimnion.

†Significantly higher mean when SM3 is compared to M5.
with a specific probe, and all were incubated for 2 h in sealed falcon tubes at 46°C. After hybridization, the filters were transferred gently to 1.5-mL microfuge tubes containing the wash solution, and incubated for 15 min at 48°C. The filters were then dried (face-up) on Whatman paper and mounted between slides and glass cover slips with 10 μL of Citifluor (mounting medium). Counts were performed under an epifluorescence microscope (Olympus BX51) equipped with the appropriate filters (for Cy3 or DAPI). At least 300 cells were counted on each section. The abbreviations BETA, GAM and PLA are used hereafter to designate the bacterial cells bound by the probes BET42a, GAM42a and PLA886, respectively.

**Statistical analyses**

Comparisons of data from the two reservoirs and from the experiments were made using the nonparametric Kruskal – Wallis (K-W) test. Sources of significant differences between the experimental treatments were detected using the Tukey – Kramer test, when the K-W test was significant. For the experiments, comparisons were made mainly on data from T48, the time point when the responses of bacterial groups to nutrient additions were examined. The relationships between variables were examined using partial correlations, and simple or multiple regressions. The data were log_{10}-transformed (except for percentages, residuals and nonlinear models, see figure legends) to stabilize the variance and to attain homoscedasticity. The outliers were identified after plotting residuals with the predicted variable. The slopes of the regressions were compared using analyses of covariance (ANCOVA).

**Results**

**In situ data**

The averages of dissolved oxygen (O₂) concentration and the proportion of heterotrophic nanoflagellates with size < 5 μm (% HNF < 5 μm) were significantly higher in the old reservoir M5 than the young reservoir SM3. The opposite pattern was found for the ratio A_250 nm:A_365 nm, ciliate abundance, bacterial production and the abundances of PLA and GAM (Table 1). For each of the other studied variables, the mean values in the two reservoirs were similar, even when some exceptionally high values recorded at certain sampling dates were excluded (e.g. 20 mg L⁻¹ for DOC at station 5048, 19 June).

**Responses of bacteria to nutrient additions, and changes in the ratio A_250 nm:A_365 nm**

**Experimental data**

The analysis was focused on T48 (the time point when changes in the abundance of bacterial groups and the absorbance of water were monitored) although all the time points are shown for total bacterial abundance and production (Fig. 1). Addition of P alone or in combination with N significantly stimulated bacterial communities from M5 (the old reservoir = Exp1 and Exp3) but not those from SM3 (the old reservoir = Exp2). The addition of N alone yielded total bacterial abundance and production generally similar to those in the controls (Fig. 1).

The responses of BET, GAM and PLA to inorganic nutrient additions are shown in Fig. 2. These responses were expressed as percentage change, chosen to reveal their magnitude. It is important to note that a high value of this variable for a group in a treatment does not necessarily mean that this group is dominant at the sampling time point T48. The across-treatment patterns in the magnitude of these responses were in agreement with those in total bacterial abundance and production in Exp1 and Exp3 (i.e. highest in +P and +NP treatments, Fig. 2a and c), but not in Exp2, where some values of the responses were higher in the control and +N treatment than in +P and +NP treatments (Fig. 2b). In Exp1 and Exp3, where nutrients stimulated bacterial communities, the response of each bacterial group was, in general, significantly different among treatments, the latter being also generally significantly different in terms of the ratio A_250 nm:A_365 nm (P < 0.05, Figs 2 and 3a–c). Note that this ratio was similar in the different treatments in Exp2 (P > 0.12). In all treatments with N alone, PLA showed the highest positive responses, which coincided with the maximum value of the ratio A_250 nm:A_365 nm in Exp1 and Exp3. Interestingly, there was a clear increasing trend in this ratio with increasing PLA abundance across treatments, a pattern that was not observed with other bacterial groups (Fig. 3d–f). After the exclusion of a group of outliers (see Fig. 3d–f), the ratio became significantly and positively related to BETA, GAM and PLA, which were also significantly correlated to each other (R = 0.64 for PLA vs. BETA, 0.67 for PLA vs. GAM and 0.91 for BETA vs. GAM). Partial correlation analyses yielded partial R higher for PLA (0.50) than for BETA and GAM (0.29 and 0.08, respectively), indicating that PLA had the strongest influence on the variations of the ratio A_250 nm:A_365 nm in the experiments.

