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# Abundance and nutrient limiting growth rate of heterotrophic bacterio-plankton in Himalayan foot hill Lake Phewa, Nepal

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## Abstract

We examined heterotrophic bacterial abundance, chlorophyll-*a* concentration and resources limiting bacterial growth from October 2004 to August 2005 in Lake Phewa. The lake has a large watershed that covers ≈435 ha of water surface surrounded by ≈23-time large catchment area that might receive up to 4850 mm annual precipitation. During the study, bacterial abundance ranged from 3.2 to $9.9 \times 10^6$ cells $mL^{-1}$, and chlorophyll-*a* varied from 2 to 32 μg $L^{-1}$. Bacterial abundance and chlorophyll-*a* weakly correlated ($r = 0.40$, $n = 77$, $P < 0.1$) in the lake. Experiments on resources, glucose (C), nitrogen (N), phosphorus (P) alone and in combination (CNP) limiting bacterial growth rate, were examined using dilution bioassays. Experimental bottles enriched with resources and controls without enrichment (in triplicate) were incubated *in situ* for 48 h at collection depth. Results showed that C, N and P in combination significantly (at 5% level) stimulated bacterial growth rate. Bioassays with single resource additions showed P as main nutrient limiting bacterial growth comparing with C and N, implying that rainfall received in the catchment might convey adequate resources causing increased P deficiency for bacterial growth in Himalayan foot hill Lake Phewa.

## Key words

**bacterial–chlorophyll-*a* coupling, dissolved organic carbon, limiting resource, monsoon, phosphorus.**

## INTRODUCTION

The abundance of heterotrophic bacteria in aquatic systems represents a balance between growth and loss rates, which are regulated by inorganic nutrients, organic substrates, predation, lysis, temperature and other factors (Elser *et al.* 1995; Gurung *et al.* 2001; Løvdal *et al.* 2007). Bacterial abundance in lakes is often tightly coupled with chlorophyll-*a* (Bird & Kalff 1984; Cole *et al.* 1988; Thorpe & Jones 2005); this pattern implies dissolved organic carbon (DOC) from autroptroic production is an important resource for bacteria (Kirchman & Rich 1997; Tanaka *et al.* 2009). Weak bacterial–chlorophyll-*a* coupling suggests allochthonous DOC from the catchment or littoral production subsidizes bacterial abundance in some systems (Findlay *et al.* 1991; Jansson *et al.* 2001; Granèli *et al.* 2004). In aquatic systems where bacterial growth is limited by inoranic nutrients (Smith & Prairie 2004; Spears & Lesack 2006), it has been shown phosphorus (P) most frequently constrains bacteria (Toolan *et al.* 1991; Caron 1994). Phosphorus is an essential nutrient required in small quantities (Karl 2000; Carlsson & Caron 2001). Recent studies suggest climatic factors, such as temperature, precipitation and runoff (Jones & Young 1998; Jansson *et al.* 2000), play an important role in regulating temporal patterns in bacterial abundance in aquatic systems.

Lake Phewa is one of the well-studied lake (Ferro 1981/1982; Lohman *et al.* 1988; Jones *et al.* 1989; Rai 2000); however, bacterial abundance and resources limiting their growth rates have rarely been examined in this Himalayan lake situated in food hills of Central Nepal.

The work was carried out in the laboratory: Fisheries Research Station, Lake Phewa, Pokhara, Nepal.

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Thus, in the present work, we studied seasonal abundance and growth limiting resources for heterotrophic bacterioplankton in Lake Phewa.

## MATERIALS AND METHODS

The watershed of Lake Phewa occupies ≈123 km$^2$ between the Greater Himalaya and the Mahabharat range (28°7′–28°12′N and 84°5′–84°10′E) with a maximum elevation of 2508 m.s.l. Lake Phewa, at 782 m, has a surface area of 4.43 km$^2$, an average depth of 8.6 m and maximum depth of 23.5 m (Jones *et al.* 1989). In this study, we selected the deepest area of the lake known as *Anadu* ≈0.5 km off shore (Fig. 1) to minimize shoreline influence. Lake Phewa a small, warm monomictic lake is used for recreation, fisheries, limited hydropower and irrigation.

