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**Title:** Epiphytic bacteria make an important contribution to heterotrophic bacterial production in a humic boreal lake

**Year:** 2017

**Version:**

**Please cite the original version:**

Vesterinen, J., Devlin, S., Syväranta, J., & Jones, R. (2017). Epiphytic bacteria make an important contribution to heterotrophic bacterial production in a humic boreal lake. *Aquatic Microbial Ecology*, 79(3), 247-257. <https://doi.org/10.3354/ame01835>

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1 **Epiphytic bacteria make an important contribution to bacterial production in a humic**  
2 **boreal lake**

3

4 RH: Littoral bacterial production

5

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15

16 Abstract

17 Bacterial production (BP) in lakes has generally been measured only in the pelagic zone  
18 without accounting for littoral BP, and studies of BP at the whole-lake scale are very scarce. In  
19 the dystrophic humic lakes which are common throughout the boreal region, low light  
20 penetration through water has been assumed to seriously limit available habitats for littoral  
21 organisms. However, many highly humic boreal lakes have extensive partly submerged  
22 vegetation around the lake perimeter which can provide well-lit substrata for highly productive  
23 epiphyton. We measured epiphytic BP on the littoral vegetation and pelagic BP in a small highly  
24 humic boreal lake in Finland during an open water season and extrapolated the BP rates to the  
25 whole-lake. Pelagic BP dominated the combined BP over the study period, but the epiphytic BP  
26 contributed an average of 24% to overall BP over the sampling period and was almost equal to  
27 pelagic BP in July. According to these results, a substantial component of BP has been previously  
28 overlooked in the lake when BP has been measured only from the pelagic. Our study  
29 demonstrates that the role of the littoral zone in bacterial production in highly humic lakes has  
30 previously been understated, and needs to be taken into account in assessments of whole-lake  
31 carbon cycling and metabolism.

32

33 Keywords: littoral, periphyton, pelagic, autotrophic, heterotrophic

34

## 35 Introduction

36 Pelagic and littoral habitats have generally been studied separately in lake ecosystem and  
37 food web research, and only very few studies have examined productivity in both habitats  
38 (Vadeboncoeur et al. 2002). Although pelagic and littoral production can be integrated by mobile  
39 consumers like fish (Schindler & Scheuerell 2002) and even zooplankton (Van De Meutter et al.  
40 2004) which utilize both pelagic and littoral resources, studies of the magnitudes of primary  
41 production (PP) and particularly of heterotrophic bacterial production (BP) at the whole-lake  
42 scale and including both pelagic and littoral habitats, are scarce. In highly humic lakes the  
43 importance of littoral benthic production has been assumed to be minor due to the very low light  
44 penetration into the water (e.g. Vadeboncoeur et al. 2002) together with very steep stratification,  
45 which restricts illuminated and oxygenated benthic habitats. However, Vesterinen et al. (2016a)  
46 showed that epiphyton on surrounding littoral vegetation dominated the whole-lake PP in highly  
47 humic Lake Mekkojärvi in southern Finland, demonstrating that macrophytes and partly  
48 submerged terrestrial vegetation can provide extensive well-lit substrata for epiphyton and make  
49 the littoral an appreciable habitat for PP in humic lakes.

50 Algae and bacteria coexist in periphytic biofilms in an association that offers space and  
51 resources to sustain production of both groups of organisms, and positive correlations between  
52 periphyton PP and BP, as well as between algal and bacterial biomass, have been well  
53 documented (e.g. Neely & Wetzel 1995, Rier & Stevenson 2002, Carr et al. 2005, Kuehn et al.  
54 2014). This can be more pronounced if light is not limiting algal growth and biomass production,  
55 when algae produce a substantial extracellular polysaccharide matrix that creates an isolated  
56 microenvironment, where inorganic nutrients can be effectively recycled (Wetzel 1993). Highly  
57 humic Lake Mekkojärvi has extensive littoral vegetation, which mostly lies just under the water

58 surface in relatively well-lit conditions where it supports thick growths of epiphyton from spring  
59 to early autumn (Vesterinen et al. 2016a). In view of the strong correlations found elsewhere  
60 between PP and BP, we can expect that littoral epiphytic BP should be high and contribute  
61 substantially to whole-lake BP in Mekkojärvi.

62 Heterotrophic bacteria are known to play a very important role in the carbon flux of  
63 aquatic ecosystems, providing a link between autochthonous and allochthonous dissolved  
64 organic matter (DOM) and bacterivores (Porter et al. 1988). In humic lakes, most of the DOM is  
65 of allochthonous origin which is an important basal resource for both pelagic (Jones et al. 1992,  
66 Pace et al. 2004, Jansson et al. 2007) and benthic (Premke et al. 2010, Karlsson et al. 2012) food  
67 webs via microbial pathways. However, most studies of bacteria and their productivity in lakes  
68 have concerned pelagic bacterioplankton alone without measuring productivity of bacteria  
69 associated with profundal sediments or with periphyton in littoral benthic habitats, where  
70 bacterial production (BP) can be of a similar magnitude to, or even higher than that in the pelagic  
71 zone (Vadeboncoeur et al. 2002 and references therein). Benthic bacteria often outnumber  
72 pelagic bacteria in lakes and rivers creating high spatial variability (Schallenberg & Kalff 1993,  
73 Fischer & Pusch 2001), and the fraction of active bacterial cells in the total number of bacteria in  
74 sediments and epiphytic biofilms can be much larger than in the pelagic (Haglund et al. 2002).  
75 Therefore, measurements of BP in these different habitats are particularly needed in humic lakes,  
76 where the importance of the littoral has been understated. Incorporation of littoral and pelagic as  
77 integrated habitats into conceptual models of lake ecosystems will contribute to a more  
78 comprehensive understanding of trophic dynamics (Vadeboncoeur et al. 2002) and of lake  
79 metabolism, which is important in resolving organic carbon budgets in lakes (Hanson et al. 2015,  
80 Solomon et al. 2015). We measured BP in the littoral epiphyton and in the pelagic water column

81 several times during an open water period in Mekkojärvi, extrapolated the results to the whole-  
82 lake scale and compared the magnitude of BP in the two habitats.

