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Limnology and trophic status of glacial lakes in the tropical Andes (Cajas National Park, Ecuador)

WILLEM R. VAN COLEN*, PABLO MOSQUERA†, MAARTEN VANDERSTUKKEN‡, KOEN GOIRIS§, MARIA-CECILIA CARRASCO¶, ELLEN DECAESTECKER*, MIGUEL ALONSO**, FABIÁN LEÓN-TAMARIZ†† AND KOENRAAD MUYLAERT*

**Department of Biology, Aquatic Biology, KU Leuven Kulak, Kortrijk, Belgium*
†Subgerencia de Gestión Ambiental, ETAPA EP, Cuenca, Ecuador
‡Department of Biology, KU Leuven Kulak, Kortrijk, Belgium
§KU Leuven Technologiecampus Gent, Ghent, Belgium
¶ETAPA, Cuenca, Ecuador
***Departamento de recursos Hídricos y Ciencias Ambientales, University of Cuenca, Cuenca, Ecuador*
††University of Cuenca, Cuenca, Ecuador

SUMMARY

1. The tropical Andes has a high density of glacial lakes that are situated in the high-altitude páramo (3500–4500 m). Ecological information about such lakes is scant despite the fact that these lakes are an important source of water for drinking, irrigation and electricity generation and feed several major tributaries of the Amazon. In this study, we provide data on a survey of 31 lakes in Cajas National Park (Ecuador). Two of the lakes were monitored monthly during one year. *In situ* nutrient addition experiments were carried out in three of the lakes.

2. Seasonal monitoring in two lakes revealed a thermal stratification of the water column between October and June, with a small temperature difference between epi- and hypolimnion (2–3 °C). Oxygen depletion of the hypolimnion towards the end of the stratification period indicated that no complete mixing of the water column occurred during stratification. There was no evidence of depletion of nutrients in the epilimnion or accumulation in the hypolimnion during stratification. There were also no clear seasonal changes in chlorophyll-*a* (Chl-*a*) concentration nor in phytoplankton community composition in the two lakes.

3. Inputs of dissolved organic carbon (DOC) from the vegetated catchment resulted in high DOC concentrations (median 2.9 mg L^{-1}) compared to temperate mountain lakes. Water transparency was relatively low, with a median extinction coefficient for photosynthetic active radiation of 0.50 m^{-1} and for UV-B radiation of 10.13 m^{-1}. Although the thermocline was deep and water transparency was low, estimates of the critical depth for photosynthesis were deeper than the mean water depth in all lakes, suggesting that phytoplankton was not light limited.

4. The phytoplankton community was dominated by chlorophytes (e.g. *Oocystis*), diatoms (small *Cyclotella* spp.) or small colonial cyanobacteria (*Aphanocapsa*, *Merismopedia*). The zooplankton community was either dominated by large cladocerans and cyclopoid copepods, or by the calanoid copepod *Boeckella occidentalis*.

5. Total concentrations of phosphorus (P) and nitrogen (N) were comparable to those in temperate mountain lakes (4–35 μg P L^{-1} and 162–758 μg N L^{-1}) while Chl-*a* concentrations were in the lower range (<1 μg L^{-1}). A large part of the total nutrient pool consists of dissolved organic N and P that appeared to have a low bioavailability to phytoplankton. The median seston N:P ratio of 44, a positive correlation between Chl-*a* and total P concentration, as well as nutrient addition assays carried out in three lakes all pointed to P limitation of phytoplankton.

Correspondence: Willem R. Van Colen, Laboratory of Aquatic Biology, KU Leuven Kulak, Etienne Sabbelaan 53, 8500 Kortrijk, Belgium.
E-mail: willem.vancolen@kuleuven-kulak.be

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Introduction

The Andes mountains have been shaped by glacial activity resulting in a high density of glacial lakes. These lakes are an important source of water for drinking, irrigation and hydroelectricity to more than 100 million people living in or near the Andes (Luteyn, 1992; Bradley *et al.*, 2006; Buytaert, Deckers & Wyseure, 2006a; Buytaert *et al.*, 2006b). They also supply water to several major tributaries of the Amazon, such as the Napo river.

Glacial lakes in the Andes are situated at high altitude (3500–4500 m) and therefore have cold water (7–12 °C). Their position near the equator results in limited seasonal variation in temperature and, hence, a weak thermal stratification (Lewis, 2000). The catchment vegetation consists of páramo grassland and scattered *Polylepis* (Rosaceae) bushes (Luteyn, 1992; Ramsay & Oxley, 1997) and the soils typically have a very high organic matter content (Buytaert *et al.*, 2005). The lakes are therefore likely to receive more significant inputs of organic matter from the catchment than temperate mountain lakes. Because of this unique combination of properties, Andean glacial lakes may differ substantially from temperate mountain lakes, which have a stable thermal stratification during summer and low inputs of organic matter from the catchment.

Tropical mountain lakes in Andes are under increasing pressure from human activities and climate change. Because the mountain climate is more favorable than the warm and humid lowlands, human activity is comparatively greater in the mountains than in the lowlands (Sarmiento, 2002; Jantz & Behling, 2012). Human activities in the Andes, including cattle raising, crop production and afforestation (Verweij & Budde, 1992; Molinillo & Monasterio, 1997; Podwojewski *et al.*, 2002; Farley & Kelly, 2004) are on the rise. In addition, the Andes appears to be warming up twice as fast as the global average (Vuille *et al.*, 2003; Bradley *et al.*, 2006). Global climate models suggest that precipitation will increase during the wet season and decrease during the dry season (Buytaert *et al.*, 2006b). It is difficult to assess how these changes will impact Andean mountain lakes because there is virtually no baseline information available on the ecology of these ecosystems.

Since the pioneering work of Myriam Steinitz-Kannan in the 1980s (Steinitz-Kannan, Colinvaux & Kannan, 1983; Miller, Kannan & Colinvaux, 1984), mountain lakes in Ecuador have barely been studied. The general aim of this study is to provide information on the limnology of glacial lakes in the Ecuadorian Andes and compare the ecological characteristics of these lakes with temperate mountain lakes. Our focus is on factors that control phytoplankton productivity and trophic status. We collected information on water column stratification, the underwater light climate (including ultraviolet radiation), nutrients, chlorophyll-*a* (Chl-*a*) and phyto- and zooplankton community composition. This study was carried out in Cajas National Park (NP) (2°50′S, 79°14′W) in Ecuador, which has a very high density of glacial lakes. A survey of 31 lakes was performed. Two of these lakes were monitored monthly over one year to study seasonal variation in stratification and phytoplankton productivity. Nutrient addition assays were performed in three lakes to determine the nutrient limiting phytoplankton productivity.

Methods

Study area

This study was carried out in Cajas NP, Ecuador, which covers 285 km^2 of páramo and contains 218 glacial lakes of >1 ha (Fig. 1). Cajas NP is the source of the Yanuncay, Tomebamba and Machangara rivers that provide drinking water to Cuenca, the third largest city in Ecuador. In the 20th century, the park was intensively used for cattle grazing. Cajas was declared a NP in 1996 and since 2001 has been managed by the local utility company ETAPA (Empresa Pública Municipal de Telecomunicaciones, Agua Potable y Alcantarillado y Saneamiento de Cuenca), that uses water from the NP for drinking water production. Cattle densities were gradually reduced and Cajas NP is now considered one of the best-managed NPs in Ecuador (Farley *et al.*, 2011). Two lakes in Cajas NP have received minor inputs of wastewater in the past (Toreadora: wastewater from a tourist lodge; Llaviucu: wastewater from a trout farm and small brewery). Irradiance, temperature and wind speed data for the year 2013 were obtained from a meteorological station situated near Toreadora Lake.

