REPORT

Forest Die-Back Modified Plankton Recovery from Acidic Stress

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Received: 10 January 2013/Revised: 15 March 2013/Accepted: 18 May 2013/Published online: 2 June 2013

Abstract We examined long-term data on water chemistry of Lake Rachelsee (Germany) following the changes in acidic depositions in central Europe since 1980s. Despite gradual chemical recovery of Rachelsee, its biological recovery was delayed. In 1999, lake recovery was abruptly reversed by a coincident forest die-back, which resulted in elevated terrestrial export of nitrate and ionic aluminum lasting ~5 years. This re-acidification episode provided unique opportunity to study plankton recovery in the rapidly recovering lake water after the abrupt decline in nitrate leaching from the catchment. There were sudden changes both in lake water chemistry and in plankton biomass structure, such as decreased bacterial filaments, increased phytoplankton biomass, and rotifer abundance. The shift from dominance of heterotrophic to autotrophic organisms suggested their substantial release from severe phosphorus stress. Such a rapid change in plankton structure in a lake recovering from acidity has, to the best of our knowledge, not been previously documented.

Keywords Atmospheric acidification · Bohemian Forest · Lake recovery · *Microcodon clavus* · Phytoplankton · Zooplankton

INTRODUCTION

Atmospheric acidification of geologically sensitive areas has represented the most adverse anthropogenic impact on both aquatic and terrestrial headwater ecosystems since the mid twentieth century (Schindler 1988; Driscoll et al. 2001). Increasing concentrations of sulfate, nitrate, and hydrogen ions in soil solutions due to long-range transport of acidic pollutants caused reduction in acid neutralizing capacity, soil depletion of base cations, and mobilization of

toxic ionic aluminum species, with adverse effects on nutrient cycling and the health of forest and aquatic ecosystems, and deleterious reduction of freshwater biodiversity (Reuss and Johnson 1986; Psenner and Catalan 1994; Driscoll et al. 2001; Vrba et al. 2003a). While deposition of acidifying pollutants has substantially declined in Europe and North America, particularly due to sulfur emission controls since 1980s, leading to partial recovery of surface waters from acid stress (Stoddard et al. 1999), biological recovery of these areas remains remarkably delayed or uncertain (Jeffries et al. 2003; Skjelkvåle et al. 2003; Yan et al. 2003; Wright et al. 2005; Nedbalová et al. 2006). Two recent reviews on plankton recovery (Graham et al. 2007; Gray and Arnott 2009) discussed this lag and its possible reasons and pointed out certain gaps in our knowledge due to a lack of data and/or methodological constraints.

In the Bohemian Forest, owing to 140 years of occasional hydrobiological research and paleolimnological studies, we were able to document significant changes in lake water chemistry and consequent changes in plankton and benthos composition, as well as extinction of fish (Vrba et al. 2003a). Moreover, the Bohemian Forest has exhibited the largest changes in acidic deposition due to 86, 53, and 35 % reduction in sulfur dioxide, nitrogen oxides, and ammonia emissions, respectively, in central Europe since the late 1980s (Kopáček et al. 2012). Due to small and geologically sensitive catchments covered with coniferous forests, the Bohemian Forest lakes have rapidly shown substantial recovery of water chemistry (Kopáček et al. 1998; Vrba et al. 2003a). The biotic response, however, has lagged for a decade or even longer (Nedbalová et al. 2006).

In this study, we present effects of rapid lake water recovery (after forest die-back and reforestation in the catchment) on biological recovery. We took advantage of

using both historical data and a long-term research approach when focusing on biological recovery of one particular lake in the region. A century ago, Rachelsee was considered as the most humic (dark brown) lake among all eight Bohemian Forest lakes (Vrba et al. 2000). Its acidic nature was also inferred from lake sediments according to several bioindicators (Steinberg et al. 1984). The estimated pH of the lake water was about 5.0 during the past centuries, with a sharp decrease to 4.5 in the second half of the last century, caused by atmospheric acidification. This trend was confirmed by the first direct pH (3.6–4.9) measurements in Rachelsee prior to the 1980s, followed by slightly increasing pH (up to 5.0) during the 1990s (Schaumburg 2000). However, Rachelsee remained the most severely acidified lake in the region even in 1999 (Vrba et al. 2003a), when its obvious trend in chemical recovery was abruptly reversed for ~5 years by a coincident natural forest die-back in the entire catchment, which resulted in high terrestrial export of nitrate and ionic aluminum. This re-acidification episode provided a unique background for the case study focused on a prompt

response of plankton, enabled by very rapid chemical recovery of the lake water after the abrupt decline in nitrate leaching from the catchment, which was much faster than the previous recovery from atmospheric acidification.