83

## 84 Material and methods

### 85 *Study lake*

86 The study was conducted at Lake Mekkojärvi (61°13'N 25°3'E), a small (0.35 ha) and  
87 highly humic headwater lake in the Evo forest region in southern Finland (Fig.1) with mean and  
88 maximum depths of 2.0 and 4.3 m. The lake is sheltered by surrounding coniferous forest and  
89 receives a high loading of terrestrial organic matter from its catchment causing high dissolved  
90 organic carbon (DOC) concentrations (30–33 mg C L<sup>-1</sup>), highly coloured water (300–800 mg Pt  
91 l<sup>-1</sup>) and low pH (5.3–5.7) (Devlin et al. 2015, Vesterinen et al. 2016a). This causes the lake to  
92 develop very steep temperature and oxygen gradients rapidly after ice-off in spring. Mekkojärvi  
93 has ice cover usually from early November until the beginning of May. During the open water  
94 period the thermocline lies between 0.5–1.0 m and anoxia occurs under that layer. Mekkojärvi  
95 becomes totally anoxic during winter ice cover and therefore cannot sustain overwintering fish  
96 populations, which has allowed development of very dense populations of the large-bodied  
97 cladoceran *Daphnia longispina* in summer. Mekkojärvi has a depth ratio ( $DR = \bar{z}/z_{max}$ ) of 0.47,  
98 so the lake is relatively steep-sided and lacks illuminated benthic surfaces due to the highly  
99 coloured water and very low light penetration (light-attenuation coefficient ranges from 4.5 to  
100 7.5). Details of the lake's physical and chemical characteristics are presented elsewhere (e.g.  
101 Vesterinen et al. 2016a). Mekkojärvi has been the subject of numerous studies, which have  
102 revealed the importance of both allochthonous C and biogenic methane to productivity of the  
103 pelagic system (e.g. Salonen & Hammar 1986, Jones et al. 1999, Salonen et al. 2005, Taipale et

104 al. 2008; 2011, Devlin et al. 2015). Bacterial densities are greater in the oxic-anoxic boundary  
105 layer in the metalimnion and in the anoxic hypolimnion than in the oxic epilimnion (Arvola et al.  
106 1992). The bacterial community in Mekkojärvi is mainly composed of heterotrophic,  
107 chemoautotrophic and photoautotrophic bacteria, including photosynthetic green sulphur bacteria  
108 (*Chlorobium* sp.) and methane-oxidizing bacteria (belonging to *Methylobacter* genus) which  
109 contribute significantly to the bacterial biomass in the meta- and hypolimnion (Taipale et al.  
110 2009). The littoral zone is not clearly defined in Mekkojärvi, but the lake has a surrounding  
111 floating moss mat (consisting mainly of *Sphagnum* and *Warnstorfia* species) lining the lake  
112 perimeter, with fallen terrestrial sedges (*Carex* sp.) and some macrophytes such as *Menyanthes*  
113 *trifoliata*, *Phragmites australis* and *Utricularia* sp. associated with the moss mat. This  
114 surrounding littoral vegetation does not extend further than ca. 1 m from the lake edge and not  
115 deeper than ca. 0.5 m, but sustains highly productive periphyton assemblages, which have their  
116 highest biomass in late-summer and can balance the whole-lake metabolism or even make the  
117 lake net autotrophic (Vesterinen et al. 2016a).

118

#### 119 *Pelagic bacterial production*

120 Pelagic sampling was carried out at the deepest point in the lake (Fig. 1). Temperature  
121 and oxygen concentrations were measured at 0.5 m intervals from the surface to the bottom with  
122 an oxygen and temperature sensor YSI 55 probe (YSI Inc., Yellow Springs, Ohio, USA) during  
123 every sampling occasion in 2015. From these measurements the water column stratification was  
124 defined as follows: 0.0–0.2 m (surface), 0.2–0.5 m (epilimnion), 0.5–1.0 (metalimnion), 1.0–3.0  
125 m (hypolimnion).

126 Pelagic bacterial production was measured five times between June and October in 2015  
127 using a [<sup>14</sup>C]-leucine uptake method (Kirchman et al. 1985) slightly modified according to  
128 Tulonen (1993). From composite water samples collected from three stratum (epi- (0–0.5 m),  
129 meta- and hypolimnion), triplicate subsamples of 5 mL were transferred to 20 mL pre-ignited  
130 glass vials containing 30 nM of [<sup>14</sup>C]-leucine (specific activity of 0.306 Ci mmol<sup>-1</sup>, Amersham  
131 Biosciences) and incubated for 60 min *in situ* in the strata from which they originated.  
132 Glutaraldehyde-killed controls were run in parallel. After incubation, all the live samples were  
133 killed with glutaraldehyde. In the laboratory, 0.5 mL of ice-cold 50% trichloroacetic acid (TCA)  
134 was added into every sample to reach a final concentration of 5%. Samples were then cooled for  
135 15 min followed by filtration onto 0.2 µm pore-size cellulose nitrate filters (Sartorius). The filters  
136 were rinsed with 1 mL of ice-cold 5 % TCA and distilled water and then dissolved in 0.25 mL of  
137 ethyleneglycolmonomethylether together with 9 mL of liquid scintillation cocktail (OptiPhase 3).  
138 The total activity of the added [<sup>14</sup>C]-leucine was counted from a subsample of 0.5 mL into which  
139 0.5 mL of 1:7-ethanolamine/ethanol absorption liquid was added together with 9 mL of  
140 scintillation cocktail. Samples were stored at room temperature for 24 h before their radioactivity  
141 was counted with a Packard Tri-Carb<sup>®</sup> liquid scintillation counter (PerkinElmer, Waltham,  
142 Massachusetts, USA).  
143 Leucine incorporation rates ([dpm sample – dpm blank]/total activity of the added leucine) were  
144 converted to biovolume by multiplying by 7.71 x 10<sup>15</sup> (µm<sup>-3</sup> mol<sup>-1</sup>) and to carbon production by  
145 multiplying by a carbon to biovolume ratio of 0.36 pg C µm<sup>-3</sup>. Both factors are appropriate for  
146 humic lakes according to their empirical determination in laboratory experiments (Tulonen  
147 1993). Daily BP rates were calculated multiplying hourly rates by 24. Areal BP values were  
148 calculated by multiplying the volumetric values by the fraction of each stratum of the water

149 column and summing over depth. These were multiplied by the area of the lake to derive the  
150 whole-lake BP values for the pelagic. To test the possible effect on anoxic hypolimnetic BP  
151 samples of oxygen contamination from air in the incubation vessels, 5 parallel samples were  
152 incubated in evacuated Labco Exetainers (Labco Limited, Lampeter, Ceredigion, UK)  
153 simultaneously with other hypolimnetic samples in September.