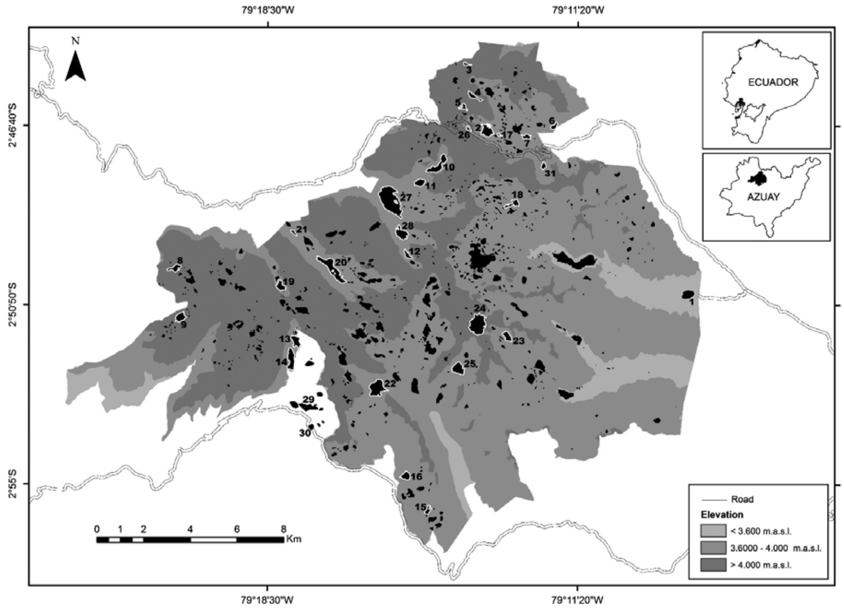

Fig. 1 Map of Cajas National Park (NP). Sampled lakes are marked with the lake number (see Table S1 for corresponding lake names). The two monthly sampled lakes were Lake Toreadora (number 2) and Lake Riñoncocha (number 4). Insets show the location of Cajas NP within the province Azuay (bottom) and the location of the province Azuay within Ecuador (top).

Sampling

A survey of 31 predominantly large (>5 ha) and deep (>10 m) lakes was carried out. These lakes were equally distributed over the major catchments of the NP. The lakes were sampled once during two sampling campaigns (August–October 2011 and August–October 2012) (Fig. 1). In addition, two lake that were representative of all lakes and that were relatively accessible (Toreadora and Riñoncocha) were sampled monthly during 1 year (January–December 2013). In 2013, nutrient limitation experiments were carried out in three lakes (Patoquinuas, Toreadora and Estrellas Cocha Izhcayrrumi). These three lakes were again selected because they were representative and accessible, as well to include lakes from the eastern and western ends of the NP. Samples for dissolved organic carbon (DOC) were collected separately in a subset of the lakes during an additional sampling campaign in January–March 2014. For all 31 lakes, the lake catchment area was calculated using GIS (software ArcMap 10.3, Esri, Redlands) on a digital elevation model obtained from the ASTER Global Digital Elevation Map. The lake volume was calculated by multiplying the lake area with average lake depth (estimated as 0.46 Z_m; Wetzel, 2001). Catchment area, lake area and lake volume were used to calculate the water residence time for each lake. Water residence time was determined based on an average rainfall of 916 mm year^{-1} (data from weather station at Lake Toreadora) and a run-off coefficient of 65% (Crespo *et al.*, 2011).

Sampling and measurements were carried out at the deepest point in the lake (determined by sonar). Temperature, conductivity and oxygen concentration were measured at 2 m depth intervals down to a maximum depth of 30 m using a submersible sensor (YSI Professional Plus and YSI ProODO probes, YSI Incorporated, Yellow Springs). pH could not be measured in a reliable way using these sensors due to the low conductivity of the water. In a subset of 19 lakes, vertical profiles of underwater irradiance were measured with a spectroradiometer (Biospherical Instruments, San Diego) equipped with a photosynthetically active radiation (PAR; 400 – 700 nm), UV (305, 320 and 380 nm) and depth sensor. Water transparency was also estimated using a 30 cm diameter Secchi disk (Wildlife Supply Co, Yulee). The euphotic depth ($Z_{1\%}$, unit: m) was calculated as $\ln(100)/K_d$.

The critical depth (Z_{cr}) concept was used to assess whether underwater light levels were sufficiently high to allow for positive net photosynthesis of phytoplankton. Z_{cr} was estimated (Kirk, 1994) for each lake from day length (N), mean surface irradiance (E_0), the vertical light extinction coefficient for PAR (K_d), the ratio of phytoplankton respiration to photosynthesis (ρ) and the irradiance at onset of light saturation (E_k):

$$Z_{cr} = \frac{N}{24 \cdot \rho \cdot K_d} \ln\left(\frac{E_0}{0.5 \cdot E_k}\right)$$

N near the equator is 12 h. E_0 was the annual average irradiance measured at the weather station near lake Toreodora, which was 130 W m^{-2} global irradiance or 60 W m^{-2} PAR (assuming 46% of global irradiance is PAR), which is equivalent to 262 μEinst m^{-2} s^{-1}. K_d was calculated by linear regression of log-transformed irradiance measurements versus depth for each lake. ρ was assumed to be 0.1 (Geider & Osborne, 1989) and E_k estimated to be around 100 μEinst m^{-2} s^{-1} as typical of cold lakes with low underwater irradiance levels (e.g. Fahnenstiel *et al.*, 1989; Markager, Vincent & Tang, 1999; Davies, Nowlin & Mazumder, 2004).

During the survey, a depth-integrated water sample was collected by pooling water samples collected at 2 or 5 m depth intervals (depending whether the maximum depth of the lake was <10 or >10 m, respectively) down to the lake bottom using a Van Dorn sampler (horizontal, 2 L content). During the monthly monitoring, water was sampled at two depths (1 m below the surface and 2 m above the bottom) and the samples were analysed separately for each depth. Water samples were subsampled for phytoplankton pigments and community composition. For analysis of nutrients, water was first screened through a 64 μm Nitex nylon mesh (for zooplankton removal). This water was sampled for total nutrients. For dissolved and particulate nutrients, this water was filtered through a pre-weighed and pre-combusted (4 h, 450 °C) GF/F filter (25 mm, 0.7 μm pore size, Whatman, Chicago). The filtrate was used for analysis of dissolved nutrients and the filter itself for analysis of particulate nutrients. Samples for DOC were filtered through a pre-combusted GF/F filter (25 mm, 0.7 μm pore size, Whatman, Chicago) into pre-combusted, acid-rinsed glass vials. After acidification with HCl (pH < 2), samples were stored at 4 °C in the dark until analysis. For analysis of pigments by high-performance liquid chromatography (HPLC), water was filtered through another GF/F filter. All filters and water samples were stored frozen until analysis. For analysis of the phytoplankton community composition, a sample was fixed with formalin (final concentration 4%). In the 25 lakes sampled in 2011, depth-integrated zooplankton samples were collected by vertical tows with a 64 μm mesh size zooplankton net down from 1 m above the lake bottom up to surface level. The zooplankton sample was fixed with formalin (final concentration of 4%). Fixed phyto- and zooplankton samples were stored in the dark at room temperature until further analysis.

Sample analyses

The nutrients N and P were fractionated into total (TN, TP), total dissolved (TDN, TDP) and particulate organic (PON, POP) fractions. Within the total dissolved fraction, a further fractionation was made between reactive [dissolved inorganic N (DIN), soluble reactive P (SRP)] and non-reactive [dissolved organic N (DON) and P (DOP)] nutrients. The non-reactive dissolved nutrients were assumed to correspond to dissolved organic nutrients (DON and DOP). DON and DOP were estimated from the difference between TDN and TDP and DIN and SRP concentrations, respectively. For analysis of TN and TP (in unfiltered water samples) and TDN and TDP (in filtered water samples), the samples were digested prior to analysis using alkaline persulphate digestion. All nutrient samples, except for PON, were analysed using a QuAAtro segmented flow analyser (Seal Analytical, Norderstedt) equipped with standard manifolds for the analysis of NH_4^+, NO_2^-, NO_3^- and PO_4^{3-}. Detection limits for these measurements were 1.5 μg L^{-1} for NH_4^+, 1.2 μg L^{-1} for the sum of NO_2^- and NO_3^- and 1.0 μg L^{-1} for PO_4^{3-}. NH_4^+, NO_2^- and NO_3^- were summed after analysis to represent all inorganic nitrogen forms (DIN). For analysis of POP, the GF/F filter was digested using alkaline persulphate digestion prior to analysis. PON was measured on the GF/F filters using a Flash HT/EA elemental analyser (Thermo Fisher Scientific, Waltham). DOC samples from the survey were analysed using TOC cuvette tests (Method 10129; Hach Lange, Düsseldorf). For two lakes, DOC measurements based on these cuvette tests were compared with analyses using a Shimadzu TOC analyser (Shimadzu Corporation, Kyoto), resulting in similar concentrations. DOC samples from the 1-year monitoring were analysed with the Shimadzu TOC analyser.

