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Cyanobacterial bloom termination: the disappearance of *Planktothrix rubescens* from Lake Bourget (France) after restoration

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SUMMARY

1. Like many large freshwater ecosystems in Europe, Lake Bourget suffered from eutrophication during the second part of the 20th century and since the 1980s has been partially restored by reductions in nutrient loadings.

2. Here, we analyse a data set comprised of field measurements of physicochemical and biological variables in Lake Bourget covering the period from 2004 to 2011 and complement this data set with laboratory experiments, to gain an understanding of the changes in phytoplankton community structure during recent years and drivers of these changes.

3. Between 1995 and 2008, Lake Bourget was characterised by the proliferation of the red-coloured filamentous and toxic cyanobacterium *Planktothrix rubescens*, comprising 34.1–52.6% of the total phytoplanktonic biomass between 2004 and 2008.

4. In 2009, although the contribution of *P. rubescens* to the total biomass was still considerable (25.3%), it was significantly lower ($P < 0.05$) compared with previous years. The cyanobacterium disappeared completely during the autumn to winter transition of 2009/2010 and has not been recorded since this time.

5. Concomitantly, total phytoplanktonic biomass declined sharply and a new phytoplanktonic community occurred consisting predominantly of mixotrophic genera, such as *Dinobryon* spp., *Rhodomonas*, *Cryptomonas* and a variety of different diatoms such as *Stephanodiscus*, *Cyclotella* and *Fragilaria*.

6. Our findings suggest declines in phosphorus concentration as a key variable in bloom termination, although a number of other factors could also be important, such as temperature-dependent water column mixing, light availability, zooplankton grazing and seasonal cyanobacterial inoculums.

Keywords: bloom termination, cyanobacteria, Lake, mid-term series, *Planktothrix rubescens*

Introduction

During the 20th century, cyanobacterial blooms have been recorded in many freshwater lakes, reservoirs and rivers worldwide as a result of eutrophication (Chorus & Bartram, 1999; Huisman, Matthijs & Visser, 2005). Because of potential toxicity and deleterious effects of blooms on ecosystem functioning and use (typically for recreational and fishing activities), this phytoplanktonic group has been the focus of a large number of studies to better understand what triggers population proliferation, toxin production and transfer through the food webs (Gallina, Anneville & Beniston, 2011; Paerl, Hall & Calandrino, 2011; O'Neil *et al.*, 2012; Posch *et al.*, 2012; Sotton *et al.*, 2014). During recent years, there has been increasing concern that cyanobacterial blooms will be promoted by global warming (Jöhnk *et al.*, 2008; Pearl, Hall & Calandrino, 2011; Trolle *et al.*, 2011; Elliott, 2012; O'Neil *et al.*, 2012; Paerl & Otten, 2013).

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The development of *Planktothrix rubescens* (reported as being meso- to eutrophic) has been related mainly to its unique physiological ability to develop and bloom at intermediate depths (typically between 10 and 20 m, i.e. above or in the upper part of the thermocline) in deep stratified lakes (Dokulil & Teubner, 2012). *P. rubescens* blooms typically form thin, dense layers in the metalimnion during late summer, where this species is able to use all available wavelengths to grow (Reynolds, 1997; Walsby *et al*., 2001; Oberhaus *et al*., 2007a), even at very low light levels (generally <1% incident PAR) (Walsby & Schanz, 2002; Jacquet *et al*., 2005; Naselli-Flores *et al*., 2007; Oberhaus *et al*., 2007a; Dokulil & Teubner, 2012). Other features that have been shown to explain the dominance of *P. rubescens* are excretion of alkaline phosphatases, in order to utilise organic forms of phosphorus when inorganic phosphate concentrations are low (Feuillade, 1994), heterotrophic nutrition (especially amino acids uptake), which confers advantages in relatively nutrient-limited conditions (Feuillade *et al*., 1988; Zotina, Koster & Jüttner, 2003; Walsby & Jüttner, 2006; Salcher, Posch & Pernthaler, 2013), allelopathy (Oberhaus, Briand & Humbert, 2008) and resistance to grazing, due to its morphology and/or toxin production (Dokulil & Teubner, 2000; Blom, Robinson & Jüttner, 2001), although some studies have also reported that grazing may impact the cyanobacterium (Kurmayer & Jüttner, 1999; Oberhaus *et al*., 2007b; Sotton *et al*., 2014). No viruses have been isolated, but this cyanobacterium has been shown to be parasitised by bacteria (Feuillade, Feuillade & Blanc, 1990) and chytrid fungi (Sontsebo & Rohrlack, 2011; Rohrlack, Christiansen & Kurmayer, 2013).

In Lake Bourget, *P. rubescens* has been the focus of several studies during the last 10 years. Vinçon-Leite, Tassin & Druart (2002) reported that *P. rubescens* contributed up to 22% of total biomass in 1996, while Jacquet *et al.* (2005) found that declining phosphorus levels combined with milder winters and favourable spring and summer meteorological conditions (i.e. a strong water column stratification) coincided with the emergence and dominance of *P. rubescens*. More recently, Savichtcheva *et al.* (2011), using a paleolimnological approach, showed that *P. rubescens* in Lake Bourget dates back to the period 1956–61, and corroborated that blooms during 1995–2009 were probably due to intermediate phosphorus concentrations (i.e. 20–50 μg P L^{-1}). Experimental studies of *P. rubescens* isolated from Lake Bourget have shown that the alga is (i) well adapted to low temperature and low intensities of green light, (ii) has strong photo-inhibition under high irradiance, (iii) grows under a wide range of light intensities and temperatures and (iv) displays allelopathic activity (Oberhaus *et al*., 2007a, 2008). Recently, Cuypers *et al.* (2011) showed that internal waves could be responsible for the variable distribution of the *P. rubescens* (the depth of the biomass peak varying up to 10 m at a given point within a single day, or between different stations in the lake at the same time). Perga *et al.* (2013) demonstrated that *P. rubescens* blooms could support a large amount of pelagic secondary production (through small zooplankton and predatory cladocerans, up to young-of-the-year perch). Moreover, in a laboratory study, Oberhaus *et al.* (2007b) showed that *Daphnia* are able to feed on *P. rubescens*, especially when characterised by small-size filaments, constituting a potential vector for the transfer of toxins into the food chain (Sotton *et al*., 2012a, 2014).

In Lake Bourget, we studied a time period during which *P. rubescens* was dominant for a few years, before rapidly disappearing (at end of 2009). The main aim of this study was to investigate what factors might have resulted in this decline. Our study was conducted over an 8-year period (2004 and 2011), with special focus on two consecutive years (2008 and 2009) to gain a better insight into factors that may have led the disappearance of *P. rubescens*. Specifically, our aims were to examine (i) whether phosphorus was the key factor explaining the disappearance of *P. rubescens*, the overall reduction in phytoplanktonic biomass and the establishment of a new phytoplankton assemblage since 2010, and (ii) whether other factors (e.g. direct and/or indirect effects of temperature, light or zooplanktonic pressure) were also important in explaining changes in the phytoplanktonic community.

Methods

Site and sampling

Lake Bourget (45°44′N, 05°51′W, 231 m a.s.l.), situated on the western edge of the Alps, is the largest natural lake in France. It is an elongated and north–south-orientated lake (length, 18 km; maximum width, 3.5 km; area, 44×10^6 m^2; volume, 3.5×10^9 m^3; maximum depth, 145 m; mean depth, 80 m; residence time, *c.* 10 years) and is classified as warm and monomictic. The catchment is about 560 km^2, with maximum and average altitudes of 1845 and 700 m a.s.l., respectively. More details are available in Jacquet *et al.* (2005). Samples of water were obtained from the reference station, located in the middle and deepest part of the northern basin. This station is more than 1.5 km from each bank, and more than

5 and 10 km from the Sierroz and Leysse rivers (the two main tributaries of the lake), respectively.