Monthly water samples were collected between October 2004 and August 2005 using a clean plastic 2 L van Dorn sampler from the water column at depths of 0, 2.5, 5, 7.5, 10, 15 and 20 m. Samples for initial bacterial counts were transferred to acid-rinsed 100 mL plastic bottles and preserved with high-grade cold glutaraldehyde at a final concentration of 2%. Preserved samples were transported in iced coolers and stored at 4°C in refrigerators until enumeration within a week. Rainfall data were collected from a meteorological station situated ≈1 km from the lake.

Water temperature was measured with a mercury thermometer. For soluble reactive phosphorus analysis, lake water was passed through precombusted GF/C filters (at 450°C for 2 h) and measured by the method of Menzel and Corwin (1965). Chlorophyll-*a* samples were obtained by filtering the lake water through Whatman GF/C glass fibre filters (≈1.2 μm pores size). Before extraction, chlorophyll samples were refrigerated in a sealed plastic container with silica gel. Chlorophyll-*a* was determined using a spectrophotometer according to the methods of Lorenzen (1967) and UNESCO (1969).

### Enrichment experiments

Potential growth limiting resources for bacterial growth were examined by performing enrichment tests following methods described in Gurung and Urabe (1999). Water samples for this purpose were taken from 2.5 m and filtered through preignited GF/F filters at low pressure. This filtration step removed protozoan and larger predators while allowing 70–90% of lake bacteria to pass into the filtrate (Gurung & Urabe 1999). The GF/F filtrate was used to quantify the initial number of bacteria for

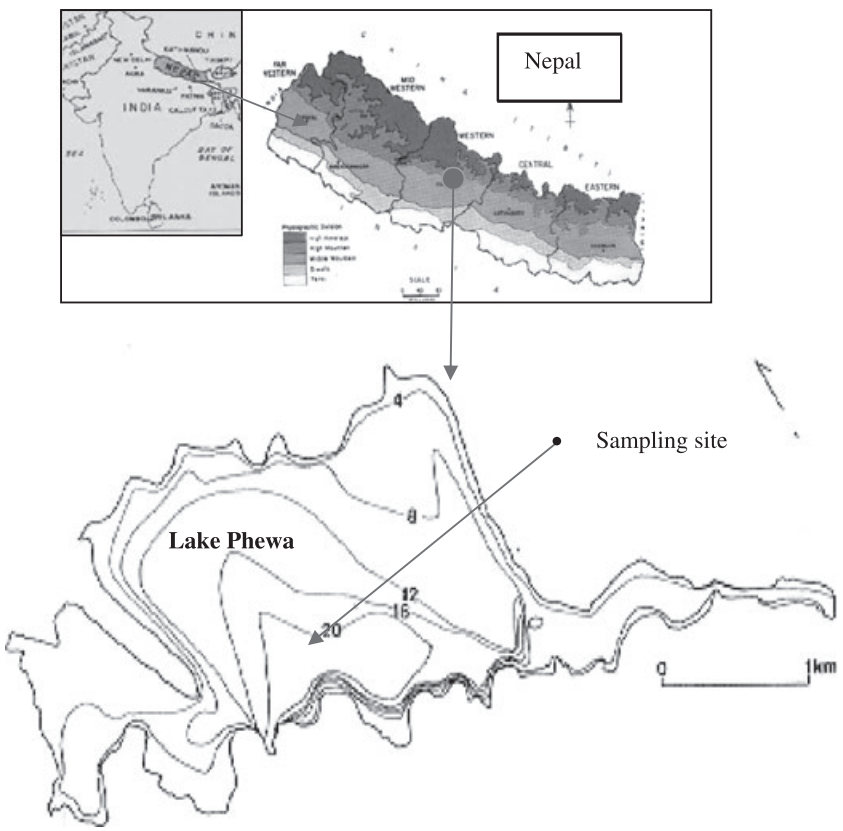


**Fig. 1.** Location and sampling site of the study area.

calculations in subsequent dilution experiments. During the experiment, filtrate was diluted to a ratio of 1:5 with 0.2 μm filtered lake water (prepared by gravity filtration of GF/F filtrate through well-cleaned 0.2 μm capsule filters [Gelman 12140] to avoid contamintation) and transferred to 150 mL acid-rinsed glass bottles.