Phytoplankton pigments (chlorophylls and carotenoids) were analysed using HPLC according to the method of Wright *et al.* (1991). Pigments were extracted from the GF/F filters in 90% methanol using ultrasonication. The carotenoid trans-β-apo-8′-carotenol was added during the extraction as an internal standard. Pigments were separated and quantified on a Waters Alliance HPLC system equipped with a Waters Spherisorb ODS2 column (Waters Corporation, Milford, USA) using a ternary gradient of 80:20 methanol: 0.5 M ammonium acetate, 90:10 acetonitrile: water and ethyl acetate. Carotenoids were identified using a PDA detector and the system was calibrated using pure standards (DHI, Hørsholm).

Phytoplankton community composition was analysed using inverted microscopy. A total of 200 units (cells or colonies) per sample were counted and identified down to genus level. Assuming basic geometric shapes, an average biovolume per genus was estimated, allowing determination of the total phytoplankton biovolume and the relative abundance of each genus. Phytoplankton community data were analysed in all survey lakes, except Cucheros, and in the epilimnetic samples of the lakes that were monitored monthly over 1 year.

For analysis of zooplankton community composition, all individuals in a subsample were counted and identified down to species level using a stereomicroscope. Copepod identification only went down to the level of order, differentiating between calanoids, cyclopoids and harpacticoids. Rotifers were observed but not quantified. Only relative abundance data were used because the tow net method cannot reliably quantify zooplankton.

Nutrient limitation experiments

To determine possible nutrient limitation of phytoplankton and to identify the main limiting nutrient, *in situ* nutrient addition assays were carried out in three lakes in 2013. For each experiment, 12 replicate 2 L polyethylene plastic bottles were filled with lake water that was filtered through a 64 μm Nitex nylon mesh to remove large zooplankton. Three replicate samples for HPLC pigment analysis were collected to determine initial pigment concentrations. Four treatments were prepared in triplicate: +P (addition of 60 μg L^{-1} P as K_2HPO_4), +N (addition of 500 μg L^{-1} N as $NaNO_3$), +NP (addition of both P and N) and a control treatment receiving no nutrient addition. The bottles were attached to an aluminum frame and incubated for 7 days in the lake at a depth of *c.* 2 m. An exploratory experiment had indicated that phytoplankton growth rates were low and that a 7-day incubation period was necessary to detect differences between nutrient treatments. After 7 days, samples for HPLC pigment analysis were collected from each bottle.

Statistical analyses

The R software was used for all statistical analysis. Principal component analysis (PCA) was used to summarise the variation in phyto- and zooplankton community composition. Phytoplankton taxa were grouped into major phylogenetic groups (chlorophytes, cryptophytes, diatoms, chrysophytes, dinoflagellates and cyanobacteria). The $\log(x + 1)$-transformed total biovolume (in 10^4 μm^3) data of each phylogenetic group was used as the input for the PCA analysis. The zooplankton dataset included the orders of calanoid and cyclopoid copepods, and cladocerans in the genera *Daphnia* (Daphniidae) and *Bosmina* (Bosminidae). $\text{Log}(x + 1)$-transformed relative abundance data were used as the input for the PCA analysis. Both PCA analyses were based on a covariance matrix in order to reduce the influence of the least abundant taxa in the analysis. The prcomp package was used for PCA analysis (package stats, R Development Core Team, 2011). We calculated correlation coefficients between Chl-*a* and the PCA axes for phytoplankton and zooplankton community data analyses with a range of abiotic variables. For analysis of the nutrient limitation assays, ANOVA was used in combination with Tukey's *post-hoc* test. To compare differences in the degree of nutrient limitation between different phytoplankton groups, we analysed not only the response of total Chl-*a* but also chlorophyll-*b* (Chl-*b*, marker pigment for chlorophytes), fucoxanthin (marker for diatoms) and zeaxanthin (marker for cyanobacteria).

Results

Lake survey

Most of the lakes studied were large and deep lakes situated at an elevation between 3680 and 4220 m and

Table 1 Minima, maxima, means and medians of the main morphological, physical, chemical and biotic variables of the survey lakes in Cajas National Park. Data for each lake individually can be found in Tables S1 and S2. DOC, dissolved organic carbon; PAR, photosynthetically active radiation; TN, total nitrogen; TP, total phosphorus; PON, particulate organic nitrogen; POP, particulate organic phosphorus.

	Minimum	Maximum	Mean	Median
Elevation (m.a.s.l.)	3160	4220	3886	3905
Lake area (ha)	1.3	77.7	15.0	10.2
Maximum depth (m)	0.3	62.0	21.7	16.0
Water residence time (year)	0.01	13.34	1.66	0.97
Water temperature (°C)	6.5	12.9	8.9	8.4
Conductivity (μS cm^{-1})	14.6	110.1	44.1	40.6
DOC (mg L^{-1})	0.5	12.3	3.8	2.9
Secchi depth (m)	1	23	6.7	5.5
K_d PAR (m^{-1})	0.16	1.74	0.59	0.50
K_d 320 nm (m^{-1})	1.30	16.53	9.73	10.13
$Z_{1\%}$ PAR (m)	2.6	28.8	7.8	9.2
$Z_{1\%}$ 320 nm (m)	0.3	3.5	0.5	0.5
Chlorophyll-*a* (μg L^{-1})	0.02	0.68	0.27	0.21
Total N (μg L^{-1})	162	758	382	349
Dissolved inorganic N (μg L^{-1})	<1.2	40	9	6
Dissolved organic N (μg L^{-1})	113	710	341	308
Particulate organic N (μg L^{-1})	5	179	47	40
Total P (μg L^{-1})	4	35	14	11
Soluble reactive P (μg L^{-1})	<1	12	6	6
Dissolved organic P (μg L^{-1})	<1	18	6	4
Particulate organic P (μg L^{-1})	<1	8	2	2
TN:TP (M)	27	62	44	44
PON:POP (M)	27	155	67	60

with a hydraulic residence time that exceeded ½ year in over two-thirds of the sampled lakes (Table 1, see Table S1 in Supporting Information). The catchment vegetation was generally dominated by páramo grasslands with small, scattered *Polylepis* bushes.

Water temperature decreased with depth in about half of the lakes, with a thermocline that was situated between 5 and 15 m (Fig. 2). Water temperature near the lake bottom was at most 4.8 °C lower than at the surface, but usually much less. Oxygen concentrations were close to saturation in all lakes (saturation corresponds to 7.01 mg O_2 L^{-1} at a temperature of 10 °C and an elevation of 3800 m) and decreased little with depth (data not shown). Conductivity of the water was low. The lakes had a relatively low Secchi depth and high extinction coefficient of PAR and UV-B, K_d320. Secchi depth and K_d PAR were correlated ($r = 0.79$; $P < 0.001$; $n = 19$). DOC concentrations were overall quite high and were below 1 mg C L^{-1} in only one out of the 31 lakes. The estimated critical depth for phytoplankton photosynthesis is deeper than or equal to the mean depth in all lakes (Fig. 3).

Total nitrogen consisted mostly of DON (on average 84% of TN) with low contributions of DIN (on average 4% of TN) and PON (on average 12% of TN) (Table 1, see Table S2 in Supporting Information). TP consisted of comparable contributions by SRP (on average 40% of TP) and DOP (on average 45% of TP) and a low contribution of POP (on average 15% of TP). The molar PON: POP ratio was generally high.