Available data

Data were obtained approximately every 2 or 3 weeks at the deepest point of the lake between 2004 and 2011. A conductivity–temperature–depth metre (CTD SBE 19 Seacat profiler, Seabird, SBE, Bellevue, WA, U.S.A.) was used to obtain vertical profiles (from the surface to the bottom) of the water temperature. Concentrations of total phosphorus (TP), P-PO_4, total nitrogen (TN), N-NO_3, N-NH_4 and Si-SiO_2 were measured at various depths (i.e. 2, 10, 15, 20, 30, 50, 80, 110, 130 and 140 m). Water transparency was measured using a Secchi disc (always measured by the same person). Underwater light intensity was measured on a few occasions using a LI-1400 current metre, and a data logger combined with an LI-193SA spherical quantum sensor (LI-COR, Lincoln, NE, U.S.A.). Meteorological data were obtained from Meteo France at a reference station located in the southern part of the lake.

For phytoplankton analysis, water samples were taken from the 0- to 20-m layer using an integrated sampler developed by Pelletier & Orand (1978). After collection, the water samples were immediately fixed with Lugol's solution. Twenty-five millilitres of each sample was decanted into an Utermöhl counting chamber (Utermöhl, 1958) and allowed to settle for at least 12 h away from light and heat. Qualitative and quantitative analyses of the phytoplankton were carried out with an inverted microscope (Zeiss, Axiovert). Abundances were converted into biomass (expressed in μg L^{-1}) using the biovolumes of each species (Druart & Rimet, 2008). Cell dimensions varied between 5 and 6 μm on average, and biovolumes were obtained from filament length using the formula PI*Radius^2*Length of the filament. Such biovolumes were then used to estimate biomass considering that 1 algal dm^3 corresponds to 1 kg (i.e. the volumetric mass of water). *P. rubescens* concentrations were also measured with a bbe Fluoroprobe after calibration (e.g. Leboulanger *et al.*, 2002; Jacquet *et al.*, 2005; Fig. S1), with cell counts made at six different depths at 2, 10, 15, 20, 30 and 50 m (as described above). Microcystin extraction and analysis were carried out as reported in Briand *et al.* (2005).

Zooplankton were collected using a net with a mesh size of 212 μm from a depth of 50 m up to the surface. The samples were fixed with formalin (37% formaldehyde) to produce a final concentration of 5% (Wetzel & Likens, 2000). Counts reported here were done using a standard microscope (Olympus BX40), with a gridded counting slide. Abundances are given as the number of individuals per m^2.

Indices and data analysis

The euphotic zone was determined as 2.5× Secchi depth (Wetzel, 2001), after verification of 55 profiles using a spherical quantum sensor (LI-1400 current meter, wavelength range 400–800 nm) between 2002 and 2005. The mixed layer depth (MLD) was determined as the deepest point at which the temperature difference from the surface was less than 1 °C. The Schmidt stability index (S) (Schmidt, 1928), reflecting intensity of stratification, was calculated with respect to the temperature dependence of density. We defined the ratio between the MLD and the euphotic zone as a proxy to infer light limitation: a ratio >1 suggests that cells are entrained by mixing below the euphotic zone, while a ratio <1 suggests light is not limiting cell growth. To prevent bias from frequently sampled layers/periods, spatial and temporal averages were calculated after linearly interpolating the data to fixed grids. Phytoplanktonic species measuring less than 20 μm in length and with a biovolume of less than 10 000 μm^3 were assigned to the nanoplanktonic class. Those over 20 μm in length and/or with a biovolume of more than 10 000 μm^3 were classified as microphytoplankton. Phytoplankton was assigned to functional groups according to Reynolds *et al.* (2002). Principal component analysis (PCA) was performed with XLStat™ on log-transformed data (log[X] + 1) in order to explore the relationships between the environmental and biological variables and to identify inter-annual patterns.

Laboratory experiment

To test the hypothesis that filament size could have been reduced as a result of phosphorus limitation, favouring zooplankton grazing, an experiment was conducted using *P. rubescens* TCC 29-1 culture strain (isolated from Lake Bourget and used in previous experimental studies, e.g. Oberhaus *et al.*, 2007a,b, 2008). The culture was first separated into four different nutrient conditions (i.e. 0, 80, 400 and 800 μg P L^{-1} based on K_2HPO_4 from initial diluted Z culture medium; P from reduction of K_2HPO_4 was not accounted for here). These cultures were incubated in 300-mL culture flasks placed under controlled light and temperature conditions (i.e. 15 °C and 20 μmol quanta m^{-2} s^{-1}, which are typical light levels measured between 15 and 20 m in Lake Bourget

during the productive season) and shaken gently every day. After 2 weeks, a 10% inoculum of each culture was incubated in triplicates in the same P conditions as described above and in 100-mL glass flasks. After 4 days (corresponding t34), sub-samples were taken to measure filament sizes. Differences between treatments were tested using a Kruskal–Wallis one-way analysis of variance on ranks followed by an all pairwise multiple comparison procedures (Dunn's method) to isolate groups that differ.

Results

The physicochemical environment

The physicochemical environment was typical of deep peri-alpine lakes located in the temperate zone, with pronounced seasonal patterns governed by the thermal stratification dynamics (Fig. 1). At the inter-annual scale, we observed two trends. (i) A decreasing trend in TP; winter concentrations of TP and P-PO_4 decreased between 2004 and 2011 from 33 to 14 μg P L^{-1} and from 29 to 8 μg P L^{-1}, respectively. This reduction in phosphorus concentration was not paralleled by total nitrogen concentrations, which remained relatively high and fluctuated during the same period between 627 and 720 μg N L^{-1}; (ii) Incomplete mixing of the water column during the winter of 2006–07 and a relatively short period of complete mixing during the winter of 2007–08. For the incomplete mixing in 2006–07, depth of the isothermal layer did not reach the bottom according to available temperature profiles. This was also supported by the finding that bottom dissolved oxygen and epilimnetic phosphorus were not replenished during this period. Possibly, as a combined effect of decreasing trends in phosphorus concentrations and the limited mixing