Triplicate water samples were spiked at final concentrations with ammonium chloride ($NH_4Cl$) at 18 μM N as the N treatment; glucose ($C_6H_{12}O_6$) was added as the organic carbon as the C treatment at 50 μM; and dipotassium hydrogen phosphate ($K_2HPO_4$) was added as *P* treatment at 2.5 μM. Enrichment was made separately or in combination as a CNP treatment. After completing all procedures within 2 h of collection, the experimental bottles were returned to the lake for incubation *in situ* for 2 days at the collection depth; bottles were suspended with support of anchors and floats at 2.5 m in the lake. After 48 h, experimental bottles were removed and transferred in an ice-cooled container to the lake shore laboratory. Samples from the experimental bottles were used for the determination of final bacterial density.

Bacterial samples were fixed with cold glutaraldehyde and stored at 4°C until enumeration using methods described in Hobbie *et al.* (1977). Aliquots ranging from 0.25 to 0.5 mL were filtered through 0.2 μm black Nuclepore filter (25 mm) (CORNING Separation Division, Tokyo, Japan) and stained with acridine orange. Bacteria were counted using an Olympus epifluorescent microscope (×1250) (OLYMPUS OPTICAL CO., Japan) with a standard B-excitation system (100 W mercury lamp, BP 420–480). At least 300 bacteria were counted from slides prepared from each subsample. Growth rate of bacteria ($\mu$ day$^{-1}$) during the 2 day incubation was calculated as follows:

$$\mu = \mathrm{LnCb} - \mathrm{Ln\ Ce}/2,$$

where Cb and Ce were densities of bacteria at the beginning and the end of the 2 day incubation.

In the bioassay experiments, significant differences in bacterial growth rates between enriched and control treatments were examined statistically by one-way ANOVA with the Tukey–Kramer HSD *post hoc* test. These statistical tests were carried out with the aid of computer package JMP software (Version 5; SAS Institute, Cary, NC, USA).

## RESULTS

### Pattern of rainfall in the watershed

From November 2004 to February 2005 rainfall was negligible (Fig. 2a). Premonsoon showers with strong evening winds began in late March and continued till May. The 2005 monsoon was relatively weak; rain peaked in August with ≈39% of the total 2542 mm precipitation (Fig. 2a). Based on rainfall, the study can be divided into premonsoon (April–May), monsoon (June–August) and postmonsoon periods (October–March).

### Water temperature and stratification

Lake Phewa has warm monomictic circulation and, during this study, water temperature ranged from 16°C to 29.5°C (Fig. 2b). Between November 2004 and February 2005, surface water temperature declined from 22°C to 18.5°C. Water temperature was nearly isothermal during this period with variation of 0.5–2.0°C in the water column. Minimum variation in the water column was measured in January. With increasing day length and temperature, stratification was established in March. The lake remained stratified till October.

### Heterotrophic bacterial abundance and chlorophyll-*a* concentration

Bacterioplankton abundance in the Lake Phewa water column varied from an average of $4.84 \times 10^6$ cells mL$^{-1}$ in January to $9.9 \times 10^6$ cells mL$^{-1}$ in March (Fig. 2c). Chlorophyll-*a* concentrations (Chl) ranged from 2 to 32 μg L$^{-1}$ (Fig. 2d). Maximum chlorophyll occurred during stratification, within the metalimnion. In April and May, Chl peaked at 5 m, whereas in June and July concentrations peaked at 7.5 and 10 m (the seasonal maximum) respectively. In August maximum Chl was 9.4 μg L$^{-1}$ at 15 m (Fig. 2d). The temporal Chl pattern at 2.5 m varied from 2.1 μg L$^{-1}$ in January to 13 μg L$^{-1}$ in June. At this depth, bacteria varied from $5.06 \times 10^6$ cells mL$^{-1}$ in January to $8.9 \times 10^6$ cells mL$^{-1}$ in March (Fig. 3). The metalimnetic Chl peak has been described previously in this lake (Lohman *et al.* 1988; Davis *et al.* 1998). Bacterial abundance and Chl in the 2.5 m samples showed close synchrony with minor variation in March and May (Fig. 3). Soluble reactive phosphorus in the 2.5 m samples peaked at 7 μg L$^{-1}$ in December and was undetectable during most of the study period.