**Ambient water data**

Ambient water data showed no significant relationship between inorganic nutrients and BETA, GAM or PLA. These three bacterial groups were again significantly related to A_250 nm:A_365 nm and, in a multiple regression, they explained 61% of the variability of this ratio (Table 2). However, for individual regressions with this ratio, the greatest R² was found with BETA, a result which somewhat contrasted with that of the experiments, even though the slopes of these individual regressions were similar (ANCOVA, Table 2).
Because GAM and PLA were, a priori, also related to the % HNF < 5 μm in the correlation matrix built with ambient water data, it was suspected that grazers might have affected the relationships of these two bacterial groups with \(A_{250\text{nm}}:A_{365\text{nm}}\). To clarify this, the relationships between \(A_{250\text{nm}}:A_{365\text{nm}}\) and the residuals obtained from the relationships of % HNF < 5 μm with PLA and GAM, respectively, were examined. No significant coupling was found. Thereafter, PLA and GAM were replaced in the multiple regression by their respective residuals, obtained as indicated above. Replacement of GAM alone did not change the \(R^2\) (0.61), while replacement of PLA alone yielded the greatest \(R^2\) (0.69) (Table 2). This indicated that, in situ, there was a negative impact of grazers on PLA (Fig. 4a) that strongly affected the coupling of this bacterial group with other groups and, ultimately, with \(A_{250\text{nm}}:A_{365\text{nm}}\).

The ambient water data also revealed that, among the targeted bacterial groups, PLA, in terms of both proportion and absolute abundance, had the strongest relationship with bacterial production (BP) (Fig. 4b), for absolute abundance equations are: \(\log[\text{BP}] = 0.64 \times \log[\text{PLA}] - 1\), \(R^2 = 0.57, P = 0.003; \log[\text{BP}] = 0.48 \times \log[\text{GAM}] - 1.1, R^2 = 0.39, P = 0.02; \log[\text{BP}] = 0.56 \times \log[\text{BETA}] - 1.2, R^2 = 0.31, P = 0.045\), without the outlier from M5-1200 June; see data in Table 1). By contrast, BP was significantly correlated across-site, positively to \(A_{250\text{nm}}:A_{365\text{nm}}\), and negatively to O2 concentrations, the latter relationship being best described by a power function (Fig. 4c and d).

Light is another factor that may cause decomposition of DOM and thus affect its structure in natural waters (e.g. Wetzel et al., 1995). This study showed that, in contrast to bacteria, \(I_n\) (subsurface light) and \(I_m\) (average light in the euphotic zone or in the epilimnion) were not correlated with \(A_{250\text{nm}}:A_{365\text{nm}}\) (\(P > 0.15\)) in the ambient water data correlation matrix. A significant, positive relationship occurred between light (only \(I_m\)) and \(A_{250\text{nm}}:A_{365\text{nm}}\) only in spring, i.e. June (\(\log[A_{250\text{nm}}:A_{365\text{nm}}] = 0.37 \times \log[I_m] - 0.29, R^2 = 0.83, P = 0.032, n = 5\), data in Table 1). Together with the fact that the experiments were conducted in the dark, the above results indicated that light was less...
important than bacteria in changing the index of DOM quality during most of this study.

**Discussion**

This study demonstrated strong coupling between the targeted *Planctomycetes* and changes in the index of DOM quality when the grazers *per se* or their negative effects on this bacterial group were removed. This is consistent with models showing that enhanced grazing on prey influences their coupling to resources (e.g. Pace & Cole, 1994). DOM composition in natural waters is complex and difficult to characterize. It is clear that the ratio \( \frac{A_{250\text{ nm}}}{A_{365\text{ nm}}} \) used here as the index of DOM quality does not tell us anything about the nature of molecules that are present in the waters; however, in this study, it gave the best relationships with bacteria, compared with the bulk DOC and the other indexes tested. In other humic waters this ratio was found to increase with the proportion of small-sized molecules in the DOM pool (De Haan, 1972). Strome & Miller (1978) also found coupling between changes in average molecular size and \( \frac{A_{250\text{ nm}}}{A_{340}} \) and \( \frac{A_{250\text{ nm}}}{A_{370\text{ nm}}} \), and suggested that shortwave/longwave absorbance ratios may provide a sensitive means for following the process of humic decomposition. In aquatic systems, across-site increases in bacterial production are generally accompanied by increases in bacterial respiration, although the relationship may not be linear (Del Giorgio & Cole, 1998). As bacterial respiration releases CO\(_2\) and consumes oxygen, the negative coupling found in this study between bacterial production and dissolved oxygen (Fig. 4d) suggests that across-site increases in the bulk bacterial activity resulted in increases in the proportion of small molecules in the DOM pool, probably through decomposition of larger molecules with consumption of dissolved oxygen. Support for this contention came from the significantly higher averages of the ratio \( \frac{A_{250\text{ nm}}}{A_{365\text{ nm}}} \) bacterial production (Table 1) and flux of CO\(_2\) to the atmosphere at the sampled stations in SM3.
Table 2. Results of the linear regressions of bacterial groups (PLA, GAM and BETA) with the ratio \( A_{250 \text{ nm}} : A_{365 \text{ nm}} \) (index of the quality of dissolved organic matter)