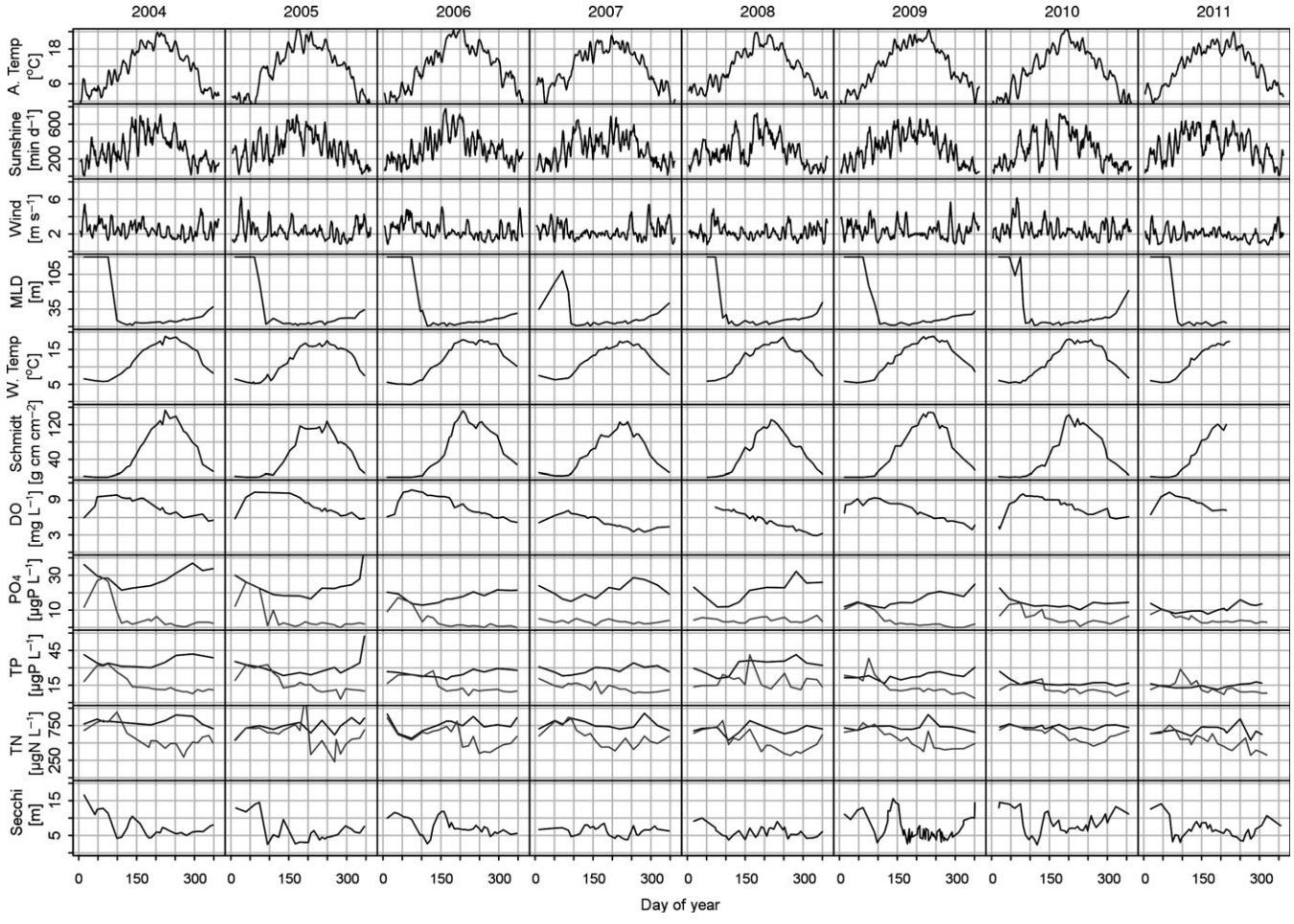

Fig. 1 Seven-day moving average of air temperature, sunshine duration and wind speed mixed layer depth (defined as the first depth, z' at which $T(z = z') - T(z = 0) < 1$ K), 0–20 m average water temperature, Schmidt stability, 100–140 m average dissolved oxygen concentration; 0–140 m (black) and 0–20 m (grey) average concentrations of P-PO_4, TP and TN, Secchi depth (m).

during the winters of 2006–07 and 2007–08, the phosphorus-depleted layer was extended from 40 to 50 m, as observed during the summers of 2004–06, to deeper layers from 2007 (Fig. S2). Full mixing of the water column occurred during the winter of 2009–10.

The PCA revealed a strong positive correlation ($P < 0.05$) between *P. rubescens* and total phytoplankton (including the cyanobacterium) abundances and between temperature and Schmidt stability (Fig. S3). A significant and negative relationship ($P < 0.05$) was also detected between *P. rubescens* and the N : P ratio (not shown) and SiO_2. Axis 1 explained 36.1% of the variance and was mainly associated with temperature and the Schmidt stability, *P. rubescens* and the total phytoplankton and to a lower extent SiO_2 and PO_4. Axis 2 explained *c.* 26% of the variance and was associated mainly with *P. rubescens*, total phytoplankton and TP. PCA clearly revealed that 2010 and 2011 separated from the other years; both 2010 and 2011, and to some extent 2009, were characterised by low *P. rubescens* biomass and low TP.

Changes in the phytoplankton community structure

From 2004 to 2006, the annual average phytoplankton biomass increased slightly from 2940 to 4346 μg L^{-1} (Fig. 2a), with biomass remaining high in 2007 and 2008. During 2004 to 2008, the proportions of cyanobacteria (mainly consisting of *P. rubescens*) increased, constituting between 43 and 53% of the total biomass. Diatoms comprised the second important algal class, represented by *Aulacoseira islandica* ssp. *helvetica*, *Cyclotella bodanica*, *C. costei*, *Diatoma elongatum*, *Fragilaria crotonensis* and *Tabellaria flocculosa*. In 2009, phytoplankton biomass declined by a factor of at least 2.5 compared with previous years (i.e. 1417 μg L^{-1}), essentially caused by the decrease and then disappearance of *P. rubescens*. Since 2010, the biomass has been relatively low, not exceeding 1100 μg L^{-1}. Both colonial and filamentous forms of cyanobacteria (picocyanobacteria are not considered here) became rare or even absent. The proportion of the total biomass constituted by diatoms and Chrysophyceae increased markedly, including species such as *Stephanodiscus alpinus*, *Fragilaria ulna* var. *angustissima*, *Fragilaria ulna* var. *acus*, *Cyclotella costei* for diatoms and some *Dinobryon* species (*D. divergens*, *D. sociale var. americanum*, *D. elegantissimum*, *D. bavaricum*) for Chrysophyceae. Interestingly, the absolute amount of eukaryotic algal groups did not markedly increase after the disappearance of *P. rubescens*. Coincident with these major changes, the proportion of microphytoplankton changed abruptly from 2010, concomitant with the disappearance of *P. rubescens* (Fig. 2b). Compositional shifts were accompanied by major changes in the proportion of the functional groups *sensu* Reynolds *et al.* (2002): the group R (covering the species in the metalimnion of stratified lakes, dominated by low light levels and relatively nutrient-rich environments) was replaced by group E, with mixotrophic taxa such as *Dinobryon* spp. characteristic of oligotrophic waters (Fig. 2c).

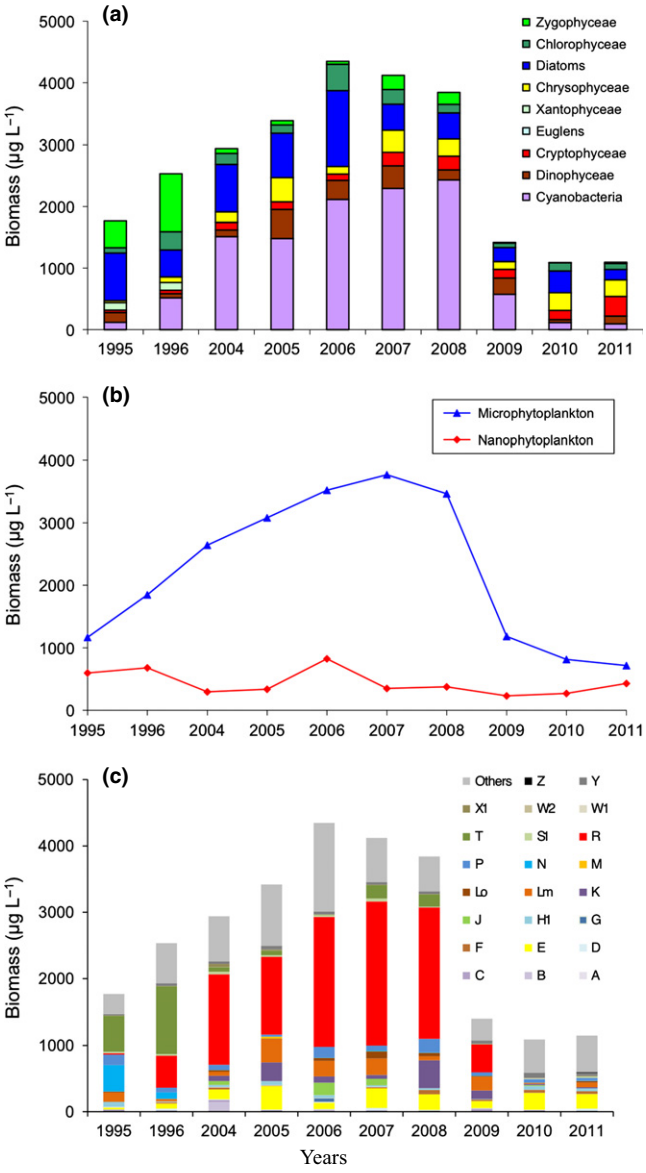

Fig. 2 Inter-annual changes in phytoplankton abundance and composition: (a) phylogenetic groups, (b) micro- and nanophytoplankton and (c) Reynolds' functional groups (Reynolds *et al.*, 2002).