### Relationship between heterotrophic bacterial abundance and chlorophyll-*a*

Heterotrophic bacteria and chlorophyll-*a* showed a weak ($r = 0.40$, $n = 77$, $P < 0.01$) relationship in Lake Phewa, across all samples (Fig. 4a). The correlation coefficient ($r$) between bacteria and Chl during the stratified premonsoon and monsoon periods was weak ($r = <0.28$, Fig 4b,d,e). In contrast, the bacteria–Chl correlation was significant during winter mixing ($r = 0.542$, $n = 28$, $P < 0.001$) and during the postmonsoon period ($r = 0.58$, $n = 42$, $P < 0.001$, Fig. 4c,f) when the water column was cooling and stratification was becoming weak.

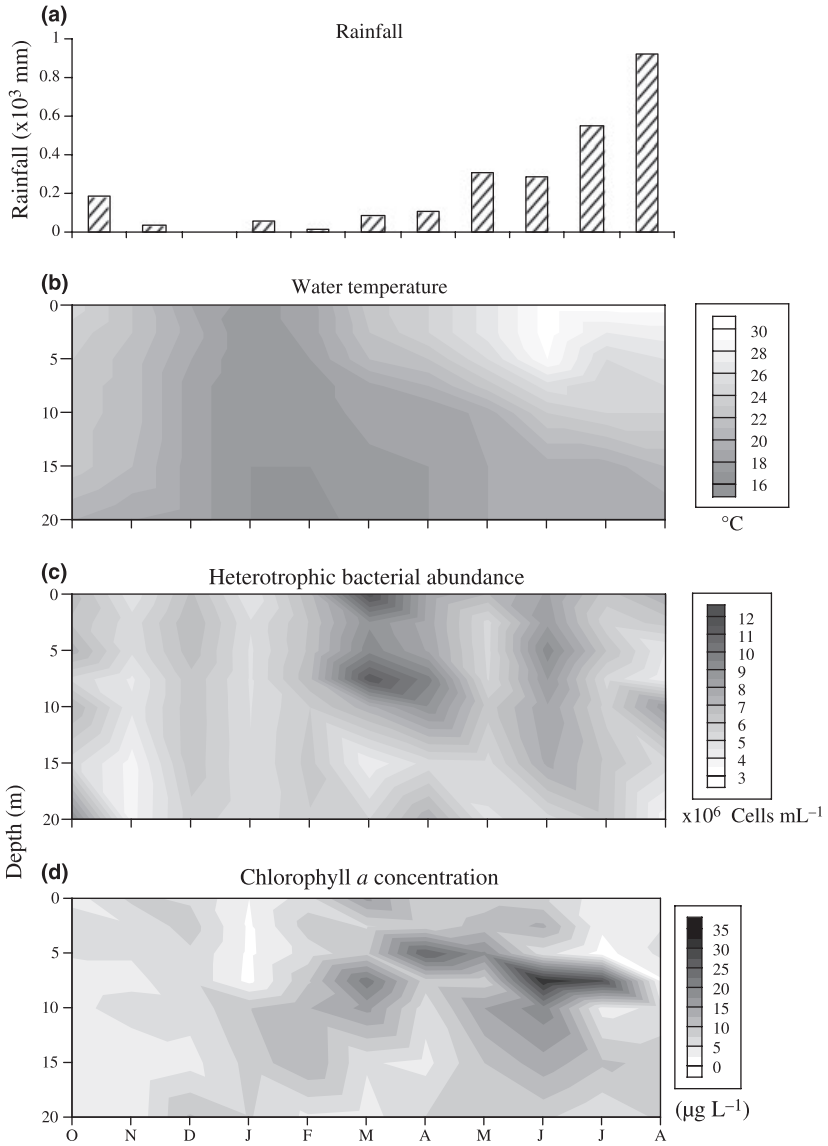


**Fig. 2.** Rainfall in catchments (a), water temperature (b), abundance of heterotrophic bacteria (c) and chlorophyll-*a* (d) in Lake Phewa.