154

#### 155 *Littoral epiphytic bacterial production*

156 Epiphytic BP was measured five times together with pelagic BP in 2015. Littoral  
157 temperatures were measured with a YSI 55 probe (YSI Inc., Yellow Springs, Ohio, USA) during  
158 every sampling occasion. Samples of littoral vegetation were collected randomly from 6 sites  
159 around the lake into 2 L plastic buckets filled with lake water from each site. As the littoral  
160 vegetation consists mainly of moss and partly submerged sedges in Mekkojärvi, these were the  
161 main representatives in the samples. Some larger plants, such as *Menyanthes trifoliata*, were not  
162 sampled, as they were difficult to process in the laboratory. Buckets were stored in a cool box  
163 containing lake water and taken to the laboratory of Lammi Biological Station, about 30 km  
164 south from Mekkojärvi. BP was measured from epiphytic biofilms using a modified version of  
165 the [<sup>3</sup>H]-leucine incorporation method described by Ask et al. (2009) based on the method  
166 originally developed by Smith & Azam (1992). [<sup>3</sup>H]-leucine was used instead of [<sup>14</sup>C]-leucine,  
167 since it was available at sufficiently higher concentrations. Six randomly selected 1 cm long  
168 subsamples of plant substratum from each sampling site were clipped and put into 1.2 mL  
169 Eppendorf tubes containing 30 µL of [<sup>3</sup>H]-leucine (specific activity of 112 Ci mmol<sup>-1</sup>,  
170 PerkinElmer, Inc.) and 70 µL of distilled water with the final concentration of 300 nM, and half  
171 of the samples were immediately killed by addition of 130 µL of 50% TCA. To determine the

172 appropriate [<sup>3</sup>H]-leucine concentration and the maximum incorporation of leucine into protein in  
 173 epiphytic biofilms, a saturation experiment was conducted once in early-June in which samples  
 174 were incubated in 7 different concentrations ranging from 30 to 1000 nM. Eppendorf tubes were  
 175 incubated outside the laboratory in an open cool box containing lake water for 60 min. The  
 176 samples were submerged at the same depth from which they originated so that they experienced  
 177 similar light conditions. The temperature of the water was measured during the incubation and no  
 178 increase above the lake *in situ* temperature was observed. Incubation was then terminated by  
 179 adding 130 µL of 50% TCA into the live samples and vortexing them. Samples were centrifuged  
 180 at 12400 rpm for 10 min and the supernatant was gently removed using a thin pipette. No marked  
 181 loss of epiphyton from the substratum was visible (although was not confirmed by microscopy).  
 182 1.2 mL of 5 % TCA was then added and the samples were again vortexed and centrifuged at  
 183 12400 rpm for 10 min. The supernatant was then removed, 1.2 mL of 80% EtOH was added and  
 184 samples were centrifuged as above. Finally, the supernatant was removed, the sample was  
 185 aerated and 1.2 mL of scintillation cocktail (OptiPhase 3) was added. Sample radioactivity was  
 186 counted with a Packard Tri-Carb<sup>®</sup> liquid scintillation counter (PerkinElmer, Waltham,  
 187 Massachusetts, USA). Leucine uptake rate was calculated as:

$$188 \text{ mmols leucine (cm substratum}^{-1}\text{) h}^{-1} = (4.5 \times 10^{-13}) \times (\text{dpm sample} - \text{dpm blank}) \times (\text{SA})^{-1} \times (\text{T})^{-1}$$

189 Eq. 1

190 , where factor  $4.5 \times 10^{-13}$  is the number of curies  $\text{dpm}^{-1}$  (a constant), SA is the specific activity of  
 191 the leucine solution in curies  $\text{mmol}^{-1}$  and T is the incubation time in hours.

192 Bacterial production was calculated as:

$$193 \text{ mg C (cm substratum}^{-1}\text{) h}^{-1} = (\text{Leucine uptake rate}) \times 132.1 \times (\% \text{Leu})^{-1} \times (\text{C:Protein}) \times \text{ID}$$

194 Eq. 2

195 , where 132.1 is the molecular weight of leucine, (%Leu) is the proportion of leucine in total  
196 protein, assumed to be 0.073 (Simon & Azam 1989), (C:Protein) is the ratio of cellular C to  
197 protein, assumed to be 0.86 (Simon & Azam 1989) and ID is the isotope dilution factor, which  
198 was assumed to be 2 for samples from oligotrophic lakes (Simon & Azam 1989).

199         Substrata were dried in an oven at 60°C for 24 h and dry-weight (DW) of substratum  
200 [mean  $\pm$  SE (g DW substratum) cm<sup>-1</sup>] was recorded ( $0.00105 \pm 0.0000876$  g, n = 25). BP values  
201 were then normalized to mg C g (DW substratum)<sup>-1</sup> h<sup>-1</sup>. Daily rates were calculated by  
202 multiplying hourly rates by 24. We examined how temperature changes during the day might  
203 affect the BP rates by using temperature data from a miniDOT Logger (PME Inc. Vista, CA,  
204 USA) which was placed in the surface water in the middle of a moss mat in the littoral in  
205 Mekkojärvi for 2 months from July to August. Littoral BP values at noon over the sampling  
206 period in 2015 plotted against the littoral surface temperature followed an exponential  
207 relationship, and that function was used to estimate BP for every hour during the incubation  
208 periods on 6 July and 5 August. These values were then summed and compared to the values  
209 derived by multiplying noon rates by 24.