Rise and fall of Planktothrix rubescens

Between 2004 and 2009, a relatively strong metalimnic *P. rubescens* biomass was recorded from spring to autumn.

During the winters between 2004 and 2009, the population was maintained in the first 50 m of the water column, at concentrations ranging between 3000 and 4000 cells mL^{-1} (Fig. 3); the highest concentration was observed during the mild winter of 2007. The year 2008 was characterised by the strong development of *P. rubescens*, in particular in summer when metalimnic abundance reached a record level (>170 000 cells mL^{-1}). *P. rubescens* then reached the epilimnion at the end of September, as observed every year, located in the 20–25 m water column until the beginning of December. Cellular concentrations averaged 20 000–30 000 cells mL^{-1}. From mid-December, it was found in large quantities to a depth of 30 m and then, by mid-January 2009, down to 50 m, and probably below. The bloom dynamics have been described elsewhere by Jacquet *et al.* (2005).

The years 2008 and 2009 were studied in greater detail to gain a better insight into factors that may have led the disappearance of *P. rubescens* by the end of 2009 (Fig. 4 & S4). The first marked difference between these

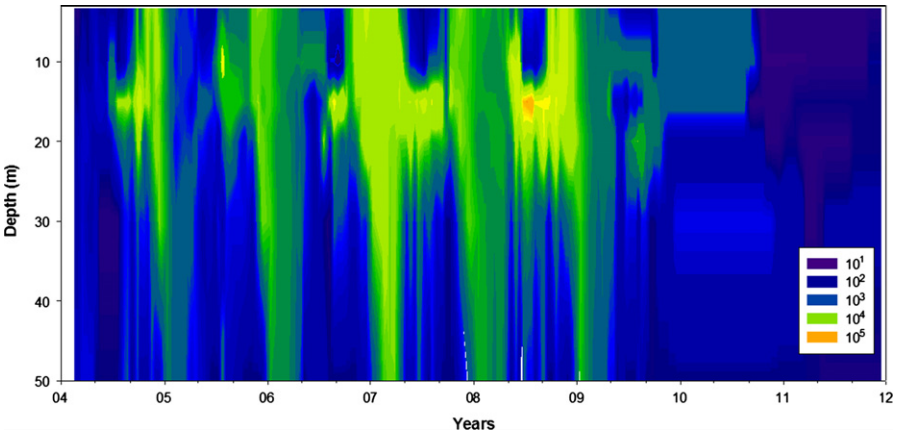

Fig. 3 Temporal change of vertical abundance of *Planktothrix rubescens* between 2004 and 2011. Data are from counts performed at discrete depths (i.e. 2, 10, 15, 20, 30 and 50 m). The colour legend refers to cells mL^{-1}. The figure and interpolation between each sampling date and depth were generated automatically using SigmaPlot™.

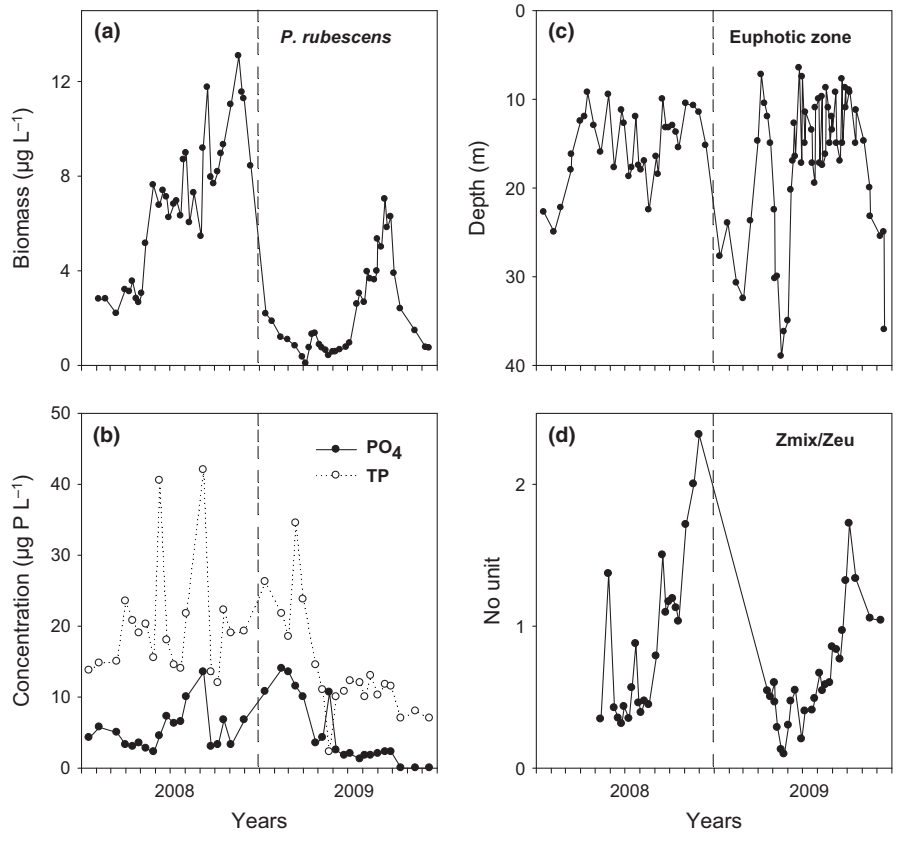

Fig. 4 Comparison between 2008 and 2009 of the seasonal variations of (a) *Planktothrix rubescens* biomass (averaged over the 0- to 20-m layer from the bbe Fluoroprobe profiles with each dot corresponding to the average value over >50 data points obtained along each profile), (b) TP and P-PO_4 (averaged over the 0- to 20-m layer) concentrations, (c) the euphotic zone (as 2.5× Secchi depth) and (d) the ratio of the mixed layer depth and the euphotic zone (Zmix/Zeu).

2 years occurred in spring, when the biomass of *P. rubescens* increased markedly in 2008, whereas it remained very low in 2009 (Fig. 4a). The biomass of *P. rubescens* decreased slightly during the summer of 2008, but increased significantly in 2009. However, levels in 2008 were always higher than those observed in 2009. Marked differences were noted later in the year, especially in autumn (from September), when the biomass remained high in 2008, but declined to very low values in 2009. Before the decline, biomasses were similar at the end of September, varying between *c.* 45 000 and 50 000 cells mL^{-1} in both years. Thus, an important event or combination of events occurred in 2009 causing *P. rubescens* biomass to decrease dramatically; afterwards, *P. rubescens* cells were only occasionally counted as part of the regular phytoplankton sampling programme in 2010, 2011 and 2012 (Jacquet, Rimet & Druart, 2014), as well as in 2013 (not shown). At the maximum biomass of *P. rubescens* (September 2009), the cyanobacterium represented *c.* 80% of the phytoplanktonic biomass; the remaining 20% comprised by Chlorophyceae (i.e. *Chlamydomonas* sp.) and Cryptophyceae (i.e. *Cryptomonas* and *Rhodomonas* sp.).