MATERIALS AND METHODS

Site Description

Rachelsee (48°58'30"N, 13°24'06"E, 1,071 m a.s.l.) is a small glacial lake (area: 5.7 ha, volume: $18 \times 10^4 \text{ m}^3$, maximum depth: 13 m) formed in the SE corrie of Grosser Rachel Mt. (1453 m) in the German part of the Bohemian Forest. Rachelsee is an oligotrophic, dimictic lake, with ~5-month ice and snow cover, and a water residence time of ~87 days. The lake was originally humic and fishless, then became strongly acidified ($\text{pH} < 4.3$) in the early 1980s, and has been slowly recovering since the late 1980s ($\text{pH} \sim 5$, Fig. 1; Schaumburg 2000; Vrba et al. 2000, 2003a).

Fig. 1 Long-term (1984–2012) trends in lake water chemistry of Rachelsee—late summer epilimnetic pH and concentrations of sulfate, nitrate, chloride, potassium, sodium, magnesium, calcium, and ionic aluminum (note different scales in $\mu\text{eq L}^{-1}$; one equivalent is mol of charge). The gray vertical bar indicates the beginning of forest die-back and plankton sampling; horizontal bars indicate two distinct periods compared in this study (values from 2000 to 2002 were not included in the statistical analyses of Fig. 2 or Table 1)

The Rachelsee catchment (58 ha) is forested (mostly Norway spruce and European beech) and situated in the core zone of the Bavarian Forest National Park, where a strict non-intervention regime has been applied since 1983. In the late 1990s, a bark beetle outbreak caused large-scale natural disturbances to sub-alpine forests in the lake catchment (Heurich 2009). Most adult Norway spruce trees underwent massive defoliation during summer 1999, when we did the first sampling. Later, the upper tree layer largely disappeared, whereas regeneration density of the young forest increased substantially during the next decade (Heurich 2009).

Sampling

We sampled the lake in late summer (August–September) in 1999 and in 2003–2011. Samples were taken with a van Dorn sampler (1 m length, 6.4 L volume) from specific depths (0–1 m from the epilimnion and other 4–5 layers along the vertical profile at the deepest point of the lake, see “Results”). Samples for chemical analyses were filtered immediately through a 200- μ m polyamide sieve. Plankton samples were preserved by either formaldehyde (for bacteria) or acid Lugol’s solution (for phytoplankton).

Small zooplankton were concentrated from the whole volume (6.4 L) of the van Dorn sampler with use of a plankton net of 40- μ m mesh size. From the quantitative data on small zooplankton only numbers from the epilimnion are presented in this study. Numbers from deeper layers were low and erratic; these data were used for the qualitative assessment only. Large zooplankton were sampled by a net of the Apstein type (200- μ m mesh size, net opening 20 cm diameter). One vertical haul was taken from the deepest point of the lake to the top. All zooplankton samples were preserved by formaldehyde.

Chemical data for 1984–1998 and 2000–2002 were from Schaumburg (2000) and L. Butz (unpublished data), respectively.

Analyses

In the laboratory, samples were filtered with 0.4- μ m glass-fiber filters (MN-5, Macherey–Nagel) for analyses of dissolved compounds. Dissolved organic carbon (DOC) and particulate carbon (C_p) were analyzed with a LiquiTOC analyser (Foss/Heraeus) for the filtrate and by combustion of the glass-fiber filter for the retained particulate organic matter. Acid neutralizing capacity (ANC, Gran titration),

Fig. 2 Principal component analysis (PCA) of surface lake water chemical characteristics in the period 1999–2009. The first two axes are plotted showing the scores of variables and sampling years and the percentage of explained variability (EV; for other abbreviations, see Table 1)

pH, total phosphorus (P_t) and dissolved P (P_d) were analyzed within 24 h of sampling. P_t and P_d were determined by the molybdate method after perchloric acid digestion. Particulate P (P_p) was the difference between P_t and P_d . Fractionation of aluminum, i.e., total reactive Al (Al_t), dissolved Al (Al_d), and organically bound Al (Al_o), was analyzed in non-filtered samples, filtered samples, and cation exchange-treated samples after their filtration, respectively. Ionic Al (Al_i) was the difference between Al_d and Al_o . Particulate Al (Al_p) was the difference between Al_t and Al_d . Concentrations of major ions were analyzed by ion chromatography. All concentrations used in this study were above the detection limits and accuracy of the analyses was checked using ion balance control including ionic Al forms and organic acid anions for each sample according to Kopáček et al. (2000). More details on the analytical methods, their accuracy, and precision are given in Kopáček et al. (2004).