210         Whole-lake estimates for epiphytic BP were derived by first calculating the BP per m  
211 lake shoreline using the average DW substratum<sup>-1</sup> m<sup>-1</sup> of lake shoreline ( $42.6 \pm 3.4$  g DW  
212 substratum<sup>-1</sup> m<sup>-1</sup>), which was calculated by entirely removing the macrophyte and moss  
213 vegetation along 40 cm of lakeshore from 24 sites around the lake (Vesterinen et al. 2016a). The  
214 whole littoral epiphytic BP estimates were then calculated by multiplying BP per m lake  
215 shoreline by the total shoreline length (320 m).

216

217 *Statistical analyses*

218 Repeated measures of analysis of variance (RMA) was used to test the differences in  
219 pelagic BP among the sampling occasions (dependent variable/within-subject variable) and  
220 between the strata (grouping variable/between-subject factor). Normality and homoscedasticity  
221 (Levene's test) of the data were tested before statistical analysis. RMA was also used to test the  
222 differences in epiphytic BP among the sampling occasions (dependent variable/within-subject  
223 variable). Independent *t*-test was used to test the possible difference in hypolimnetic BP in oxic  
224 and anoxic vials. Regression analysis was used to test the relationships between surface  
225 temperatures and epilimnetic and epiphytic BP. All the statistical tests were conducted with IBM  
226 SPSS Statistics (version 20.0.0.2; IBM, Armonk, New York, USA). All the descriptive statistics  
227 are means  $\pm$  SE if not expressly noted.

228

## 229 Results

### 230 *Pelagic bacterial production*

231 The mean O<sub>2</sub> concentrations over the study period were  $4.1 \pm 0.7$  mg L<sup>-1</sup> in the  
232 epilimnion,  $1.2 \pm 0.5$  mg L<sup>-1</sup> in the metalimnion and  $0.8 \pm 0.1$  mg L<sup>-1</sup> in the hypolimnion.  
233 Total pelagic BP was highest in early summer, and decreased steadily towards autumn (Fig. 2).  
234 After the early summer peak, BP remained under 20.0 mg C m<sup>-2</sup> d<sup>-1</sup> (Fig. 2). Epilimnetic and  
235 metalimnetic BP together constituted 85 % of the total pelagic BP in early-June. In July the rates  
236 were similar in all the three strata. Hypolimnetic BP increased slightly towards autumn and  
237 constituted the largest fraction of total pelagic BP in late-summer and autumn (56–63%). The  
238 mean BP over the sampling period was  $11.6 \pm 2.0$  mg C m<sup>-2</sup> d<sup>-1</sup> in the epilimnion,  $5.7 \pm 2.0$  mg C  
239 m<sup>-2</sup> d<sup>-1</sup> in the metalimnion and  $6.0 \pm 1.8$  mg C m<sup>-2</sup> d<sup>-1</sup> in the hypolimnion. The change in BP over  
240 time was significant (RMA,  $F_{4, 24} = 6.0$ ,  $p < 0.01$ ), as were the interactions between time and

241 strata (RMA,  $F_{8,24} = 4.0$ ,  $p < 0.01$ ). Tukey's HSD test revealed significant differences in BP  
242 between meta- and hypolimnion. Epilimnetic BP appeared to be generally related to the surface  
243 temperature (Fig. 3A), but a high value in early summer prevented a significant correlation  
244 (exponential regression,  $F_{1,3} = 6.320$ ,  $R^2 = 0.678$ ,  $p = 0.087$ ). No statistically significant  
245 difference ( $t$ -test,  $t_6 = -0.606$ ,  $p = 0.606$ ) was found between values of hypolimnetic BP  
246 measured in oxic or anoxic vials (mean values  $2.9 \pm 1.3 \text{ mg C m}^{-3} \text{ d}^{-1}$  in the oxic and  $2.0 \pm 1.0$   
247  $\text{mg C m}^{-3} \text{ d}^{-1}$  in the anoxic).

248

#### 249 *Littoral epiphytic bacterial production*

250         Based on the test conducted in early June, saturation of leucine incorporation into protein  
251 appeared at 300 nM concentration (Fig. 4) and this concentration was therefore applied in the  
252 production measurements. Variability among replicates may be the result of patchy occurrence of  
253 periphyton on the substrata or variability of chlorophyll *a* (chl *a*) along the substrata, assuming  
254 that there was a positive relationship between the periphyton chl *a* and BP. Epiphytic BP was  
255 highest in summer (June and July) and decreased towards autumn (August, September, October;  
256 Fig. 5). The change in BP over time was significant (RMA,  $F_{4,68} = 17.6$ ,  $p < 0.01$ ), and Tukey  
257 HSD revealed significant differences between summer and autumn. Epiphytic BP correlated  
258 significantly with littoral surface temperature (Fig. 3B; exponential regression,  $F_{1,3} = 21.7$ ,  $R^2 =$   
259  $0.878$ ,  $p = 0.019$ ).

260         Daily epiphytic BP in July calculated from the exponential function of BP and  
261 temperature (Fig. 3B) and hourly temperatures from the *in situ* data logger was  $2.8 \text{ mg C (g DW}$   
262  $\text{substratum})^{-1} \text{ d}^{-1}$ , which is only ca. 10% higher than the value estimated multiplying hourly  
263 leucine incorporation rates by 24 ( $2.6 \pm 0.4 \text{ mg C [g DW substratum]}^{-1} \text{ d}^{-1}$ ). In August the similar

264 comparison was 2.0 versus 1.5 mg C (g DW substratum)<sup>-1</sup> d<sup>-1</sup>, a difference of 15%. According to  
265 these comparisons, multiplying noon BP h<sup>-1</sup> by 24 gives slightly lower, and thus more  
266 conservative, estimates of the daily epiphytic BP.

267

#### 268 *Whole-lake pelagic and littoral bacterial production*

269 Whole-lake estimates for pelagic and littoral epiphytic BP revealed that the pelagic  
270 dominated the combined BP over the open-water period, contributing over 80% to whole-lake  
271 BP in early-June and in October (Fig. 6). Littoral epiphytic BP made the highest contribution to  
272 overall BP during summer with the highest value (34.8 g C d<sup>-1</sup>) and contribution (45%) in early-  
273 July. The lowest littoral value (4.1 g C d<sup>-1</sup>) and contribution (6%) occurred in October. The mean  
274 values of pelagic and littoral epiphytic BP over the sampling period were 63.6 ± 15.6 and 20.5 ±  
275 5.4 g C d<sup>-1</sup>, respectively, and their respective mean proportions of the overall BP were 76 and  
276 24%.