Total phosphorus and $P\text{-}PO_4$ concentrations differed between 2008 and 2009 (Fig. 4b). TP was below 10 μg L^{-1} in 2009 and remained around 20 μg L^{-1} in 2008; $P\text{-}PO_4$ reached rapidly undetectable concentrations in 2009 and was between 3 and 7 μg L^{-1} in 2008. Light availability also differed between the 2 years (Fig. 4c), with the euphotic zone fluctuating between 7.7 and 11 m from 22 September until 10 October, while in 2008, there was almost no variation (the euphotic depth varied between 13 and 13.8 m from 24 September until 15 October). Finally, the ratio between the MLD and the euphotic zone, considered as an index of the combination of light extinction and the structure of the metalimnetic layer (Sverdrup, 1953), revealed that it was on average >20% higher in 2009 than in 2008, during the same period as mentioned just above. Indeed, this ratio varied between 1.09 and 1.19 in 2008 and between 1.31 and 1.72 in 2009.

The importance of the initial biomass of *P. rubescens*, which can provide the inoculum for the rest of the year (Walsby, Avery & Schanz, 1998; Jacquet *et al.*, 2005), was also tested (Fig. 5). While no clear relationship was found between winter months (considered as DJF or JFM) and summer (JJA or JAS), autumn (SON or OND) or with the whole year, we did find significant ($P < 0.05$) positive relationships between winter and spring biomass ($r = 0.65$, $n = 13$, Fig. 5a), spring biomass and blooms in summer ($r = 0.74$, Fig. 5b) and autumn ($r = 0.67$, Fig. 5c), suggesting that the spring inoculum was indeed important in explaining the success of the cyanobacterium during subsequent months. A positive relationship was also found between summer and autumn months ($r = 0.81$, $P < 0.01$, Fig. 5e) and between these seasons and the all year ($r = 0.85$ or $r = 0.92$, $P < 0.01$, Fig. 5e, 5f). In 2009, the spring biomass was relatively low compared with previous years, but *P. rubescens* still succeeded in blooming during the summer months, suggesting that the inoculum was high enough to permit cyanobacterium development. In 2010 and 2011, *P. rubescens* was detected only occasionally during spring (0 and <130 cells/mL in 2010 and 2011, respectively), leading to a total absence or the presence of only a few filaments of the cyanobacterium in summer and autumn. Note that it was also the case in 2012 and 2013 (not shown).

Zooplankton dynamics and effect of phosphorus on Planktothrix rubescens filament size

Over the period 2004–11, analysis of the pelagic crustaceans revealed a very dynamic pattern (Fig. 6). The greatest zooplankton biomass (especially herbivorous zooplanktonic forms, such as *Daphnia longispina*, *D. brachyurum* or *D. hyalina*, and cyclopoid nauplii, c1, c2 and c3) was recorded in spring and/or autumn 2009, coinciding with the decline of *P. rubescens*. However, comparison of 2008 and 2009 from September to December showed no clear relationship between the cyanobacterial biomass and zooplankton in 2008. Positive (but not significant at $P > 0.05$) relationships were recorded between *P. rubescens* and herbivorous cyclopoids ($r = 0.76$, $n = 6$), herbivorous calanids ($r = 0.67$, $n = 6$) and Cladocera ($r = 0.4$, $n = 6$) in 2009.

In the laboratory study, we found that although filament size was relatively homogeneous in P-rich conditions, under more P-limiting conditions, filaments displayed a variety of sizes, and filament size reduced significantly at lower levels of P ($P < 0.05$, Fig. 7). Note, we did not measure phosphorus concentrations at the end of the experiment.

Discussion

Changes in phosphorus and phytoplankton community structure

Efforts to reduce nutrient contributions in Lake Bourget via the main tributaries (i.e. the rivers Leysse and Sierroz) have been successful, with TP reduced from *c.* 300 tons in 1974 to less than 30 tons in 2011 (Bryhn *et al.*,

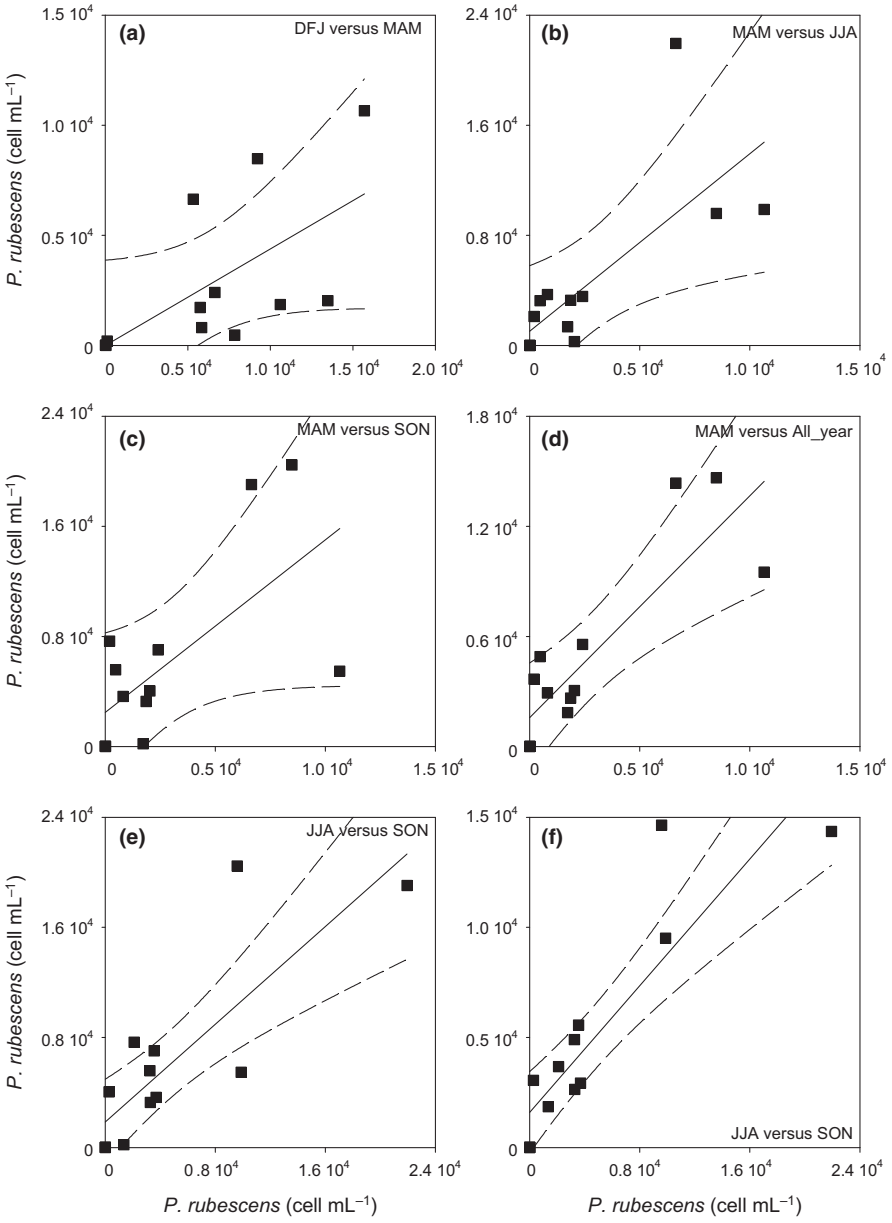

Fig. 5 Relationships ($P < 0.05$) between *Planktothrix rubescens* concentrations (cells mL^{-1}) during different seasons (DJF = winter, MAM = spring, JJA = summer, SON = autumn) and for the whole year. Each dot is the seasonal average for each year (2000–12). The dashed lines symbolise the confidence intervals at 99%.

2010; Jacquet, Domaizon & Anneville, 2012), resulting in a shift from eutrophic to oligo-mesotrophic in *c.* 30–40 years. However, between 1995 and 2009, this ongoing nutrient reduction led to an apparent paradoxical situation with the development of blooms of the toxic and filamentous cyanobacterium, *Planktothrix rubescens* (e.g. Jacquet *et al.*, 2005, 2012). Moreover, Savichtcheva *et al.* (2011) showed that *P. rubescens* developed and bloomed in typical mesotrophic conditions several times during the 20th and 21st centuries in Lake Bourget. Such episodic occurrences of *P. rubescens* were also observed in Lake Nantua and Mondsee (Feuillade, 1994; Dokulil & Teubner, 2012). By the end of 2009, *P. rubescens* disappeared in Lake Bourget, accompanied by a dramatic change in the composition and structure of the phytoplanktonic community.