<table>
<thead>
<tr>
<th>Regression</th>
<th>( y )</th>
<th>( x_1 )</th>
<th>( x_2 )</th>
<th>( x_3 )</th>
<th>Constant</th>
<th>( R^2 )</th>
<th>( R^2 ) adjusted</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>( A_{250 \text{ nm}} : A_{365 \text{ nm}} )</td>
<td>0.005 (PLA)</td>
<td>0.014 (GAM)</td>
<td>0.051 (BETA)</td>
<td>0.486</td>
<td>0.61</td>
<td>0.49</td>
<td>0.029</td>
</tr>
<tr>
<td>II</td>
<td>0.021 (PLA)</td>
<td>– 0.01 (ResGAM)</td>
<td>0.051 (BETA)</td>
<td>0.488</td>
<td>0.61</td>
<td>0.48</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>– 0.041 (ResPLA)</td>
<td>0.022 (GAM)</td>
<td>0.075 (BETA)</td>
<td>0.488</td>
<td>0.69</td>
<td>0.58</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>–</td>
<td>–</td>
<td>0.068 (BETA)</td>
<td>0.481</td>
<td>0.58</td>
<td>0.481</td>
<td>0.0025</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>–</td>
<td>–</td>
<td>0.043 (GAM)</td>
<td>–</td>
<td>0.489</td>
<td>0.41</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>0.053 (PLA)</td>
<td>–</td>
<td>–</td>
<td>0.497</td>
<td>0.49</td>
<td>0.49</td>
<td>0.0075</td>
<td></td>
</tr>
</tbody>
</table>

Acronyms for bacterial groups are as in Table 1. ResPLA and ResGAM are residuals of the relationships of grazers (% HNF < 5 \( \mu \text{m} \)) with PLA and GAM, respectively. Data were log_{10}-transformed, except for residuals. \( n = 13 \) for all. The outlier from SM3-5048 was not included in the regressions. I, II and III are multiple regressions while IV, V and VI are simple regressions.

Fig. 3. Across-treatment variations in the ratio \( A_{250 \text{ nm}} : A_{365 \text{ nm}} \) (a–c) and its relationships with PLA, BETA and GAM, respectively (d–f), across replicates during the three experiments, all data at T48 combined. For (d)–(f), the x-axis was left on a linear scale because of the narrow range of \( A_{250 \text{ nm}} : A_{365 \text{ nm}} \) values. The arrow in (f) indicates a group of outliers, which were excluded (as well as their corresponding points in d and e) during partial correlation analyses (see text). Acronyms for bacterial groups are as in Fig. 1.

(young reservoir, 8497 mg m\(^{-2}\) day\(^{-1}\)) compared with M5 (old reservoir, 1848 mg m\(^{-2}\) day\(^{-1}\)), and the significant and positive relationship found between these fluxes and \( A_{250 \text{ nm}} : A_{365 \text{ nm}} \) in several reservoirs (including M5 and SM3) in mid to north Quebec (R.D. Tadonléke et al., unpublished data). Previous findings have shown that small molecules are generally less recalcitrant to bacterial breakdown than large molecules (Saunders, 1976) and that simple molecules are more rapidly metabolized by bacteria than recalcitrant DOM (Wetzel et al., 1995). Significant coupling has also been found between \( A_{250 \text{ nm}} : A_{365 \text{ nm}} \) and CO\(_2\) partial pressure in Swedish lakes (Sobek et al., 2003). That
Planctomycetes abundance and proportion had the strongest relationships with bacterial production and that Planctomycetes response in +N treatments coincided with peaks of $A_{250} - A_{365}$ support the idea that members of the Planctomycetes were strongly involved in changes in DOM size structure during this study, probably through decomposition.