277

#### 278 **Discussion**

279 Pelagic BP dominated the combined (pelagic + littoral epiphytic) BP in Mekkojärvi  
280 during the study, but the littoral epiphytic BP contributed appreciably, particularly in summer.  
281 According to our estimates of the mean whole-lake rates over the sampling period in 2015,  
282 around one quarter of the combined (pelagic + littoral epiphytic) BP in Mekkojärvi has been  
283 previously overlooked when the epiphyton has not been taken into account. However, the  
284 complete whole-lake BP also include BP of sediment bacteria, which was not measured in this  
285 study but can be assumed to make an appreciable contribution to the total BP of the lake, as their  
286 production rates can be several times higher than in the overlying water (Sander & Kalff 1993,

287 Ask et al. 2009). In subarctic oligotrophic Swedish lakes, BP from allochthonous OC by sediment  
288 bacteria was found to exceed the combined PP and BP in the pelagic (Ask et al. 2009). In our  
289 study, littoral BP is represented as epiphytic BP, but it should be noted that the true littoral BP  
290 also includes BP in the surrounding water in the littoral, which we did not measure. Considering  
291 the higher surface water temperatures in the littoral than in the pelagic and the positive  
292 relationship between temperature and BP together with potentially higher quantities of labile  
293 organic compounds, such as periphytic algal exudates, and nutrients, BP in the in the littoral  
294 water may be higher than that in the pelagic and can potentially increase the contribution of  
295 littoral to whole-lake BP in Mekkojärvi. The strictly anaerobic green sulphur bacterium  
296 *Chlorobium* is also abundant in the deeper layers in Mekkojärvi (Taipale et al. 2009, Karhunen et  
297 al. 2013). As the BP samples in this study were exposed to O<sub>2</sub>, the contribution of *Chlorobium* to  
298 BP was probably underrepresented in our measurements. However, both the high production of  
299 *Chlorobium* and potentially high production of sediment bacteria probably contribute the very  
300 high community respiration rates reported from Mekkojärvi (Salonen et al. 2005, Vesterinen et  
301 al. 2016). Despite these gaps, which do not allow us to report total whole-lake BP values, our  
302 results clearly highlight how epiphytic BP can be a major part of the whole-lake BP in small  
303 humic lakes.

304         The high BP measured in the epilimnion in spring weakened the correlation between  
305 epilimnetic BP and the surface temperature, but indicated an association between BP and the  
306 phytoplankton PP spring maximum, which has been documented in earlier studies in Mekkojärvi  
307 (Salonen et al. 2005, Vesterinen et al. 2016a). These apparently related production maxima of  
308 both groups of organisms may reflect exploitation by both groups of a pulse of nutrients from the  
309 catchment with snow-melt in spring. Alternatively or additionally, it may be a result of bacterial

310 stimulation by labile autochthonous OM released by phytoplankton, which couples BP with PP.  
311 Such positive relationships between pelagic BP and PP and chlorophyll are well-documented  
312 (e.g. White et al. 1991, Cole et al. 1988, Kritzberg et al. 2005). However, in DOC-rich  
313 Mekkeljärvi, the low concentrations of inorganic nutrients, and thus restricted resource  
314 stoichiometry (i.e. high C:N:P ratio), for actively growing bacteria have been suggested to limit  
315 the bacterial production on labile carbon substrates, such as algal exudates, in the pelagic  
316 (Dorado-García et al. 2016). Therefore, higher nutrient availability, reflected also in enhanced  
317 PP, appears the more likely explanation for high pelagic BP in spring. Generally the temperature  
318 dependence of BP and growth is modulated by other environmental conditions, such as  
319 availability of inorganic nutrients and quality and quantity of organic matter substrates (Apple et  
320 al. 2006). The hypolimnetic fraction of the total pelagic BP was clearly higher than the  
321 epilimnetic and metalimnetic fractions through the autumn, which is partly explained by higher  
322 volume of water in the hypolimnion, but probably also reflects higher nutrient concentrations in  
323 the hypolimnion as reported in previous studies (e.g. Vesterinen et al. 2016a). Temperature in the  
324 hypolimnion remains around 4 °C through the summer whereas surface temperature often rises  
325 above 20 °C. Nutrient concentrations, in turn, remain rather constant in the hypolimnion through  
326 the stratification period (Vesterinen et al. 2016a). As only a small part of the total epiphyton  
327 biomass in the littoral is grazed by littoral invertebrates during the summer (Vesterinen et al.  
328 2016b), the remaining biomass is presumably decomposed in the water column and may  
329 contribute to the relatively high hypolimnetic BP in the autumn.

330         Epiphytic BP in the littoral correlated positively with the surface temperature, and the  
331 correlation was stronger than between pelagic BP and temperature in the epilimnion. As the  
332 temperature logger data from the sampling occasions on July and August allowed us to calculate

333 the BP estimates for each hour on those days, which were within 10–15% of those calculated  
334 multiplying by 24, the surface temperatures at noon were apparently close to the average daily  
335 surface temperatures. However, the production by epiphytic heterotrophs is also light-mediated  
336 and associated strongly with the epiphytic PP. Kuehn et al. (2014) found 60% higher production  
337 rates in litter-associated bacteria which were exposed to light than those which were in dark. If  
338 we assume a similar relationship between PP and BP and that a similar difference is applicable to  
339 periphyton in Mekkojärvi, then the epiphytic BP rates would be 60% lower during the night.  
340 However, day lengths in our study area range from 19.5 h in June to ca 10 h in October, so the  
341 photoperiod is long during summer months and epiphyton is exposed to light for most of the day.  
342 In autumn, in turn, day lengths are shorter but also the PP by epiphyton is low (Vesterinen et al.  
343 2016a) and, like the epiphytic BP, shows a trend of decreasing towards autumn. How much this  
344 light-dependent variation might truly affect epiphytic BP in Mekkojärvi, and in highly humic  
345 lakes in general remains speculative.