Teubner *et al.* (2003) and Dokulil & Teubner (2005) reported that *P. rubescens* could develop considerable biomass at TP around 10 μg P L^{-1}, so it is surprising that *P. rubescens* did not develop at similar concentrations in Lake Bourget. However, by the end of 2009 (from early September to the end of the year), TP never exceeded 10 μg P L^{-1}, and P-PO_4 was below the detection limit in the 0- to 20-m surface layer. In fact, from 2007, a clear deepening of the P-depleted layer was observed (Fig. S2); a phenomenon that has been observed elsewhere (e.g. Lake Geneva) coincident with re-oligotrophication (e.g. Anneville & Leboulanger, 2001;

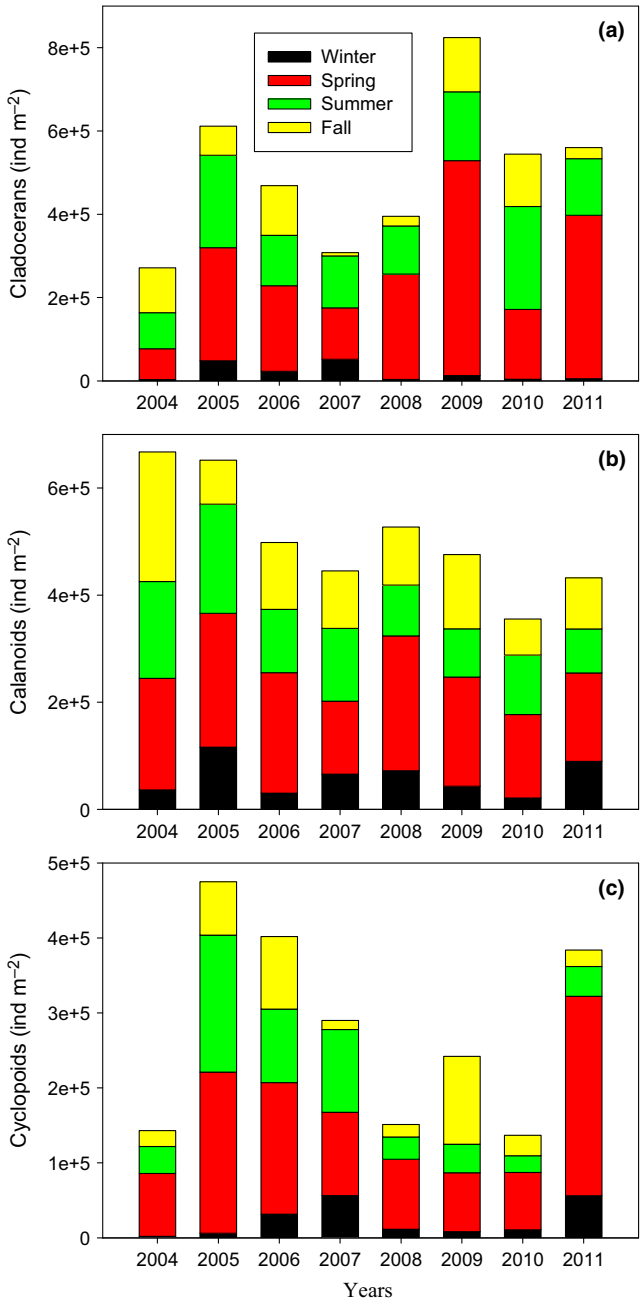

Fig. 6 Herbivorous zooplankton abundances: cladocerans (a), calanoids (b) and cyclopoids (c) for different seasons from 2004 to 2011.

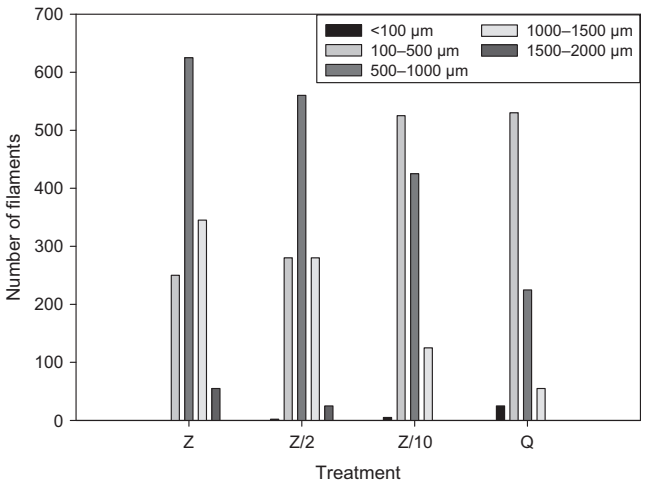

Fig. 7 *Planktothrix rubescens* filament length distribution for different KH_2PO_4 concentrations.

Anneville *et al.*, 2004). Comparatively, TP exceeded 20 µg P L^{-1} and P-PO_4 was detected in the autumn of 2008, when the bloom was still present. These data, and the relationship between TP (or P-PO_4) and *P. rubescens* (Fig. 8), confirmed the preference of *P. rubescens* for mesotrophic conditions and suggest that P depletion was the main factor contributing to the decline of *P. rubescens*. This finding agrees with other studies on the disappearance of *P. rubescens* from Lake Mondsee (Dokulil & Teubner, 2012) and cyanobacterial forms in general in Müggelsee (Wagner & Adrian, 2009; Huber *et al.*, 2012). Our results therefore support the conjecture that the trophic state is important and that *P. rubescens* is characteristic of mesotrophic conditions (Chorus & Bartram, 1999; Jeppesen *et al.*, 2005; Dokulil & Teubner, 2012). In contrast to the findings of Posch *et al.* (2012) and the role of nitrogen in Lake Zurich, we did not find any evidence that *P. rubescens* biomass was maintained despite higher N : P ratios (following the reduction of P, but stable N concentrations) that could have favoured this non-N_2-fixing cyanobacterium.

Phytoplankton biomass has been shown to display delayed responses to environmental change (Jeppesen *et al.*, 2005; Duarte *et al.*, 2009; Kerimoglu, Straile & Peeters, 2013), due to the 'portfolio effect' (i.e. compensatory trajectories of individual populations make the total biomass appear unresponsive) (Gonzalez & Loreau, 2009; Jochimsen, Kümmerlin & Straile, 2013). Phytoplankton dynamics in Lake Bourget appear to be another example of the portfolio effect; during re-oligotrophication of Lake Bourget, the phytoplankton biomass did not decline as predicted due to the proliferation of *P. rubescens*. In 1994–95, the total biomass was 2051 µg L^{-1}, in 2004–05, it was 3180 µg L^{-1}, with contribution by *P. rubescens* of 40% (Jacquet *et al.*, 2005; Humbert *et al.*, 2006), while during 2004–08, the total biomass varied between 2940 and 4346 µg L^{-1}, with still a large proportion due to *P. rubescens*. However, when this species disappeared in 2010, so did the portfolio effect, as reflected by the abrupt decline in phytoplankton biomass (to *c.* 1030 µg L^{-1}).