Members of the Planctomycetes are reported to be ubiquitous (Fuesrt, 1995; Gade et al., 2004; Fuerst, 2005) and some of them have been found to follow or to accompany cyanobacterial or algal blooms (see Fuesrt, 1995). Planctomycetes have been suggested to be involved in the degradation of recalcitrant compounds, based on the strong difference in their growth in activated sludges with contrasting biochemical oxygen demand (BOD)$_5$ (Neef et al., 1998). However, I am not aware of any cross-system and experimental study that has showed significant coupling between them, N and changes in DOM quality in natural samples as found here. This coupling indicates that Planctomycetes may be involved in the degradation of DOM, particularly when supplied with N in nutrient-poor waters. Laboratory studies based on Planctomycetes strains support these findings. It has been shown, for example, that some strains of Planctomycetes play a role in N cycling (Strous et al., 1999) and in the degradation of carbohydrates as analysed by a proteomic approach (Rabus et al., 2002). A recent study of the genome of a Planctomycetes strain has shown the presence of a large number of open reading frames (ORFs) encoding enzymes required for the degradation of plant detritus and algal polysaccharides (Glöckner et al., 2003). Dissolved carbohydrates and plant detritus are generally highly abundant in newly flooded reservoirs (e.g. Richardot et al., 2000), which seems to be consistent with the finding that PLA were significantly more abundant in SM3 than in M5 (Table 1), even though part of this difference may be due to grazers. The proteomic and the genomic approaches are increasingly used in microbial ecology. It is thus useful to link the genomic potential found within environmentally relevant bacteria with data regarding their abundances and function in natural samples.

Members of the Betaproteobacteria have been found to catabolize riverine DOM (Kisand & Wikner, 2003), to decompose organic matter (release of amino acids) on lake snow aggregates (Schweitzer et al., 2001), or to be abundant in lake water enriched with DOC containing high-molecular-weight humic substances (Eilers et al., 2003). That BETA was also coupled to changes in DOM quality in the present study seems to be consistent with these previous findings. Members of the Gammaproteobacteria have often been reported as opportunist bacteria that grow rapidly, especially during confinement (e.g. Eilers et al., 2000).
The proportions of Planctomycetes detected by the probe PLA886 in this study (2–10%, mean = 6%, Fig. 4b) are comparable with those found in lakes by the few studies on this group (0.6–11%, mean = ∼5%; Gade et al., 2004). Genetic sequences affiliated with Planctomycetes have also been found to account for 3% and 4% of the clone libraries in two Swedish forest lakes (Eiler & Bertilsson, 2004). In addition to this similarity in the quantitative importance of Planctomycetes among systems, it appeared that the residuals derived from the PLA–grazer relationship increased the ability to explain variability in A250 nm:A365 nm (Table 2) and that PLA best explained bacterial production, as stated earlier (Fig. 4b), and did not show a positive link with flagellate abundance. These results suggest that unspecific binding to eukaryotes (plants, fungi and protists such as Acanthamoeba spp., Cryptosporidium spp. or Toxoplasma spp.) that posses the identical target region for probe PLA886 (Neef et al., 1998) was not an important problem in this study and probably did not affect the presented data. In fact, the experimental inocula were free of microorganisms with size > 0.8 µm due to filtration on 0.8-µm pore-size filters, and all the counts clearly distinguished bacteria from eukaryotes when present. It should be noted, however, that this filtration might have excluded free-living members of the Planctomycetes that have size > 0.8 µm. On the other hand, the probe PLA886 does not target all Planctomycetes. Hence, the abundance of all the members of the phylum Planctomycetes in the study sites might be higher than that recorded here with this probe.

We are aware that the sensitivity of the standard FISH method used here may be a problem in nutrient-poor aquatic systems (see Pernthaler & Amann, 2005), where the ribosome content of bacteria is often low. More sensitive FISH methods (e.g. catalyzed reporter deposition-FISH) have been developed to improve the detection rate in such conditions, i.e. when the nucleic acid content of cells is low (Speel et al., 1998; Pernthaler et al., 2002b). In the present study, it is unlikely that the sensitivity problem significantly affected the results, because (1) the experimental samples were nutrient-amended and (2) in situ, the proportion of prokaryotic cells with high nucleic acid content, thought to be among the most active (e.g. Lebaron et al., 2001; Tadonléke et al., 2005), was generally > 59% (Table 1), suggesting that prokaryotic cells with high ribosomal content were abundant in situ in most cases.