Bacterioplankton were counted and sized after DAPI staining on black polycarbonate filters (0.2- μ m pore size, Osmonics), using an epifluorescence microscope (Olympus BX-60 or AX-70) and an image analysis system (Lucia G/F 4.11 or NIS-Elements 3.0, Laboratory Imaging, Prague). We quantified single unicellular bacteria separately, and used the line intercept method for quantification of the total length of bacterial filaments (Nedoma et al. 2001). After a pre-sedimentation of samples (5 $\times$), phytoplankton were counted in Utermöhl's sedimentation chambers on an inverted microscope (Nikon Diaphot). Total biovolume of filamentous cyanobacteria was estimated in sedimentation chambers using the line intercept method (Nedoma et al. 2001). Cell volumes were estimated by shape assimilation to simple geometric forms and converted to carbon using a factor of 200 fg C μ m⁻³ (Straškrabová et al. 1999). Zooplankton samples were counted in chambers, using an inverted microscope (Nikon Diaphot). The small zooplankton were counted from the whole volume of the sampler (6.4 L) in most cases. Subsampling was carried out in 2006 and 2008 when the numbers of *Microcodon* were high. The numbers counted in subsamples exceeded 1000 specimens. Numbers of large zooplankton taken by net hauls were always very low, sometimes zero. Therefore, only the presence or absence of species was evaluated.

Statistics

To explore the temporal patterns of lake water features during the decade of lake recovery, a principal component analysis was run in Canoco 4.5 (ter Braak and Šmilauer 2002) using epilimnetic values of 16 chemical characteristics (Fig. 2). All the variables except pH were $\log(x + 1)$ transformed prior to the analysis. Based on the first axis

scores of individual sampling years, the dataset was divided into two periods with apparently distinct summer values (1999–2005, with negative scores; and 2006–2009, with positive scores). The differences between these two periods were tested by the non-parametric Mann–Whitney test (Prism 5, GraphPad Software, Inc.).

RESULTS

Long-Term Trends in Lake Chemistry

The long-term trend in summer epilimnetic concentrations of major ions in Rachelsee shows that sulfate concentrations have gradually decreased by 70 % of their maximum values in the mid-1980s (Fig. 1), following sulfur emission control. Nitrate concentrations decreased

Table 1 Comparison of limnological parameters in the epilimnion of Rachelsee in two distinct periods after forest die-back in the lake catchment. Values are medians (minimum–maximum) of four late summer values (1999 and 2003–2005 vs 2006–2009); significant differences between the periods (*p* values of Mann–Whitney test) are in bold

Parameters	1999–2005	2006–2009	<i>p</i> Value
z_s (m)	7.2 (3.1–8.5)	3.4 (2.2–3.7)	0.144
Cond. (μ S cm ⁻¹)	21 (19–23)	13 (12–17)	0.029
ANC (μ eq L ⁻¹)	−8 (−20 to −6)	0 (−8 to 8)	0.104
pH	4.94 (4.90–5.14)	5.26 (4.86–5.50)	0.343
SO ₄ ^{2−} (μ eq L ⁻¹)	55 (52–63)	50 (48–53)	0.057
NO ₃ [−] (μ eq L ⁻¹)	73 (47–93)	29 (13–42)	0.029
Cl [−] (μ eq L ⁻¹)	13 (11–15)	9.4 (7.4–11)	0.029
Ca ²⁺ (μ eq L ⁻¹)	41 (34–56)	36 (32–54)	0.772
Mg ²⁺ (μ eq L ⁻¹)	36 (28–43)	26 (25–29)	0.029
Na ⁺ (μ eq L ⁻¹)	26 (24–33)	22 (21–23)	0.029
K ⁺ (μ eq L ⁻¹)	15 (9.6–18)	8.1 (7.1–9.6)	0.042
NH ₄ ⁺ (μ eq L ⁻¹)	4.7 (1.0–15)	0.46 (0.41–0.53)	0.029
Al _i (μ eq L ⁻¹)	6.5 (3.5–13)	1.1 (0.2–7.4)	0.114
Al _o (μ g L ⁻¹)	24 (5–38)	61 (39–95)	0.029
Al _p (μ g L ⁻¹)	92 (64–153)	145 (68–188)	0.483
Al _t (μ g L ⁻¹)	279 (164–358)	250 (209–296)	0.686
P _t (μ g L ⁻¹)	3.7 (2.7–5.3)	6.8 (2.4–7.9)	0.343
P _p (μ g L ⁻¹)	2.9 (0.8–3.5)	4.1 (1.2–4.3)	0.189
P _d (μ g L ⁻¹)	1.4 (0.4–2.1)	2.7 (1.2–3.6)	0.110
TOC (mg L ⁻¹)	2.0 (1.4–3.6)	4.2 (3.4–5.4)	0.057
DOC (mg L ⁻¹)	1.4 (0.9–2.7)	3.3 (2.6–3.9)	0.057
C _p (mg L ⁻¹)	0.63 (0.49–0.87)	0.92 (0.69–1.44)	0.114
PB (μ g C L ⁻¹)	32 (10–95)	178 (105–211)	0.029