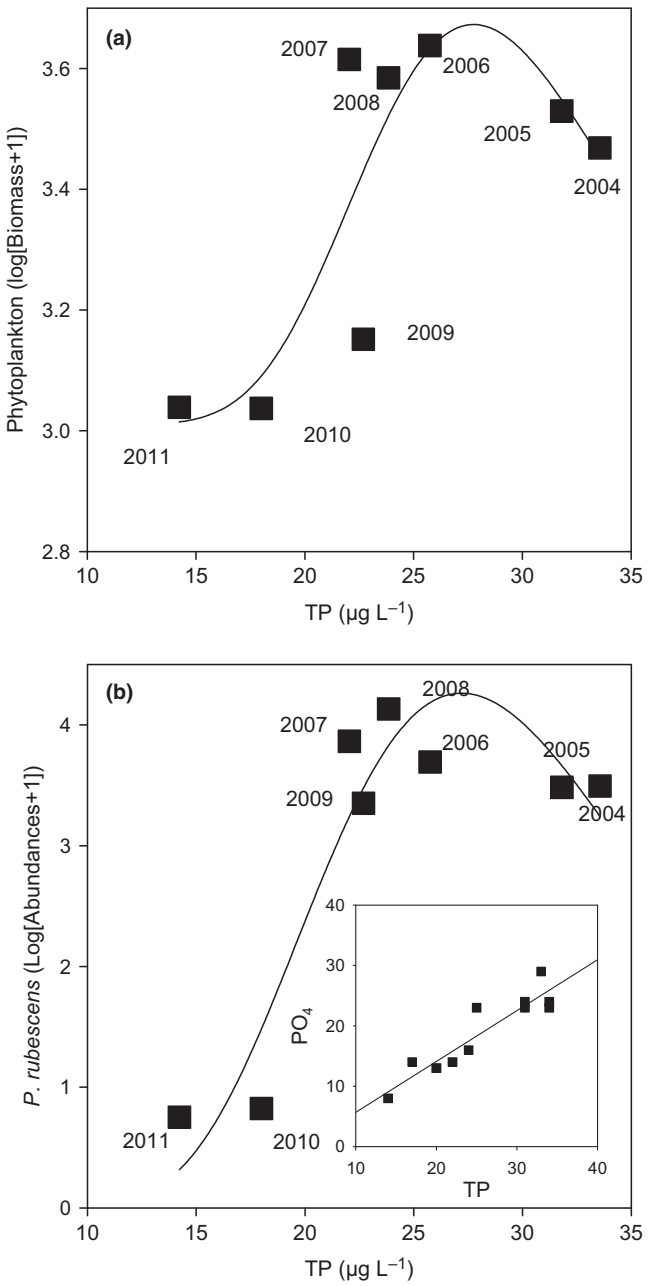

Fig. 8 Relationships between the winter (February–March) water column average concentrations of TP (µg L^{-1}) and the total phytoplankton biomass (a) or *Planktothrix rubescens* cell abundances (b) averaged over the year (without the winter months). The best fit to the data corresponded to a polynomial relationship. The insert panel represents the relationship between winter average values of TP and P-PO_4 (in µg L^{-1}) for 2000–11 ($r = 0.92$, $n = 12$, $P < 0.01$). The data were temporally, and in the case of phosphorus data vertically, interpolated to a fixed grid prior to averaging, in order to avoid potential biases due to the non-homogeneous representation of time and space in the data sets.

As *Planktothrix rubescens* is a nuisance species (Feuillade, 1994), such dynamics have important implications for management practices. In Lake Bourget, decreasing nutrient loads did not result in immediate reductions in phytoplankton biomass and might have led to the occurrence of harmful algal blooms, but with further reductions, harmful algal blooms disappeared, and total phytoplankton biomass declined. Following the disappearance of *P. rubescens*, and hence, decline in the share of microphytoplanktonic forms, the new dominant forms that emerged comprised diatoms (*Stephanodiscus*, *Cyclotella*, *Fragilaria*), cryptophytes (*Rhodomonas* and *Cryptomonas*) and chrysophytes, with mixotrophic taxa such as *Dinobryon* spp., and also *Ceratium* (Dinoflagellate). Such changes are in agreement with the general expectation that the small cells and mixotrophic forms are more competitive under nutrient-limited conditions (Reynolds *et al.*, 2002).

Temperature and mixing

Temperature can affect phytoplankton succession through differential effects on metabolic rates (Butterwick, Heaney & Talling, 2005) and vertical niche separation (Lindenschmidt & Chorus, 1998). For *P. rubescens*, temperature can act directly (i.e. on the growth capacities of the species) and/or indirectly (by stabilising the water column). Oberhaus *et al.* (2007a) showed that *P. rubescens* sustained high growth rates at temperatures between 15 and 25 °C. They also showed that the species was very competitive compared to *P. agardhii* at low temperature (15 °C) and in green light, that is the conditions encountered by *P. rubescens* in Lake Bourget at the base of the epilimnion, where it develops and blooms. Jacquet *et al.* (2005) also pointed out the importance of temperature through prolonged stratification, following warmer-than-average winter/spring periods (during recent decades), allowing earlier water stratification and thus conferring a competitive advantage on *P. rubescens*, the metalimnic development of which requires water column stability. Several authors have proposed that in deep lakes, the effect of lake warming on the dynamics of *P. rubescens* is likely to favour the cyanobacterium by reducing the winter mixing depth (Walsby *et al.*, 1998; Anneville *et al.*, 2004; Salmaso, 2010; Posch *et al.*, 2012; this study). Posch *et al.* (2012) also showed that the physical entrainment of filaments into deeper waters can be a significant mortality factor for the cyanobacterium, because gas vesicles within the cells collapse as a result of hydrostatic pressure, and because even if some filaments remain buoyant, their ascension velocity is too slow for them to reach the upper sunlit layers.

Initial population size of competing species might determine the fate of planktonic succession (Sommer,

1989). From our data for 2004 to 2011, we found a good correlation between winter air temperature and the spring biomass of *P. rubescens* ($r = 0.79$, $P < 0.05$), suggesting that the onset of stratification is decisive. However, we did not find any clear relationship between the Schmidt stability index and the *Planktothrix* biomass. As water column stability occurs every year in this lake, slight inter-annual variability might be not a relevant factor to consider here with regard to the disappearance of the cyanobacterium (see also Dokulil & Teubner, 2012). In 2009, no such spreading of the cyanobacterium was recorded, and the peak remained relatively stable and was located in the 15- to 20-m layer from early summer to mid-October. Full mixing of the water column occurred during the winter of 2009–10. In large and deep ecosystems, net growth is negative when stratification breaks down, and the depth of the mixed layer falls below a critical depth (Salmaso, 2010; Dokulil & Teubner, 2012). *P. rubescens* in Lake Bourget did not grow when the water was fully mixed (the population collapsed during February–March). Such a collapse is probably related to light limitation (Halstvedt *et al.*, 2007), but also to vesicle damage owing to hydrostatic pressure (Walsby, Schanz & Schmid, 2005).

Light availability

It is likely that *P. rubescens* was limited by light availability at the depth where it occurred (below 15 m) at the end of September and early October 2009. Such a limitation could be inferred from the transparency data and estimated euphotic zone that was largely above the peak of the cyanobacterial biomass at this period. The low value of transparency found during this period of 2009 (around 10 m, range 7.7–11 m) was significantly different from previous years ($P < 0.05$), not only 2008, for which Zeu was always deeper (>12.4 m on average, range 12.4–14.6 m). Low transparency in 2009 was not related to algal biomass since the bbe fluoroprobe did not reveal higher phytoplankton levels in the 0- to 10-m layer in early autumn of 2008 compared with 2009 that could have resulted in higher shading. It is thus possible that detritus, inert particles were responsible for lower transparency and euphotic zone reduction in 2009 at the end of September until mid-October. As a result, *P. rubescens* was largely situated under the light level required for growth.

Previous studies on the ecophysiology of *P. rubescens* have highlighted the importance of light (Bright & Walsby, 2000; Walsby & Schanz, 2002; Blikstad Halstvedt *et al.*, 2007). For example, Walsby & Schanz (2002) showed that light limitation could explain the gradual decrease of the population in winter in the neighbouring Lake Zurich. These authors also showed that population growth halted when the mixed depth exceeded the critical depth for growth in autumn (Sverdrup's principle). We compared 2008 and 2009, from the end of September to the end of October, for the combination of light extinction and the structure of the metalimnetic layer. The ratio of the mixed layer and the euphotic zone was 40% higher in 2009 than 2008, suggesting that population changes could also be determined by interactions of light and depth distribution.