346       The extent to which benthic bacteria in lakes use organic C of allochthonous or  
347 autochthonous origin remains poorly known. In periphytic matrixes, the dissolved organic  
348 carbon pool is a mixture of extracellular release from macrophytes, excretion of both attached  
349 algae and bacteria, decomposition products following autolysis of epiphytes and dissolved  
350 carbon compounds of both autochthonous and allochthonous origin (Allen 1971, Attermeyer et  
351 al. 2014). The relative importance of these compounds likely varies between periphytic groups  
352 colonizing different habitats, e.g. between epiphyton, epilithon and epipsammon. Ask et al.  
353 (2009) showed that, although sediment bacteria in clear-water Swedish lakes were mainly fuelled  
354 by benthic PP, allochthonous C made a substantial contribution to the benthic BP. In contrast,  
355 Rodríguez et al. (2013) reported that benthic autochthonous OC supported pelagic BP in a small

356 clear-water lake. Allochthonous C has higher accessibility to sediment bacteria than to epiphytic  
357 bacteria due to high burial rates of allochthonous OM to lake sediments particularly in smaller  
358 lakes (Cole 2013). In small humic lakes, where non-illuminated sediments lack benthic  
359 autotrophic production, all potential autochthonous C for sediment bacteria comes from the  
360 upper water layers and will have been at least partly decomposed by pelagic bacteria. Therefore,  
361 allochthonous C is presumably more important for sediment bacteria in small humic lakes.  
362 Wetzel & Søndergaard (1998) described how macrophytes provide an extensive and diverse  
363 three-dimensional habitat for microbial colonization, which results in a shift from dominance of  
364 the macrophytes to the very high productivity of the attached microbiota. Theil-Nielsen &  
365 Søndergaard (1999) described epiphytic biofilms as “hotspots” for BP, exploiting exudation of  
366 DOC from macrophytes and epiphyton. Photolysis of recalcitrant allochthonous DOM can  
367 produce labile organic molecules that are more available for heterotrophic bacteria (Wetzel et al.  
368 1995, Paul et al. 2012). Since we only measured production of the epiphytic bacteria in  
369 Mekkojärvi, we cannot distinguish between autochthonous and allochthonous C sources  
370 supporting BP. As the littoral ambient water is brown with high quantities of allochthonous DOM  
371 (Kairesalo et al. 1992) bacteria may utilise that directly and after photolysis. However,  
372 considering the probable substantial release of labile autochthonous C from the highly productive  
373 epiphytic biofilms in the littoral (Vesterinen et al. 2016a), the bacteria are likely to rely heavily  
374 on autochthonous C. A light-mediated biotic decomposition process via algal stimulation of  
375 litter-associated microbial heterotrophs has recently been recognized (e.g. Francoeur et al. 2006,  
376 Danger et al. 2013). Kuehn et al. (2014) studied this process and concluded that periphytic algae  
377 function as a photosynthetic conduit for labile carbon supply to microbial heterotrophs (bacteria

378 and fungi) over very short time intervals, demonstrating the important role of bacteria and fungi  
379 in this light-mediated carbon cycling process.

380 Vesterinen et al. (2016a) demonstrated how the littoral in Mekkojärvi was strongly net  
381 autotrophic and reported  $364 \pm 66 \text{ mg C (g DW substratum)}^{-1} \text{ d}^{-1}$  as the mean PP by epiphyton in  
382 Mekkojärvi in 2012. Comparison to the mean daily epiphytic BP of  $1.52 \pm 1.36 \text{ mg C (g DW}$   
383  $\text{substratum)}^{-1} \text{ d}^{-1}$  in this study reveals the strong dominance of autotrophic production in the  
384 biofilms in the littoral and large quantities of autochthonous C potentially available for secondary  
385 production. A similar comparison between pelagic PP (in 2012) and BP (in 2015) reveals that the  
386 PP during the phytoplankton spring maximum can be 10 times higher than BP but the rates later  
387 in summer and autumn can be very even. Since the strong overall net heterotrophy and very high  
388 bacterial respiration has been demonstrated in the pelagic in Mekkojärvi (Salonen et al. 2005,  
389 Vesterinen et al. 2016a), it is likely that both anaerobic bacteria (e.g. green sulphur bacteria) and  
390 sediment bacteria contribute strongly to the whole-lake metabolism. However, comparison of PP  
391 and BP measured in different years can only be considered indicative. We did not measure  
392 epiphytic PP or chl *a* in this study, but comparison between the PP rates and chl *a* in the  
393 epiphyton in Mekkojärvi in 2012 (Vesterinen et al. 2016a) and BP in this study reveals a similar  
394 trend of increase from spring to late summer and then decrease towards autumn. This also  
395 indicates the possible positive relationship between the epiphytic BP and the autochthonous C  
396 produced by epiphyton. However, the relative concentrations of OC originating from internal net  
397 primary production (NPP) versus allochthonous OM loading to lake metabolism remains  
398 unresolved (Hanson et al. 2015).

399 There are various sources of error and uncertainty included in any studies which attempt  
400 to upscale rate estimates made in bottle incubations to the whole-lake scale (Hanson et al. 2015).

401 The estimation of the variability in whole-lake extrapolation is challenging due to high spatial  
402 heterogeneity and complex interactions (Pace 2001). Pelagic production rates can have high  
403 spatial variability (Van de Bogert et al. 2007), and a particular challenge for estimating the error  
404 for littoral epiphytic BP is associated with the variability of substrata around the lake.  
405 Mekkojärvi, however, is a very small lake and has a relatively uniform basin morphometry, so  
406 whole-lake extrapolations are likely to yield better constrained estimates than for larger lakes  
407 with highly variable morphometry. As the quantification of available substrata for epiphyton  
408 along the lake was done from 24 sites, which can be considered a rather high number of  
409 replicates around this small lake (Vesterinen et al. 2016a), it can be expected to have yielded a  
410 rather reliable estimate for the mean substratum weight per m lake shore ( $42.6 \pm 3.2$ ). Although  
411 the BP samples were collected only from six randomly selected sites around the lake to keep the  
412 workload reasonable, the spatial distribution of different plant species around the lake was well  
413 represented in the samples, which consisted mainly of two dominant plant groups, sedges and  
414 mosses. Some larger plant species (such as *Phragmites australis* and *Menyanthes trifoliata*),  
415 which have a patchy appearance around the lake shore, were not sampled due to their large size  
416 and the difficulties of incubating representative tissue samples with attached epiphyton in  
417 Eppendorf tubes.