z_s Transparency (Secchi depth); Cond. conductivity; ANC acid neutralizing capacity (alkalinity); Al_i, Al_o, Al_p, Al_t ionic, organic, particulate, and total aluminum; P_t, P_p, P_d total, particulate, and dissolved phosphorus; TOC, DOC total and dissolved organic carbon; C_p particulate carbon; PB phytoplankton biomass (as carbon)

Fig. 3 Changes in chemistry and plankton in the epilimnion of Rachelsee in late summer of 1999 and 2003–2011 (*horizontal bars* two distinct periods, cf. Fig. 2; Table 1): acid neutralizing capacity (ANC), dissolved organic carbon (DOC), dissolved (P_d) and particulate (P_p) phosphorus, phytoplankton and bacterioplankton biomass (as carbon), and rotifer abundance (*bottom* logarithmic scale); *n.d.* not determined

less steeply from 1989 to 1997 and then markedly increased in the period 1999–2005, when nitrate became the dominant strong acid anion in the lake water. The high terrestrial export of nitrate resulted from a large-scale die-back of spruce forest in the catchment and reversed the ongoing lake water recovery from acid stress until 2005 (Fig. 1). The elevated concentrations of nitrate were compensated for by elevated terrestrial export of base cations and especially Al_i . Al_i concentrations showed a remarkable peak from 2000 to 2002 contrary to its overall decreasing trend during the past 25 years (Fig. 1). In the period 2006–2010, nitrate and Al_i exports declined to their pre-disturbance levels (Fig. 1), enabling immediate and rapid recovery of many limnological parameters compared to the 1999–2005 period (Fig. 2; Table 1). The forest die-back thus reversed the lake water recovery (especially Al_i concentrations) back to values occurring in the early 1980s (Fig. 1). During this short-term acidification, the lake water remained clear and bluish owing to low DOC concentrations as well as low phytoplankton biomass in the epilimnion (Figs. 3, 4). In the following 2006–2009 period, summer epilimnetic values of several limnological parameters showed sudden changes (cf. Table 1; Figs. 2, 3), reflecting predominately the rapid decline in terrestrial nitrate export (Fig. 1). When comparing the parameters of the two distinct periods, we found a marked drop in concentrations of most major ions (nitrate, base cations, and Al_i), as well as in the Secchi depth, and obvious increase in pH, ANC, concentrations of DOC, C_p , P forms, and phytoplankton biomass, even though not all changes were significant due to few data ($n = 4$ for either period, cf. Table 1; Fig. 3). In contrast, sulfate and chloride concentrations exhibited no evident changes and continued in their previous decreasing trends (Fig. 1).

Plankton Development

The plankton of Rachelsee exhibited conspicuous changes both in space and in time during the decade under study. Bacterial filaments dominated (>80 %) plankton biomass in 1999 and 2003, when phytoplankton and rotifers were scarce in the epilimnion. Both phytoplankton biomass and rotifer abundance increased after 2005. Conspicuous changes in bacterial morphology suggested that a certain shift in the bacterioplankton composition was very likely (Fig. 3).