Chl-*a* concentration was below 1 μg L^{-1} in all lakes (Table S2) and significantly correlated with total phytoplankton biovolume estimated from microscopic cell counts ($r = 0.36$; $P = 0.048$; $n = 30$). A total of 43 phytoplankton genera were encountered, with diversity ranging from 5 to 20 genera per sample (see Table S3 in Supporting Information). In the PCA analysis based on major phytoplankton groups, the first axis (explaining

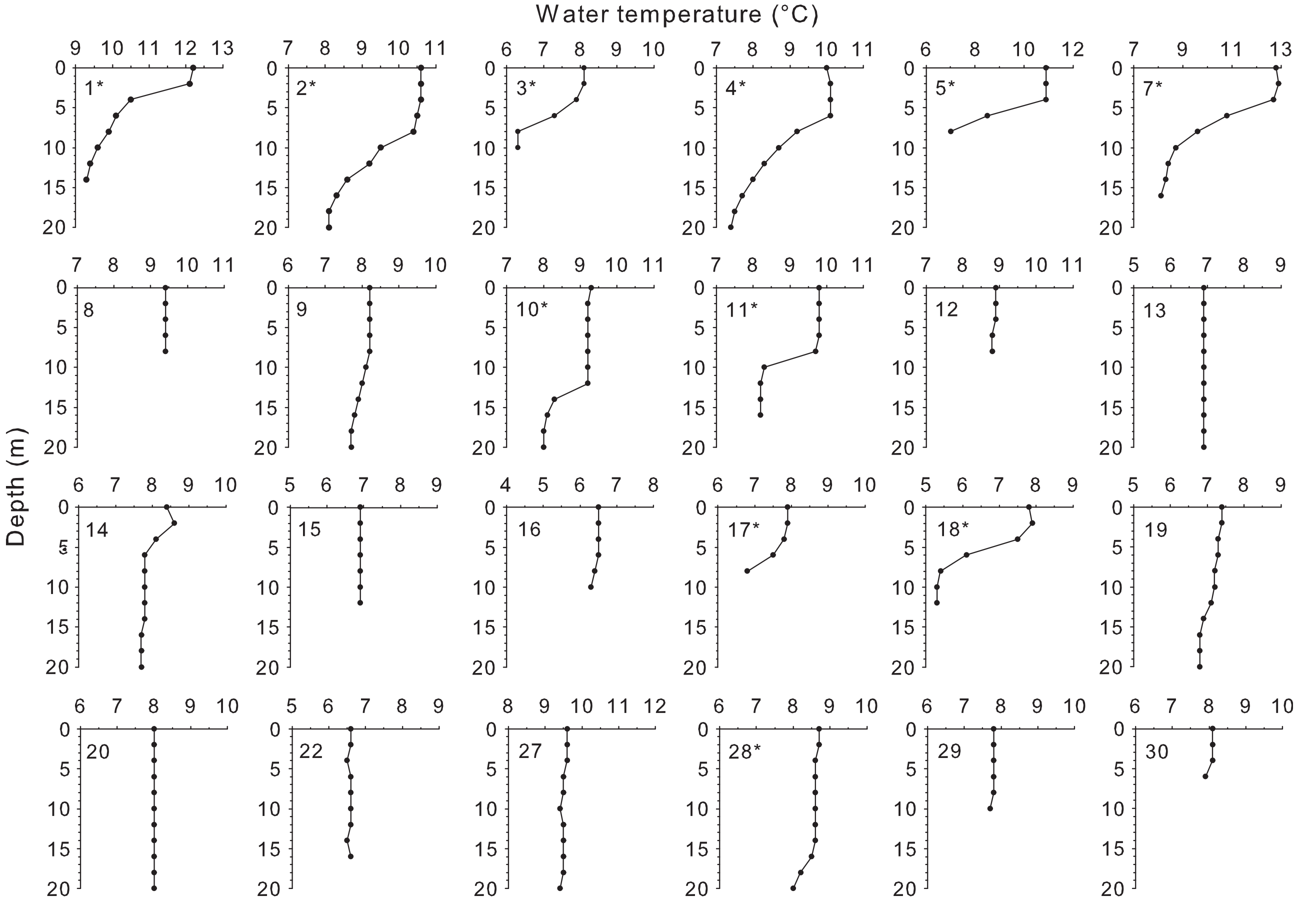

Fig. 2 Vertical temperature profiles (to a maximum depth of 20 m) from all lakes deeper than 2 m. Lake number is given in the top left corner of each graph. Lakes 01–22 were sampled in August–October 2011, lakes 27–30 in August–October 2012. *Presence of thermal stratification (see Table S1 for corresponding lake names).

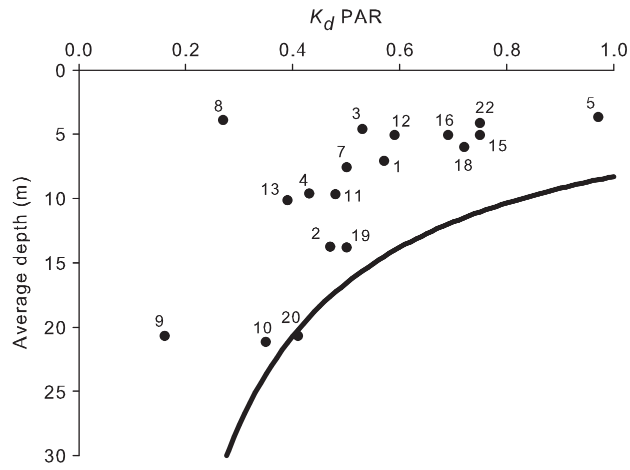

Fig. 3 Plot of each lake (lake numbers are used), based on the their average depth and K_d PAR, upon the theoretical critical depth (Z_{cr}, indicated by the black line). Above the Z_{cr} there is net photosynthesis, below the Z_{cr} there is net respiration and hence light limitation. Average depth for each lake was calculated as 0.46 Z_m (see Table S1 for corresponding lake names).

33% of total variation) separated lakes in terms of biovolume while the second axis (explaining 30% of total variation) separated lakes dominated by diatoms, usually, where the main cladoceran genera encountered were *Bosmina* and *Daphnia* (*D. laevis*, *D. pulex* and *D. longispina*). Among calanoid copepods, the most abundant species were *Boeckella occidentalis* (Centropagidae) and an undescribed diaptomid. Among the cyclopoids, *Microcyclops* spp. (Cyclopidae) and *Metacyclops* spp. (Cyclopidae) were dominant. In the PCA analysis of major zooplankton groups, the first axis (explaining 53% of total variation) separated lakes dominated by *Daphnia*, *Bosmina* and cyclopoid copepods from lakes dominated by calanoid copepods while the second axis (explaining 23% of total variation) distinguished between lakes in terms of zooplankton density (Fig. 4b).

Chlorophyll-*a* only showed a weak positive correlation with TP, and no significant correlation with any other parameter (Table 2). The PCA axes that summarised changes in community composition of phytoplankton (PC2 Phyto) and zooplankton (PC1 Zoo) both showed a significant negative correlation with surface water temperature. PC2 Phyto was also significantly positively correlated with elevation and Secchi depth, while PC1 Zoo was correlated with Chl-*a*.