418 In conclusion, our study shows that littoral epiphytic bacteria can make a significant  
419 contribution to whole-lake BP in humic lakes and, together with previous findings of highly  
420 productive photosynthetic epiphyton in the littoral in Mekkojärvi (Vesterinen et al. 2016a),  
421 demonstrates the importance of the littoral zone in the biomass production and C cycle in highly  
422 humic lakes, at least in the small humic lakes like Mekkojärvi that are so abundant throughout  
423 the boreal region and contribute substantially to greenhouse gas emissions (Raymond et al. 2013,

424 Holgerson & Raymond 2016). Although it is reasonable to suppose that in Mekkojärvi labile  
425 autochthonous C produced by epiphytic algae is an important source for closely associated  
426 bacteria, our study does not provide direct evidence of this phenomenon. This question merits  
427 future study in which more sophisticated whole-lake scale approaches, which account for both  
428 pelagic and littoral habitats, can address the role of the littoral zone in humic lakes.

429

#### 430 Acknowledgements

431 We thank Lammi Biological Station for both financial support to JV and for  
432 accommodation and laboratory facilities. We also acknowledge financial support from the Maj  
433 and Tor Nessling Foundation, the Doctoral Programme in Biological and Environmental Science  
434 of the University of Jyväskylä, The Academy of Finland (projects 137671 to RJ and 296918 to  
435 JS) and the Marie Skłodowska-Curie actions (660655 to JS) under the EU Horizon 2020.

436

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610

611 Figure 1. Location and bathymetry of Lake Mekkojärvi in southern Finland. Open circle denotes  
612 the sampling point for the pelagic measurements. Numbers refer to depth in meters.

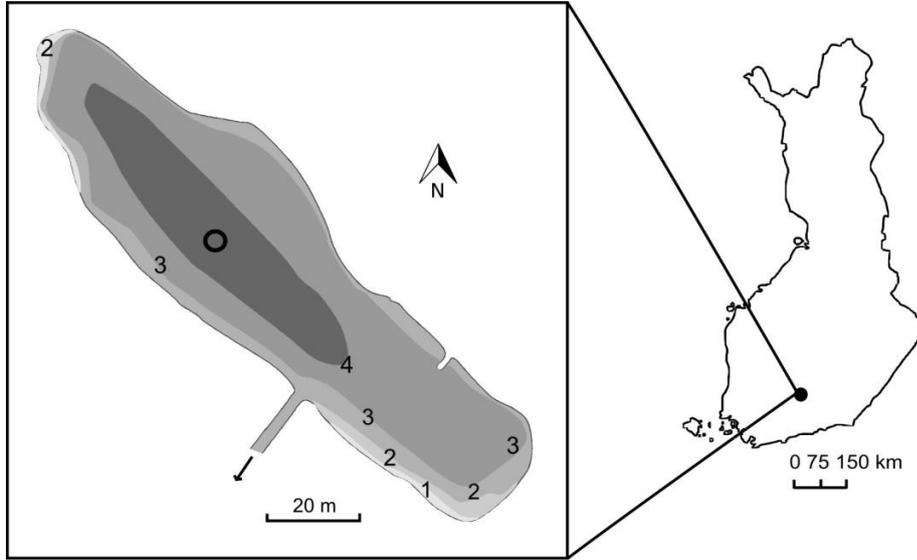
613 Figure 2. Pelagic bacterial production (BP) per unit area (mean  $\pm$  SE) in three different strata in  
614 2015. The dotted line expresses the areal BP in the whole water column as the sum of values  
615 from three strata.

616 Figure 3. Exponential relationships between (A) epilimnetic (pelagic) and (B) littoral epiphytic  
617 BP and surface temperature.

618 Figure 4. The mean  $\pm$  SE uptake of leucine for epiphytic bacteria in 7 different leucine  
619 concentrations.

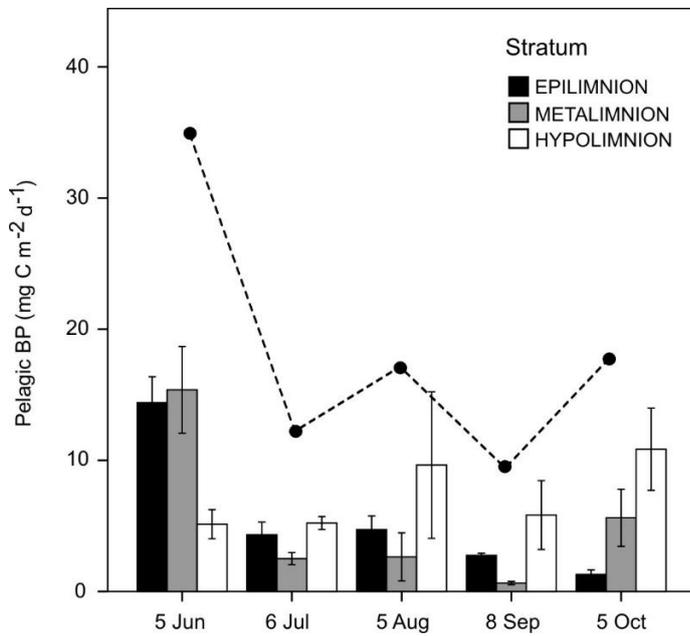
620 Figure 5. Littoral daily (mean  $\pm$  SE) epiphytic BP in Mekkojärvi, derived from the noon rates by  
621 multiplying by 24 and then normalized to g dry-weight of substratum.

622 Figure 6. A) Whole-lake estimates for BP of pelagic bacterioplankton and littoral epiphytic  
623 bacteria and B) their relative proportions.