Species composition of both phytoplankton and zooplankton remained almost unchanged since our first (1999) and second (2003) sampling of the lake (cf. Vrba et al. 2003a and Nedbalová et al. 2006, respectively). Besides the significant increase in epilimnetic phytoplankton biomass (Table 1), the most remarkable changes were documented

in the vertical distribution of the phytoplankton as well as in its relative composition in the 2006–2009 period (Fig. 4). Until 2005, the phytoplankton formed deep water maxima typical for a clear-water lake. While *Carteria* sp. (Chlorophyta) dominated the bottom maximum in 1999, *Peridinium umbonatum* (Dinophyceae) peaked above the bottom in 2003. The latter species and/or another dinoflagellate, *Gymnodinium uberrimum*, were the common algal dominants of phytoplankton during the whole study (Fig. 4). In addition, filamentous cyanobacteria (mostly *Pseudanabaena* sp.) and chrysophytes (*Dinobryon* spp., *Synura sphagnicola*) have increased their contribution to phytoplankton biomass at certain depths since 2006 (Fig. 4).

Zooplankton were very species poor and scarce in abundance at the beginning of this study (in 1999 and 2003). Besides several littoral chydorid and copepod species (see Table 4 in Vrba et al. 2003a), crustacean open-water dwellers were entirely absent until 2009, when a few specimens of *Ceriodaphnia quadrangula* were sampled for the first time and confirmed again in 2011. Until 2009, the only zooplankters of the open water were the acidotolerant rotifer species *Keratella serrulata*, *Brachionus sericus*, and *Microcodon clavus*, occurring in low numbers in 1999 and 2003 (no data available from 2004 to 2005). Yet, we have recorded outbreaks of the latter species since 2006, when *M. clavus* abundance increased by several orders of magnitude (Fig. 3) and formed a rather unique zooplankton assemblage in the epilimnion. Two other acidotolerant species, *S. tremula* and *Collotheca pelagica*, have occasionally been observed in deeper layers since 2008 (not shown).

DISCUSSION

Effects of Forest Die-Back on Lake Chemistry

Our study presents the effects of a large-scale natural disturbance on both chemical and biological recovery of a lake ecosystem during a period of considerably reduced acidic deposition (Kopáček et al. 2012). The obvious trend in recovery of lake water chemistry was interrupted by the forest die-back in the entire catchment of Rachelsee in 1999, which caused the temporary peaks in concentrations of nitrate, Al_i , and base cations (Fig. 1). On the other hand, the rapid improvement in lake water quality in the mid-2000s was likely triggered by intensive natural forest regeneration (Heurich 2009). This study indeed suggests an instant response of plankton to the rapidly recovering lake water chemistry.

Among the Bohemian Forest lakes, a similar temporary increase in nitrate concentrations related to lowered

Fig. 4 Changes in the vertical distribution and taxonomic composition of phytoplankton biomass (as carbon) in Rachelsee during the studied period. Note the different scaling of either top panel compared to the same scaling of all others; vertical bars the Secchi depth on sampling dates

vegetation vigor was described by Veselý et al. (1998a) in Prášílské Lake, because of extensive forest die-back and salvage logging that occurred in its catchment in the late

1980s. The authors suggested that reduced adsorption surfaces of broken and defoliated canopy could have substantially reduced atmospheric inputs of acidic pollutants to

the lake catchment. Moreover, logging and deterioration of forest vitality led to a decrease in biological consumption of N and an increase in mineralization of litter within the catchment, explaining the increase in nitrate concentrations in Prášilské Lake after 1990 (Veselý et al. 1998a). While this case occurred in the contemporary managed spruce forest and only in a part of the Prášilské catchment during the peak of acidic depositions, our present study focused on the large-scale natural disturbance in the Rachelsee catchment during the period of already reduced acidic depositions. Thus, there were remarkable differences in the effects of forest die-back on lake water chemistry in either case. Veselý et al. (1998a) observed a ~ 10 -year increase in nitrate concentrations by a factor of ~ 2 , but a stable decrease in Al concentration (Veselý et al. 1998b) in Prášilské Lake, whereas we recorded a ~ 5 -year nitrate peak by a factor of ~ 3 in Rachelsee, accompanied with elevated aluminum concentrations (Fig. 1). Why did the similar effects of forest die-back on lake chemistry differ?