## Enrichment experiments

In dilution bioassays, bacterial growth rates in the control ranged from 0.29 $day^{-1}$ in July to 0.78 $day^{-1}$ in April (Fig. 5). Interestingly, neither C nor N additions showed significant growth stimulation during the study. The most frequent limiting resource for bacterial growth was P. Bacterial growth rates in P treatments were significantly ($P < 0.05$) larger than controls, except in February and April. Stimulation by P enrichment resulted in bacterial growth rates twice the control value in 9 of 11 experiments. The largest growth stimulation by P enrichment occurred in October (1.018 $\mu$ $day^{-1}$) and the smallest (0.413 $\mu$ $day^{-1}$) in February.

In CNP treatments bacterial growth was significantly ($P < 0.01$) larger than the control in 9 of the 11 incubations (exceptions were in February and April). Bacterial growth rates in the CNP treatment were always larger than the P treatment. The largest stimulation in the CNP treatment occurred in August (1.518 $\mu$ $day^{-1}$) and the smallest (0.456 $\mu$ $day^{-1}$) in February.

## DISCUSSION

The mean heterotrophic bacterioplankton abundance in Lake Phewa was $6.39 \times 10^6$ $mL^{-1}$ (Fig. 2). According to trophic state boundaries based on mean bacterial abundance by Cotner and Bidanda (2002) and Thorpe and Jones (2005), Lake Phewa is eutrophic. The lowest bacterial abundance occurred in January that coincided with the lowest Chl value, low temperature and a mixed water column. Bacterial abundance peaked in March, shortly after the lake stratified, but did not coincide with peak

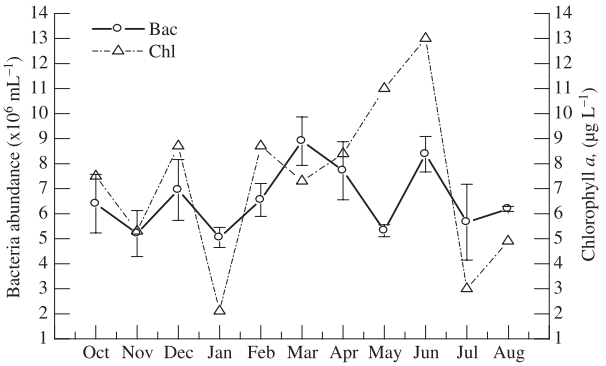


**Fig. 3.** Changes in chlorophyll-*a* concentration and bacterial abundance at 2.5 m in Lake Phewa.

Chl in the water column (Fig. 2). The metalimnitic Chl peak (Fig. 2d) during summer stratification was most pronounced during the monsoon from June to August (Fig. 2a,d).

The bacteria–Chl coupling in Lake Phewa was weak (Fig. 4a). Weak bacteria–Chl relations have been described in heterotrophic systems where allochthonous organic carbon was supplied from the catchment (Findlay *et al.* 1991; Le *et al.* 1994). In this study, bacteria–Chl coupling was most strong when the water column was not stratified (Fig. 4c) and during the postmonsoon period when stratification was weakening (Fig. 4f). Both are periods of low rainfall and runoff. This empirical evidence