The results presented here suggest that in nutrient-poor systems, differences in the type of inorganic nutrient supply will ultimately cause differences in the quality of DOM, as a consequence of differential responses of bacterial groups or lineages. When bacterial groups responded positively to nutrient additions (Exp1 and Exp3), both these responses and the index of DOM quality were generally significantly different among the treatments +N, +P and +NP (Figs 2, 3a and c). The index of DOM quality was higher in the +N (where total bacterial growth was low and PLA had the highest positive responses) than in the two other treatment (where numerous bacteria were stimulated). This suggests that pulses of N alone led to relative accumulation of small molecules, while pulses of the limiting P, alone or in combination with N, caused the small molecules to be rapidly taken up by the growing bacteria. How the inorganic nutrient availability or type affects changes in the DOM quality by bacteria is poorly known. Most studies dealing with this aspect have often considered only the bulk DOC. The growth of a microorganism is affected by both the external and the internal nutrient concentration (Vadstein et al., 1988) and ratios [e.g. C:N:P (Vrede et al., 2002)]. This growth, in turn, affects the environment surrounding the microorganism. It has been shown that different bacterial groups or lineages grow differently on DOM of different concentration or quality (Eilers et al., 2000; Kisand et al., 2002; Eiler et al., 2003; Kisand & Wikner, 2003) and can have different elemental content (Troussellier et al., 1997) and growth efficiencies (Heijnen & Dijken, 1992), which themselves are known to be influenced by the nutrient ratio in the substrate (Del Giorgio & Cole, 1998; Vrede et al., 2002). Then, that the responses observed in +P vs. +NP treatments were different although N alone did not stimulate the whole bacterial community (Figs 1, 2, 3a and c) is probably partly due to differences in the initial N : P ratios in these treatments. In natural environments, seasonal succession has been observed for members of the different bacterial groups (see, for example, Brümmer et al., 2004, for Planctomycetes), indicating that different lineages in each bacterial group may respond differently to environmental changes. Recent work has shown that different members of the Planctomycetes exhibited different vertical distribution in relation to nitrate vs. ammonium concentrations in the Black Sea (Kirkpatrick et al., 2006). Finally it is known, for example, that some members of the Betaproteobacteria are autotrophic ammonia-oxidizing bacteria (Head et al., 1993) whereas some others are nitrate-reducing bacteria. Based on these differences in the properties of species or lineages, the differential changes in DOM quality observed here in relation to the supply of different types of inorganic nutrient could be expected; however, they have seldom been shown in natural samples. If the higher responses of Planctomycetes (as compared with the other groups) in the +N treatments reflected their greater ability to process DOM in the presence of N, this would imply that, in such conditions, this bacterial group may be very important in supplying the other groups with small-sized molecules, given that the ability to utilize low- vs. high-molecular-weight DOM may differ among bacterial phylogenetic groups (Cottrell & Kirchman, 2000). It would have been more interesting to use species- or genera-specific probes, or other approaches
allowing a higher resolution of the bacterial composition (e.g. 16S rRNA gene sequence analyses) during this study.

The responses of bacterial groups to nutrients in Exp2 were unclear and no significant difference was found between treatments in terms of DOM quality (Figs 2b and 3b). This might be due, at least partly, to the fact that bacteria were not nutrient limited in Exp2 (Fig. 1). In such conditions, some opportunistic bacteria not detectable in natural samples may grow strongly during confinement (e.g. Eilers et al., 2000; Haukka et al., 2005), which may hamper our understanding of the responses of other bacterial groups or species to the actual treatment (Eilers et al., 2000). It has been argued that nutrient limitation of bacteria may be a prerequisite to observe nutrient effects on bacterial groups or species clearly using dilution culture experiments (Pinhassi & Berman, 2003).

Conclusions

The results presented here show strong coupling between members of the Planctomycetes and changes in the quality of DOM in natural samples in the absence of grazers. These changes seem to vary with the type of inorganic nutrient supply, suggesting that taking into account the interactions between inorganic nutrients, bacterial groups or species and DOM may help to improve our understanding of the functioning of aquatic systems, particularly those that are nutrient-depleted.

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