Seasonal changes in two lakes

Air temperature was *c.* 6 °C year-round but dropped by about 2 °C between June and September (Fig. 5). During the same period, wind speed increased. Precipitation showed no clear seasonal pattern. For most of the year, both lakes showed a vertical gradient in water temperature ranging from 10 to 11 °C at the surface to 8 °C near the bottom, with a thermocline that was situated at 8–12 m depth. Water column stratification disappeared between June and September. Oxygen concentrations were near saturation in the epilimnion and were depleted below the thermocline. This depletion reached a maximum at the end of the stratification period in May. Mean DOC concentration was 6.3 mg L^{-1} (ranging from 3.8 to 9.0 mg L^{-1}) in Toreadora Lake and 5.4 mg L^{-1} (ranging from 4.1 to 9.2 mg L^{-1}) in Riñoncocha Lake and displayed no clear seasonal variation (data not shown). Mean Secchi depth was 7.5 m in

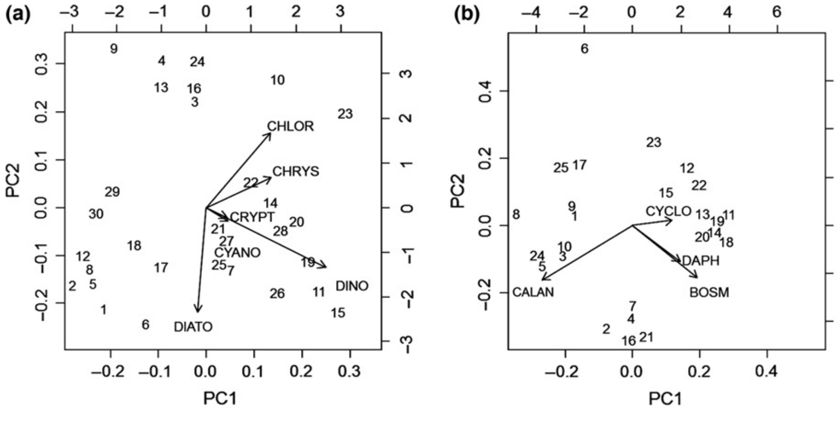

Fig. 4 Ordination diagram based on PCA analysis of (a) phytoplankton and (b) zooplankton community data, showing major phytoplankton/zooplankton groups (vectors) and lakes (numbers). Phytoplankton groups include diatoms (DIATO), chlorophytes (CHLOR), chrysophytes (CHRYS), cyanobacteria (CYANO), cryptophytes (CRYPT) and dinoflagellates (DINO). Zooplankton groups include *Daphnia* sp. (DAPH), *Bosmina* sp. (BOSM), cyclopoid copepods (CYCLO) and calanoid copepods (CALAN) (see Table S1 for corresponding lake names).

Table 2 Correlation coefficients between abiotic parameters and the first (PC1 Zoo) and second (PC2 Phyto) axes of PCA analysis of zooplankton and phytoplankton community data, respectively, as well as with chlorophyll-*a* (Chl-*a*) concentrations. DOP, dissolved organic phosphorus; TN, total nitrogen; TP, total phosphorus; PON, particulate organic nitrogen; POP, particulate organic phosphorus; DON, dissolved organic nitrogen; DOP, dissolved organic phosphorus; SRP, soluble reactive phosphorus; DIN, dissolved inorganic nitrogen.

	Chl-*a*	PC2 Phyto	PC1 Zoo
Area	0.18	0.07	0.18
Depth	0.09	0.34	0.06
Elevation	−0.04	0.41*	−0.05
Water temperature	−0.35	−0.49*	−0.46*
Secchi depth	−0.25	0.38*	−0.26
TN	0.13	0.14	0.19
TP	0.39*	0.39*	0.23
DIN	−0.29	−0.17	−0.30
SRP	0.16	0.26	0.03
DON	0.01	0.14	0.09
DOP	−0.04	0.19	0.22
PON	0.34	0.27	−0.02
POP	0.33	0.29	−0.18
Chl-*a*	–	0.05	0.51*

*Significant correlation ($P < 0.05$).

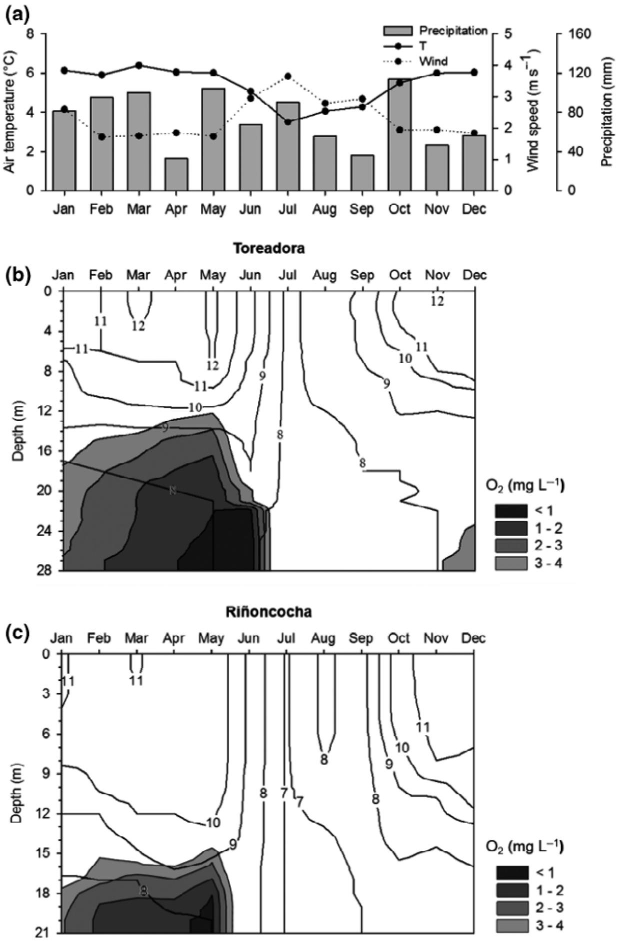

Fig. 5 Results of monthly monitoring of Lake Toreadora and Lake Riñoncocha (January–December 2013). Figure shows variation in average wind speed, air temperature and precipitation throughout the year measured at a weather station near Lake Toreadora (a), and shows a contour plot of seasonal changes in vertical temperature and oxygen profiles in Lake Toreadora (b) and Lake Riñoncocha (c).

Toreadora (ranging from 6.0 to 9.0 m) and 6.2 m in Riñoncocha (ranging from 5.0 to 7.5 m) showing no clear seasonal changes in either of the two lakes (data not shown).

Total nutrient concentrations in the epilimnion were on average 173 μg L^{-1} TN and 5.6 μg L^{-1} TP in Toreadora and 237 μg L^{-1} TN and 8.0 μg L^{-1} TP in Riñoncocha Lake (Fig. 6). Average DIN and SRP concentrations were substantially lower: 5.2 μg L^{-1} DIN and 3.1 μg L^{-1} SRP in Toreadora and 7.6 μg L^{-1} DIN and 4.1 μg L^{-1} SRP in Riñoncocha Lake. Again, no clear seasonal variation in nutrient concentrations was apparent. Differences between epi- and hypolimnion were marginal and not significant, except for DIN concentrations in Toreadora being significantly higher in the hypolimnion compared with the epilimnion (paired *t*-test, $P = 0.001$, $n = 12$).

Chl-*a* concentration was on average 0.35 μg L^{-1} in Toreadora and 0.25 μg L^{-1} in Riñoncocha Lake. In both lakes, Chl-*a* concentrations were slightly lower during the period of the thermocline turnover. Phytoplankton community composition in both lakes did not change substantially throughout the year (Fig. 7). The phytoplankton community in Toreadora Lake was dominated by chlorophytes (mainly *Oocystis* spp.) and small diatoms (mainly *Cyclotella* spp.), whereas small colony-forming cyanobacteria (mainly *Merismopedia* spp. and *Aphanocapsa* spp. [Merismopediaceae]) were dominant in Riñoncocha Lake.

Nutrient limitation experiments

In the three lakes where nutrient limitation experiments were carried out, no significant growth occurred in the control treatments that received no nutrient additions (Fig. 8). In Patoquinuas and EC Izhcayrrumi, Chl-*a* concentrations were significantly higher in the P and NP treatments compared with the control and N treatment, but no difference was found between the P and NP treatments, indicating P limitation. In Toreadora, Chl-*a* concentration in the P treatment was