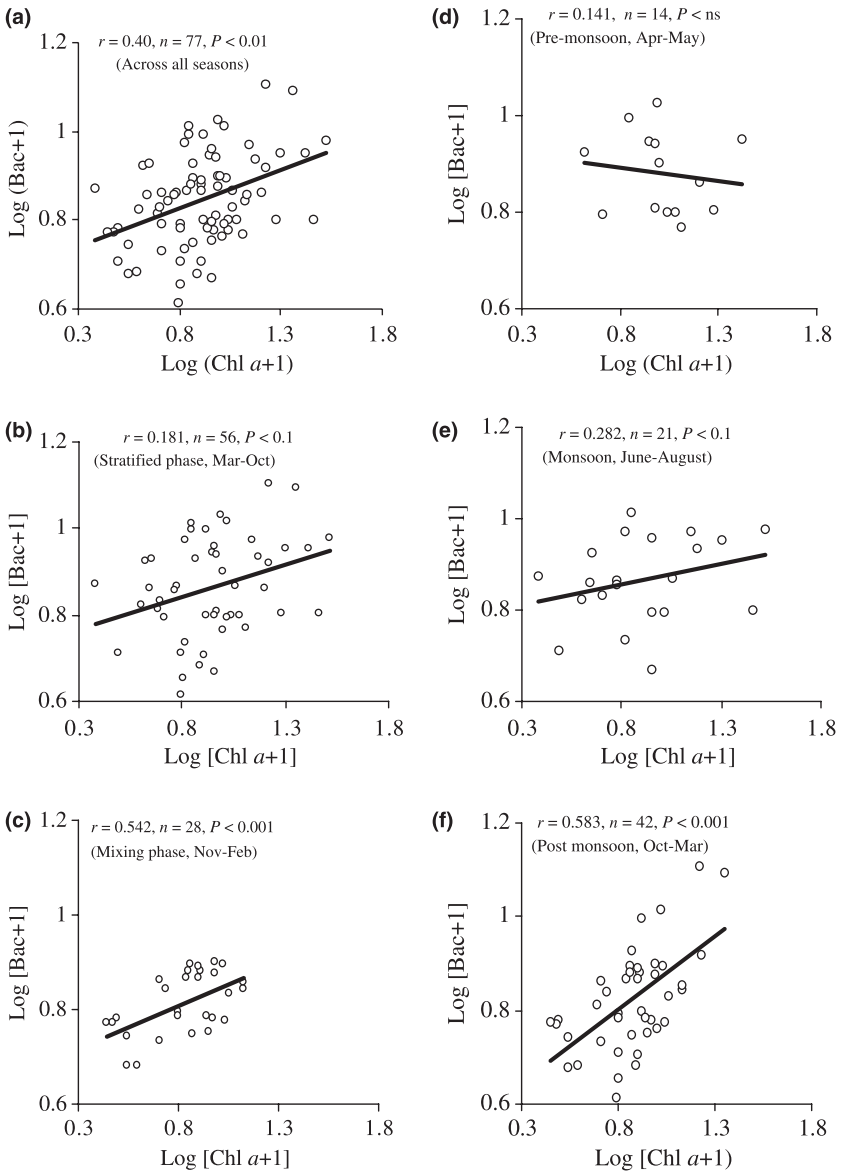


**Fig. 4.** Relationship between heterotrophic bacteria and chlorophyll-*a* concentration, (a) across the study period, (b) stratified, (c) mixing, (d) premonsoon, (e) monsoon and (f) postmonsoon phase.

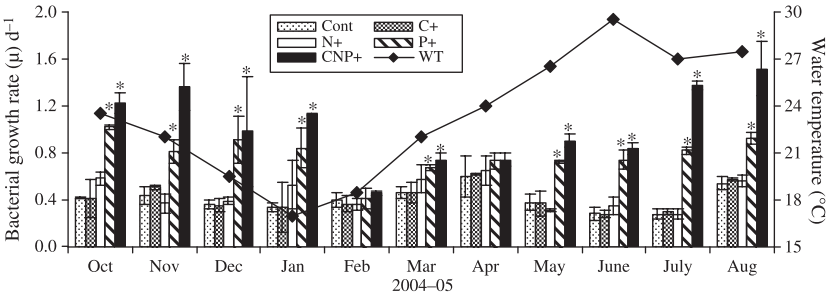


**Fig. 5.** Changes in water temperature and bacterial growth rate in organic carbon (C), nitrogen (N), phosphorus (P) and combination of all treatments (CNP) and control at 2.5 m. Treatments with significantly (at 5% level) higher growth rate than control have been marked by *.

suggests heterotrophic bacteria depend on phytoplankton for organic carbon supply in these months. Bacterial abundance at 2.5 m was synchronous with Chl during most months of the study, exceptions were March and May (Fig. 3), suggesting that bacterial abundance tracked Chl in the mixed surface layer of Lake Phewa during much of the study period.

Factors other than autochthonous production, as measured by Chl, are likely important in determining BA in Lake Phewa during some seasons. Top-down control could be temporally important; as this lake has been heavily stocked with plankton feeding fish for open water production and cage aquaculture (Swar & Pradhan 1992; Gurung *et al.* 2005). Premonsoon rain and strong storms, often combined with hail (Paudel & Thapa 2001; Das 2005), would deliver allochthonous materials to the water column during these months. During the peak of the monsoon, it is common for runoff to enter the lake as an interflow (Davis *et al.* 1998). During premonsoon and monsoon periods, DOC was likely supplied in runoff from the large watershed that is dominated by forests, agriculture and an extensive urban area. During monsoon storms, factors such as interflows, water column cooling and seiche activity would also be a potential mechanism to distribute DOC in the water column from internal sources (Davis *et al.* 1998).