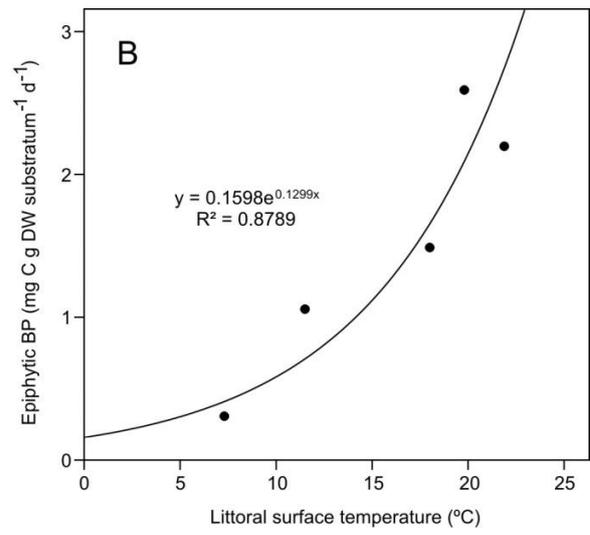
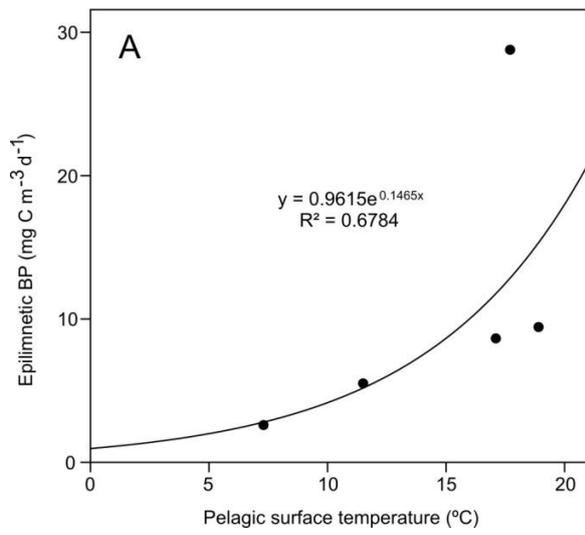


624  
625 Fig 1.

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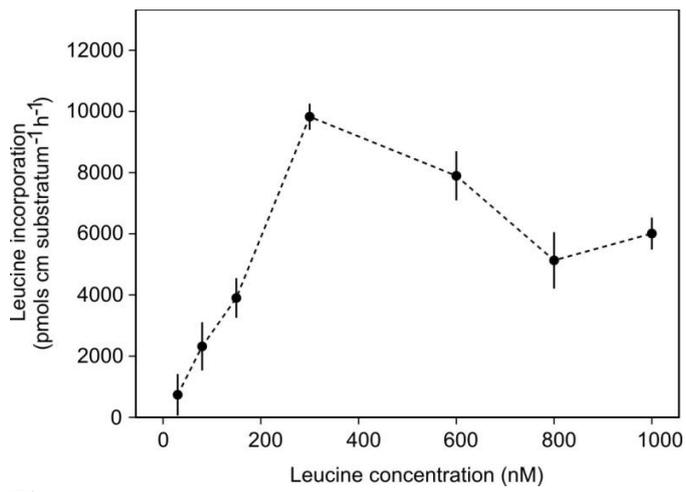
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630 Fig 2.  
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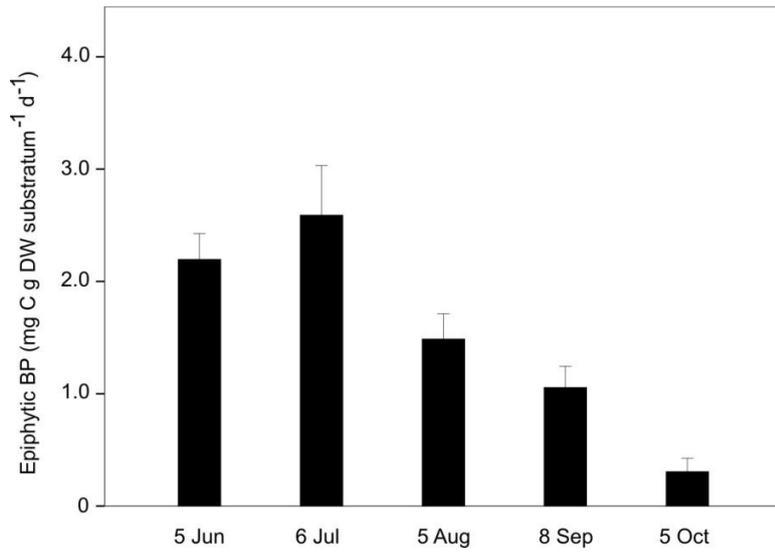
633 Fig 3.

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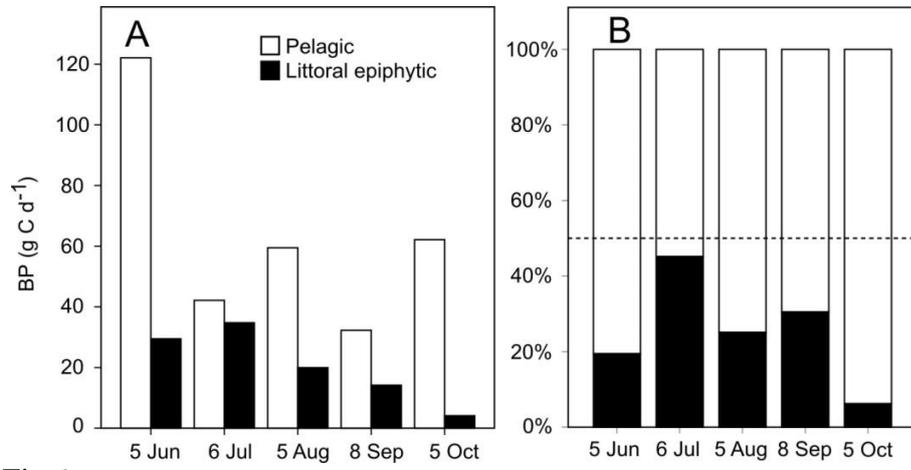
636 Fig 4.



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639

Fig 5.

640



641  
642

Fig 6.