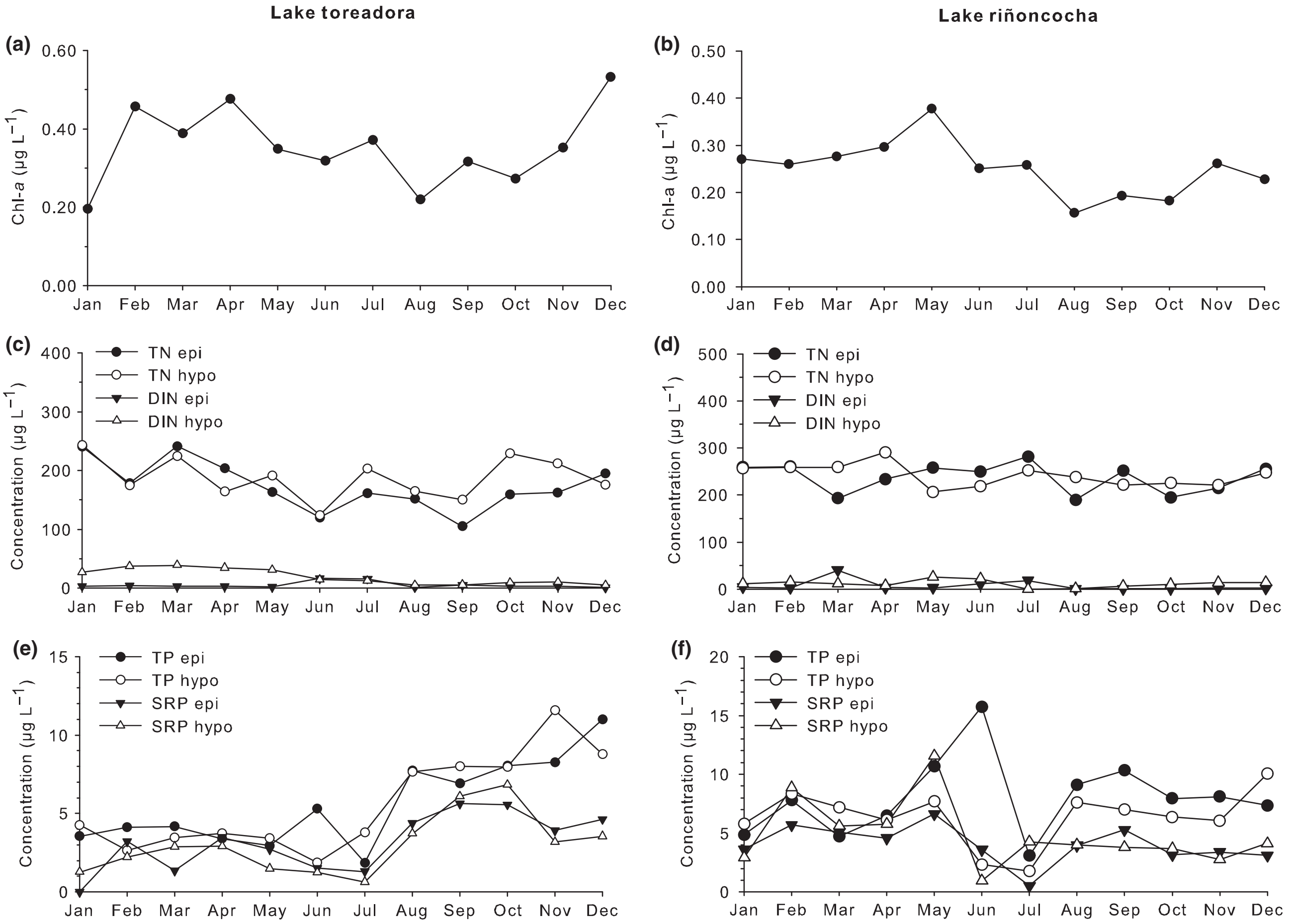

Fig. 6 Seasonal changes in surface chlorophyll-*a* concentrations (a, b), nitrogen concentrations (c, d) and phosphorus concentrations (e, f) for Lake Toreadora (left) and Lake Riñoncocha (right) (January–December 2013). For nutrient concentrations a differentiation was made between the total (TN, TP) and dissolved (DIN, SRP) fraction, as well as between concentrations in the epilimnion (epi) and hypolimnion (hypo).

significantly higher than the control and the NP treatment was also significantly higher than the P treatment, indicating serial P and N co-limitation. Analysis of the accessory pigments fucoxanthin, zeaxanthin and Chl-*b* suggested that different phytoplankton groups responded differently to nutrient additions. Zeaxanthin (an indicator pigment for cyanobacteria) never increased in response to nutrient additions but Chl-*b* (indicator for chlorophytes) and fucoxanthin (indicator for diatoms) responded in the same way to nutrient additions as Chl-*a*.

Discussion

In tropical lakes, thermal stratification of the water column is typically weak as a result of limited seasonal variation in temperature (Lewis, 2010). In tropical mountain lakes, stratification is weakened further because differences in water density are small at low water temperature. Nevertheless, the two lakes that were monitored monthly over 1 year displayed a thermal stratification of the water column, with a temperature difference of up to 4 °C between epi- and hypolimnion. Stratification occurred between October and June and the thermocline was situated between 8 and 12 m (Fig. 5). The fact that oxygen concentrations declined in the hypolimnion throughout the stratification period suggests that this thermal stratification was stable, although partial mixing of epi- and hypolimnetic waters may occur during the stratification period. Destratification occurred during the southern hemisphere winter in July to September, when wind speed was higher and air temperature lower. Almost half of the lakes that were sampled once during the survey lacked stratification lacked stratification (Fig. 2). This may be due to the fact the survey was carried out during the winter period,

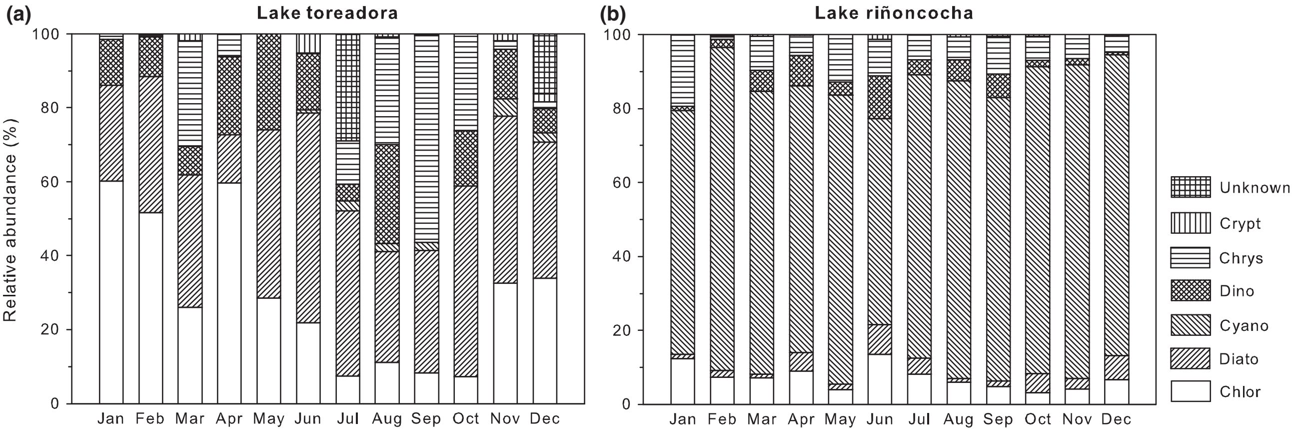

Fig. 7 Changes in phytoplankton community composition throughout the year (January–December 2013) for (a) Toreadora and (b) Riñoncocha. The relative abundance of the major phytoplankton groups is given for each month: chlorophytes (Chlor), diatoms (Diato), cyanobacteria (Cyano), dinophytes (Dino), chrysophytes (Chrys) and cryptophytes (Crypt). Unidentified algae species are indicated by 'unknown'.