Small lakes can depend on allochthonous DOC for metabolic activities (Wetzel 1984, 1995; Urabe *et al.* 2005; Wetzel & Tuchman 2005). Lake Phewa has a small volume relative to its catchment area, and the monsoon-dominated climate can deliver up to 4846 mm of rainfall per year (Paudel & Thapa 2001; Gurung *et al.* 2006), causing lake water to be exchanged many times during summer (Ferro 1981/1982; Lohman *et al.* 1988). During the monsoon, phytoplankton in Lake Phewa can be reduced by silt-laden inflows that dilute lake water and reduce available light (Davis *et al.* 1998; Gurung *et al.* 2006). Presumably DOC is delivered to the lake with monsoon inflow. In Lake Phewa, 2.52 mg $L^{-1}$ DOC had been reported (Lohman *et al.* 1988; McEachern 1996). In other lakes with similar DOC concentrations, bacterial growth has been P deficient (e.g. Morris & Lewis 1992; Gurung & Urabe 1999).

Enrichment experiments showed that P was a limiting resource for bacterial growth in Lake Phewa (Fig. 5). This result was not surprising given that soluble P was largely undetected in our samples. The C, N, P independently or in combination did not stimulate substantial growth comparing with control in February and April (Fig. 5). There is no clear explanation for this outcome but high bacterial growth rates in the control during February and April suggest resources were available and heterotrophic bacteria were not constrained in those samples.

We expected that bacterial resource limitation would vary across seasons in Lake Phewa; however, this was not the case, and P limitation was most frequent in limiting bacterial growth. Bacteria are known to have a high P requirement (Karl 2000; Spears & Lesack 2006). Carlsson and Caron (2001) determined temperature was not an important determinant of bacterial growth rates at values >12°C. Subtropical Lake Phewa had a minimum water temperature of 16°C during the study (Fig. 5) suggesting that temperature was not a key factor influencing bacterial growth.

Generally, short-term bacterial *in situ* dilution bioassy experiments are criticized for reliance on only initial and final observations. Increasing the duration of experiments can effect growth patterns and rates, and alter the natural species composition (Gurung & Urabe 1999). In our dilution bioassays, internal nutrient cycling by grazers was eliminated by prefiltration, which also decreased in ambient nutrients for bacterial uptake. To minimize this potential decrease in nutrient concentration during incubation, bacterial density was reduced by dilution.

In general in this study, the bacterial abundance did not correlate with chlorophyll-*a* strongly, suggesting that there could be other alternative sources of organic substrate for bacterial growth. It is known that the phototrophic picoplankton can predominantly occur and contribute substantial concentration of Chl in aquatic environments (Johnson & Sieburth 1979; Callieri & Stockner 2002; Wakabayashi & Ichise 2004). The phototrophic picoplankton, which has not been included in this study, might explain the tight correlation between chlorophyll-*a* and bacterial abundance in the lake.

In Lake Phewa, the bacterial–Chl linkage seems most tight during seasons when the lake is mixing or the water column is weakly stratified and there is low rainfall and runoff (Fig. 4c,f). During periods of stratification and monsoon inflow, there is circumstantial evidence that allochthonous organic matter from the catchment area enters into the lake causing severe P-limitation to bacterial growth. This trend indicates that with increasing rainfall recieved into the catchment, probably higher amount of organic substrate conveyed into the lake. As the catchment area, especially western shoreline, has been heavily urbanized and many sewarage drained into the lake. Thus, it is possible that supply of resources like C and N were transported in the lake with increasing rainfall in catchment, which in turn had been reflected in terms of P limitation to bacterial growth. Supporting to this trend, several other studies have also revealed similar pattern of P limitation to bacterial growth, especially in coastal areas (Cotner *et al.* 2000; Farzalla *et al.* 2006) likely to have higher anthropogenic activities in near by shoreline. The present scenario implies that biogeochemistry studies of lakes need to be assessed in relation to meteorological events concerning to climate change and global warming.

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