Importance of zooplankton grazing

Induced defence in highly productive systems is a common paradigm (e.g. Leibold, 1989; Elser & Goldman, 1991). The proposed role of herbivorous zooplankton and/or pelagic fishes on cyanobacterial (e.g. *P. rubescens*) toxin transfer has been recently challenged in Lake Bourget (Sotton *et al.*, 2012a,b; Perga *et al.*, 2013) and elsewhere (Sotton *et al.*, 2014). Our analysis revealed that the highest herbivorous zooplankton biomass (during autumn 2009) coincided with the decline of *P. rubescens*. One may argue that the herbivorous zooplanktonic forms could benefit from the lower cyanobacterial biomass, but we suggest that they probably grazed on the cyanobacterium and thus contributed to its decline. This conjecture is supported from our experiment (with reduced filament length), the positive relationship in 2009 between herbivorous zooplankton and *P. rubescens* (and not in 2008) and from the study of Perga *et al.* (2013). It is also noteworthy that effective and significant grazing of *Daphnia* on *P. rubescens* has been demonstrated recently by Shams *et al.* (2013).

We found that bottom-up factors might have amplified top-down forcing: filaments may have indeed become shorter due to the increasing P limitation during the study period. Kamenir & Morabito (2009) showed that decreasing size and biovolume were observed in *P. rubescens* in Lago Maggiore (Italy) during re-oligotrophication. Although we did not measure total filament length from the outset of the survey, we propose that *P. rubescens* could indeed have experienced filament length reduction as P-limiting conditions intensified in Lake Bourget and may have been more efficiently grazed by the zooplankton, as shown under experimental conditions (Oberhaus *et al.*, 2007b; Shams *et al.*, 2013). Such length reduction could have also increased the grazing capacity of ciliates, which have recently been shown to feed on toxic cyanobacteria, typically *Planktothrix*

(Combes *et al.*, 2013), but also by rotifers and copepods as suggested by Perga *et al.* (2013).

We found that the microcystin toxin concentrations decreased significantly during the study period (following biomass reduction), with the exception of one date (29 September, 5 μg L^{-1} of microcystin) when levels were very low or not detected in 2009, in contrast to previous years (Fig. 9). This loss or severe reduction of toxin

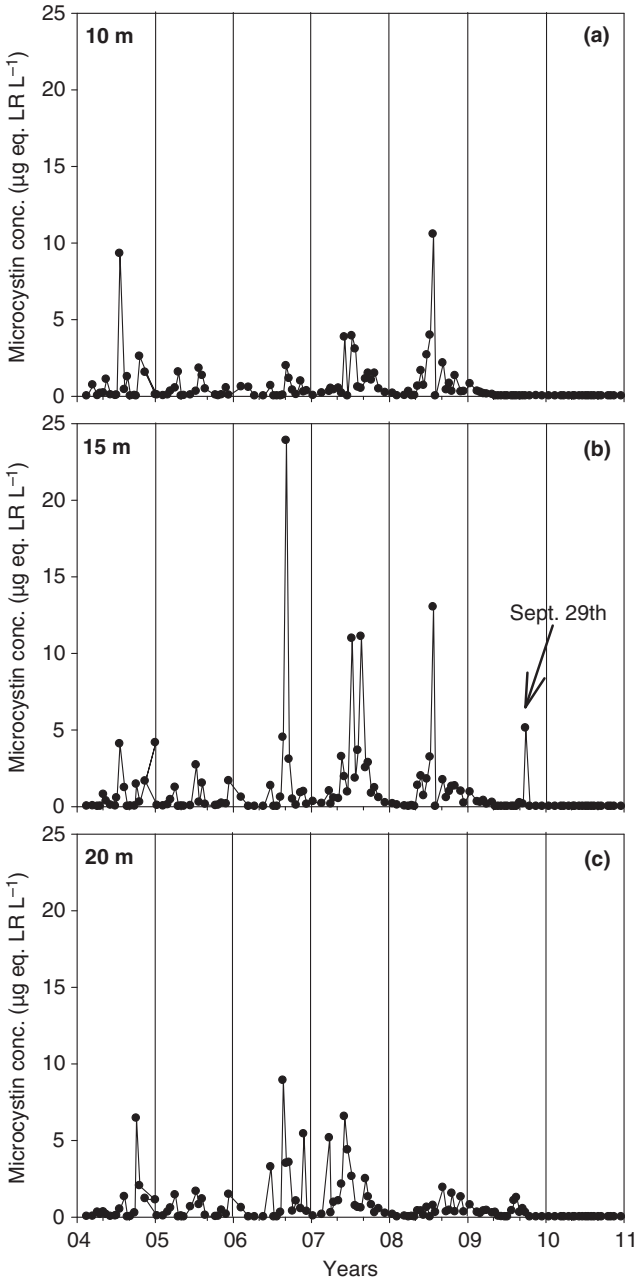

Fig. 9 Evolution at three different depths: 10 m (a), 15 m (b) and 20 m (c) of the intracellular microcystin (LR + RR, given as μg LR equivalent per L) concentration of *Planktothrix rubescens*. The last relatively important concentration was recorded on 29 September 2009, prior to *P. rubescens* bloom termination and definitive decline.

production could also have favoured zooplanktonic grazing (Rohrlack *et al.*, 2001). Furthermore, it is possible that *Daphnia* acquired resistance and better detoxification mechanisms (e.g. Jiang *et al.*, 2013). Chislock *et al.* (2013) have recently shown that *Daphnia* can increase in abundance and suppress phytoplankton biomass, despite high initial levels of cyanobacteria and microcystin, indicating that the latter do not prevent strong control of phytoplankton biomass by *Daphnia* genotypes that are adapted to environments with abundant cyanobacteria and associated cyanotoxins. It is also possible that the proportion of *P. rubescens* clones devoid of microcystins was greater in 2009 than in previous years. As the concentration of toxin has been shown to be related to the growth rate of *P. rubescens* (Briand *et al.*, 2005), this conjecture is supported since the growth rate was indeed probably lower at the end of 2009. In other words, it is possible that reduced growth rate reduced the production of toxins and/or that the relative abundance of *P. rubescens* filaments do not produce microcystin (Garneau, Posch & Pernthaler, 2013). Lastly, the reduction or loss of oligopeptide production (e.g. microcystins, microviridins and anabaenopeptins) in *P. rubescens* due to unfavourable growth conditions could favour parasitic chytrid fungi that are able to inflict significant mortality on this species when it is not able to protect itself by producing oligopeptides (e.g. Rohrlack *et al.*, 2013).

In conclusion, a conjunction of events (involving several factors or processes) was probably responsible for the disappearance of *P. rubescens* in Lake Bourget, including interactions for nutrients, light availability, temperature and water column stability (Jacquet *et al.*, 2005; Taranu *et al.*, 2012). Field and laboratory studies also indicate that zooplankton grazing pressure could have enhanced bloom termination. Finally, full mixing of the water column, enhancing dilution of the population throughout the water column, may affect *P. rubescens* population size due to light limitation and vesicle damage.

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Supporting Information

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

Figure S1. Relationship between cell counts of *P. rubescens* and the bbe Fluoroprobe (data in μg chlorophyll equivalent per L).

Figure S2. Evolution of P-PO_4 in the 0–20 m layer below the surface (above panel) and thickness of the P-PO_4 depleted layer, i.e. below 10 μg P L^{-1} (bottom panel), depending on the year and the month of sampling.

Figure S3. PCA of the environmental parameters in Lake Bourget.

Figure S4. Comparison of the seasonal variations of *P. rubescens* in 2008 (A) and 2009 (B) from all data obtained with the bbe Fluoroprobe profiles.

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