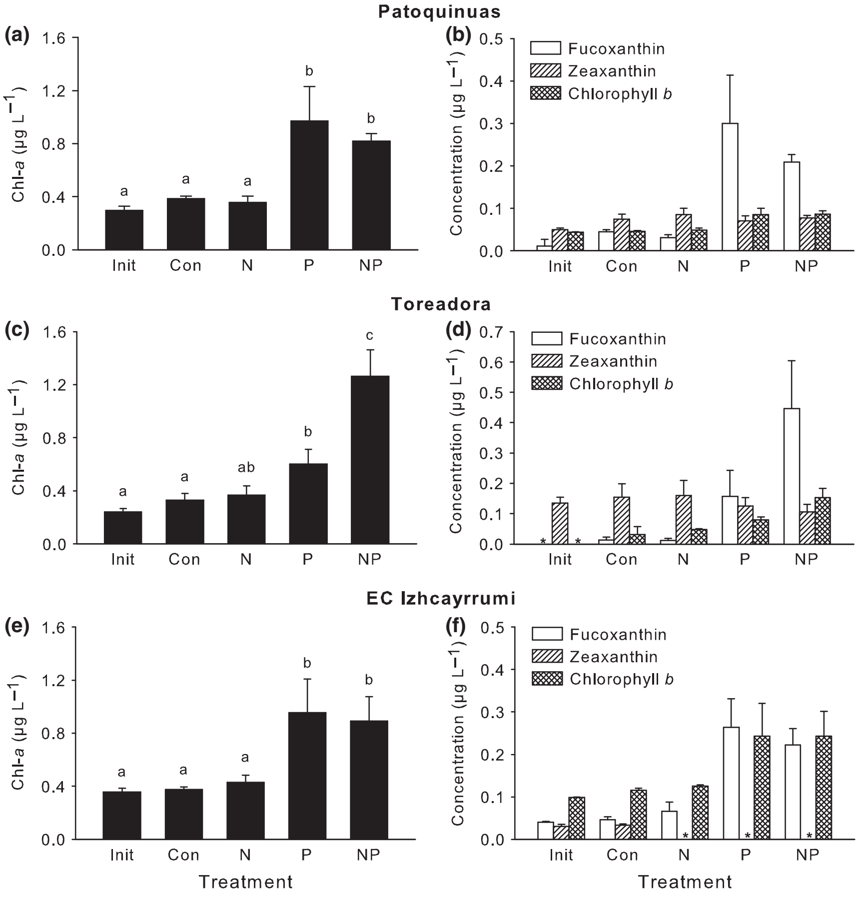

Fig. 8 Results of nutrient limitation experiments performed in Patoquinas (a, b), Toreadora (c, d) and EC Izhcayrrumi (e, f). For each experiment the graph on the left shows the chlorophyll-*a* response to the different treatment, the graph on the right shows the response of the three marker pigments fucoxanthin, zeaxanthin and chlorophyll-*b*. Init: initial, before incubation; Con: control, no nutrients added; N: nitrogen added; P: phosphorus added; NP: nitrogen and phosphorus added. Letters above the bars indicate significant differences ($P \leq 0.05$) between treatments. *Absence of the pigment in the analysed sample.

when destratification was observed in the two lakes that were monitored monthly. When present, the thermocline was situated between 5 and 15 m.

The water transparency in the lakes was relatively low, with a median K_d PAR of 0.50 m^{-1}. This value for K_d PAR is higher than in European (0.27; Laurion *et al.*, 2000) or North American mountain lakes (0.33; Rose *et al.*, 2009) situated above the tree line. The low water transparency can be ascribed to relatively high DOC concentrations in the lakes. The median DOC concentration was 2.9 mg L^{-1} (mean: 3.8 mg L^{-1}), whereas DOC concentrations in mountain lakes situated above the tree line in Europe or North America are generally <1–2 mg C L^{-1} (Table 3). In temperate mountain lakes DOC concentration is related to the vegetation cover and soil development in the catchment, and is higher in lakes with a forested catchment than lakes with a catchment of grassland and bare rock (Sommaruga, 2001). Despite the fact that the lakes are situated above the tree line, they are surrounded by a well-developed vegetation of páramo grassland that is productive year-round. Moreover, organic matter produced by this vegetation accumulates in the soils of the páramo because low temperature, high humidity and a high metal content limit mineralisation of organic matter in the andosol soils (Buytaert *et al.*, 2006a). The high DOC concentrations in the lakes can therefore be ascribed to inputs of organic matter from the catchment into the lake. The lakes also had a low transparency for UV radiation, with UV-B radiation being absorbed in the top 0.5 m of the water column. Therefore, UV is probably not an important stressor in these lakes, or at least not as important as in many temperate mountain lakes or in the large Andean lake Titicaca (Villafañe *et al.*, 1999; Sommaruga, 2001). A recent study on lakes situated above the tree line in Bolivia also noted relatively high DOC concentrations and a low UV transparency (Aguilera, Lazzaro & Coronel, 2013).

Table 3 Review of studies performed on high-elevation lakes worldwide. Parameters given here are mean values for the total nitrogen (TN) and phosphorus (TP) concentrations, the chlorophyll-*a* (Chl-*a*) concentration as a measurement of the phytoplankton biomass and dissolved organic carbon (DOC). Data from this survey are added as well. When a range is given, the mean value (when available) is provided in parentheses. –, no data.

Mountain range	Number of lakes studied	TN (μg L^{-1})	TP (μg L^{-1})	Chl-*a* (μg L^{-1})	DOC (mg L^{-1})	Reference
Europe	235	381	0.7–78 (5.6)	0.0–29 (2.2)	0.003–9 (0.9)	Catalan *et al.* (2009)
Alps	57	–	2.9	0.2–10.6	0.24–2.48 (0.7)	Sommaruga *et al.* (1999)
	40	105–397*	1.0–6.9	–	0.4–2.1	Schmidt *et al.* (2004)
	2	264–830	1–8	0.1–14.5	–	Tait & Thaler (2000)
Pyrenees	82	44–968	0.94–19.3	0–19.07	0.01–3.7	Buchaca & Catalan (2008)
Sierra Nevada	31	107–930	4–31	0.2–4.1	–	Morales-Baquero *et al.* (1999)
	4	–	–	–	0.44–0.83	Reche *et al.* (2001)
Tatra Mountains	84	212–897 (556)	0.7–51.6 (6.2)	0.12–34.2 (2.2)	–	Kopáček *et al.* (2000)
	91	–	–	–	0.40	Kopáček, Stuchlik & Hardekopf (2006)
North America						
Rocky Mountains	41	4–350 (161)	1.6–8.7 (4.8)	0.3–14.3 (4.3)	–	Elser *et al.* (2009b)
	8	163–344	9.9–19.1	0.7–5.6	–	Morris & Lewis (1988)
	5	–	–	–	0.4–1.5	Hood, Williams & Caine (2003)
	7	–	–	–	1.5–2.0	Goodman, Baker & Wurtsbaugh (2011)
	6	130–470 (224)	6.7–29.3 (13.4)	1.0–7.0 (3.1)	–	Hundey *et al.* (2014)
Central America						
Mexican Altiplano	1	316	8.7	3.8	–	Chacon-Torres & Rosas-Monge (1998)
South America						
Andes – Argentina	18	50–400 (172.2)	2–31 (9.1)	–	–	Rogora *et al.* (2008)
	35	702	7	2.2	–	Quirós & Drago (1999)
Andes – Bolivia	26	–	<2.44	1.1–28.6	1.0–5.4	Aguilera *et al.* (2013)
Andes – Ecuador	31	162–758 (382)	4–35 (14)	0.02–0.68 (0.27)	0.5–12.3 (3.8)	This study
Asia						
Himalaya	17	162–172	5.5–9.1	1.3–3.0	–	Lacoul & Freedman (2005)
	31	118–751 (296.3)	2–52 (6.4)	–	–	Tartari, Tartari & Mosello (1998)
	1	–	–	–	2.0	Mitamura *et al.* (2003)
Africa						
Rwenzori	17	155–696	3–68	0.3–10.9	3.0–13.6	Eggermont *et al.* (2007)
Australia						
New Zealand Highlands	21	100–390	6–14	0.5–4.0	–	Spencer (1978)

*Measured as dissolved nitrogen.

A deep thermocline combined with a low water transparency results in a low underwater light availability to phytoplankton and may cause light limitation (e.g. Loch Ness; Jones, Young & Hartley, 1996). The low underwater light availability is not compensated by a high surface irradiance. Global irradiance in Cajas NP measured 60 W m^{-2} PAR or 23 Einst m^{-2} day^{-1}, which is comparable to the annual average PAR irradiance at temperate latitudes of 50–60° (Lewis, 2011). The low irradiance is due to a nearly constant cloud cover at this elevation in the equatorial Andes (Emck & Richter, 2008). The Z_{cr} was used to assess whether underwater light levels were sufficiently high to allow for positive net photosynthesis of phytoplankton. The estimated critical depth exceeded the average depth in all lakes, indicating that positive net photosynthesis rates are possible, even when the lakes are fully mixed (Fig. 3). *In situ* measurements of photosynthesis, however, are needed to confirm this.

If phytoplankton productivity is not limited by light it might be limited by nutrients. In phytoplankton, the intracellular nutrient ratio is generally a good indicator of the limiting nutrient and this can be estimated from the N:P ratio in the particulate matter (Healy & Hendzel, 1980). The N:P ratio in the particulate matter was high (median 60, minimum 27) and points to P as the limiting nutrient in most lakes. The positive relation between TP and Chl-*a* also points to P as the limiting nutrient. P limitation was also observed in the nutrient limitation assays carried out in three of the lakes (Fig. 8). In the experiment in Toreadora Lake, a higher response was observed to N + P addition than to P addition alone, indicating serial co-limitation of P and N (as defined by Harpole *et al.*, 2011). This serial co-limitation suggests that small inputs of P may cause a shift from P to N limitation, at least in some of the lakes.

Soluble reactive P concentrations were often several μg L^{-1} above the detection limit. Possibly, the SRP measured here did not consist of free ortho-phosphate but of DOP that was detected as SRP by the molybdenum blue method (Jansson *et al.*, 2012). The volcanic andosol soils that occur in the catchment of the lakes are rich in humic-metal-P complexes (Gerke, 2010) that may end up in the lakes. Despite the fact that P from these humic-metal-P complexes has a low bioavailability to phytoplankton (Li & Brett, 2013), the low pH during the molybdenum blue analysis causes dissociation of P from these humic-metal-P complexes and detection of this DOP as SRP (Rigler, 1968; Koenings & Hooper, 1976).

It is not clear whether P limitation is the pristine condition in these lakes or whether it is the result of anthropogenic impacts on these lakes. If P limitation in the lakes is the pristine condition, this may result from a combination of low P inputs from the catchment caused by high P retention by the páramo soils (e.g. Yang & Post, 2011; Hashimoto *et al.*, 2012) and relatively high N inputs from N_2 fixation by the páramo vegetation [e.g. by *Lupinus* spp. (Fabaceae) that are common in the páramo vegetation, Martineau & Saugier, 2007]. In temperate mountain lakes, N limitation is often the pristine condition but many lakes have shifted to P limitation as a result of atmospheric N deposition originating from industrial activities and traffic (e.g. Bergström & Jansson, 2006; Elser *et al.*, 2009a). Atmospheric N deposition in the Andes mountains is caused by forest fires in the Amazon and rates measured in montane forests just below the elevation of the lakes in this study are about 4 kg N ha^{-1} $year^{-1}$ (Fabian, Kohlpaintner & Rollenbeck, 2005; Boy *et al.*, 2008). Although this is lower than in the Alps in Europe (up to 25 kg ha^{-1} $year^{-1}$; Rogora *et al.*, 2006, 2008) or the Colorado Mountains in North America (up to 8 kg ha^{-1} $year^{-1}$; Baron *et al.*, 2000; Burns, 2004; Elser *et al.*, 2009b), these rates are well above N deposition rates measured in pristine ecosystems in the southern Patagonian Andes when lakes are N limited (<1 kg N ha^{-1} $year^{-1}$; Pedrozo *et al.*, 1993). The lakes may also have been influenced by nutrient inputs from the catchment caused by cattle grazing and associated burning of the páramo vegetation, although it is not clear how this would influence the N:P stoichiometry of the lakes.

In stratified lakes, nutrient limitation can be enhanced by losses of nutrients from the epilimnion to the hypolimnion. In both lakes, P concentrations were comparable between epi- and hypolimnio, apart from a slightly higher concentration of DIN in the hypolimnion compared to the epilimnion in Lake Toreadora. Although we did not study a detailed vertical profile of Chl-*a*, it is unlikely that the lakes have a deep chlorophyll maximum due to the limited difference in nutrient concentrations between epi- and hypolimnion. Water column stability is low at low water temperatures (Wetzel, 2001) and sinking of cold surface water during the night may cause erosion of the thermocline. This would equal out differences in nutrient concentrations between the epi- and hypolimnion. Turnover of the thermocline between July and September did not cause a pronounced increase in nutrients in the epilimnion and a concomitant increase in Chl-*a* (Fig. 6). On the contrary, a small decrease in Chl-*a* concentration was observed during thermocline turnover in both lakes. This was probably as a result of reduced underwater light availability to phytoplankton caused by an increase in mixing depth. This suggests that phytoplankton productivity is not controlled by sequestration of nutrients in the hypolimnion.

The lakes studied here can be considered as extremely oligotrophic (Chl-*a* < 1 μg L^{-1}). Chl-*a* concentrations were near the lower range of concentrations found in lakes of other mountain ranges around the world (Table 3). Total nutrient concentrations, on the other hand, were comparable to other mountain lakes. PON and POP represented only a small fraction of TN and TP, which indicates that phytoplankton uses only a small fraction of the total nutrients. This may be because a substantial part of the total nutrients in the lakes consists of dissolved organic nutrients that may have a low bioavailability. TN was indeed mostly composed of DON. TP was composed of about equal amounts of DOP and SRP. However, as argued above, part of this SRP may consist of humic-metal-P complexes with low bioavailability. Dissolved organic nutrients often represent a large proportion of the total nutrient pool in pristine lakes (Berggren *et al.*, 2015). In the lakes studied here, however, the total concentration of dissolved organic nutrients was higher than in other pristine lakes around the world (Meybeck, 1982; Hedin, Armesto & Johnson, 1995; Kaushal & Lewis, 2003; Rogora *et al.*, 2008). The high DON and DOP concentrations can be ascribed to the high DOC concentration.

Microscopic analyses showed that the phytoplankton community in the lakes was dominated by chlorophytes, small diatoms or, as in Lake Riñoncocha, by small colonial cyanobacteria (Fig. 4a). HPLC analysis of marker pigments confirmed that chlorophytes (marker: Chl-*b*), diatoms (marker: fucoxanthin) and cyanobacteria (marker: zeaxanthin) were important in the lakes. High concentrations of zeaxanthin were often detected in lakes where no cyanobacteria were observed during microscopic analyses. Probably, this zeaxanthin was derived from picocyanobacteria such as *Synechococcus* spp. that were too small to be detected by inverted microscopy. The phytoplankton taxa that were observed belonged to the classes A (the small diatom *Cyclotella* spp.), F (the colonial chlorophyte *Oocystis*) and Z (prokaryotic picoplankton) in the functional classification of Reynolds *et al.* (2002). These classes are characteristic of deep, clear and nutrient-poor lakes. Small colonial cyanobacteria such as *Merismopedia* and *Aphanocapsa* belong to classes K and L_0 that are typical of more eutrophic waters. Nevertheless, other studies have also reported small colonial cyanobacteria from oligotrophic tropical mountain lakes, e.g. lake Mucubajii in Venezuela (Lewis & Weibezahn, 1976).

The zooplankton community was dominated by either calanoid copepods or by *Daphnia*, *Bosmina* and cyclopoid copepods (Fig. 4b). The calanoid copepod *B. occidentalis* that was common in many lakes is a genus that is typical for cold waters in South America (Menu-Marque, Morrone & de Mitrovich, 2000). It is unlikely that this zooplankton controlled phytoplankton by grazing because in the control treatments of the nutrient limitation experiments, where zooplankton was removed by screening, we did not observe an increase in Chl-*a* concentration upon removal of the zooplankton.

In conclusion, the glacial lakes of Cajas NP are cold water lakes with a weak but stable thermal stratification. Inputs of DOC from the vegetated catchment result in a lower water transparency than in temperate mountain lakes situated above the tree line, as well as a low underwater UV radiation. Total nutrient concentrations are generally higher than in other mountain lakes while Chl-*a* concentrations are lower, probably because the water contains a high concentration of dissolved organic nutrients with a low bioavailability. External inputs of bioavailable nutrients or an increase in the mineralisation of dissolved organic nutrients may result in a rapid increase in phytoplankton productivity, because Chl-*a* concentrations are very low and phytoplankton is limited by nutrients (mainly P).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Measurements of limnological parameters per lake.

Table S2. Detailed nutrient measurements per lake.

Table S3. Phytoplankton species composition per lake.

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