The forest die-back caused a significant reduction in horizontal sulfur and nitrogen depositions in the Prášilské catchment, because dry deposition to the canopies exceeded wet deposition of sulfur and nitrogen by factors of 3.7 and 2.4, respectively, in the 1980s (Kopáček et al. 2012). Thus, reduced acidic deposition to a large extent mitigated the elevated terrestrial export of nitrate and the resulting leaching of strong acid anions (sum of sulfate, nitrate, and chloride) slowly decreased, as well as concentrations of Al_i . In addition, human interventions, such as quick reforestation and partial bark beetle combat, in the remnant forests in the Prášilské catchment may have partly diminished the severity of nitrate leaching.

In contrast, the effect of massive defoliation on acidic deposition was substantially lower in the Rachelsee catchment almost two decades later, when dry deposition represented only circa 70–100 % of wet deposition of sulfur and nitrogen compounds (Kopáček et al. 2012). Under such conditions, elevated nitrate leaching had a relatively high effect on terrestrial export of strong acid anions, which rose from 120 to 195 $\mu\text{eq L}^{-1}$ between 1999 and 2002. Almost 70 % of this difference was compensated for by elevated Al_i concentrations in Rachelsee. Because sulfate concentrations continued in their gradual decrease throughout the study (Fig. 1), the abrupt drop in nitrate leaching associated with natural forest regeneration after 2005 (Heurich 2009) has “switched off” the elevated terrestrial export of strong acid anions and Al_i , and was followed by an immediate response of the lake ecosystem.

Biological Recovery of Rachelsee

Based on the surveys of all Bohemian Forest lakes in 1999 and 2003, Rachelsee was included in the group of strongly

acidified lakes with severe phosphorus limitation and characterized by very low plankton biomass with dominance of heterotrophic filaments (Vrba et al. 2003a; Nedbalová et al. 2006). This study documents the sudden change in its lake water chemistry, as well as in its plankton biomass structure (Figs. 2, 3). As bacteria are considered to be superior competitors to phytoplankton for phosphorus (Currie and Kalff 1984), the shift from the dominance of heterotrophic to autotrophic organisms indicates that the plankton has been substantially released from severe phosphorus stress due to reduced aluminum–phosphorus interaction (Kopáček et al. 2004; Vrba et al. 2006). To our knowledge, such a rapid change in plankton structure in a lake recovering from acidity has not been previously documented.

A substantial loss of heterotrophic filaments was observed despite the absence of cladoceran filtration (cf. Vrba et al. 2003b, 2006; *Ceriodaphnia quadrangula* was first recorded in Rachelsee as late as 2009 and has never been abundant). Therefore, it was probably associated with changes in lake water chemistry and/or with the hundred-fold increase in rotifer abundance since 2006 (Fig. 3; unfortunately, rotifers were not quantitatively sampled in the critical years 2004 and 2005). Yet mixotrophic flagellates from the genus *Dinobryon*, which can induce filament formation (cf. Vrba et al. 2003b), were present on all sampling dates. The possible effect of the rotifer *M. clavus* upon bacteria and small algae is not known, but its abundant population (grazing on small bacteria) could have caused more intense phosphorus regeneration in the epilimnion and, consequently, its better availability for phytoplankton (cf. Vrba et al. 2006) since 2006. An additional supply of phosphorus might have come from higher terrestrial export as recent volume-weighted mean concentrations of P_t in the inlet water increased by some 50 % compared to the period 1999–2005. Similar elevated phosphorus export from catchments affected by forest die-back has recently been observed elsewhere in the Bohemian Forest (Kopáček, unpublished data).

The increase in phytoplankton biomass was not accompanied by changes in species composition (cf. taxonomic resilience of recovering lakes in Graham et al. 2007). The dinoflagellates *P. umbonatum* (often reported as *P. inconspicuum*, which is currently regarded as a taxonomic synonym of *P. umbonatum*) and *G. uberrimum* are common dominants in phytoplankton of acidified lakes elsewhere (e.g., Almer et al. 1978). However, the importance of cyanobacteria and chrysophytes in the period 2006–2009 could indicate changes in phytoplankton structure in relation to chemical recovery and replenished DOC level (cf. Graham et al. 2007). It was experimentally shown that *P. umbonatum* can rapidly decline in response to increasing pH and phosphorus availability (Avalos Perez

et al. 1994). In addition, the shift from hypolimnetic to epilimnetic phytoplankton maxima can also be regarded as a clear sign of lake recovery, likely reflecting both the above-mentioned increase in phosphorus availability and an obvious decrease in light availability (see z_S in Table 1). As in other Bohemian Forest lakes, aluminum plays the key role in controlling phosphorus availability for algae (Vrba et al. 2006), rather than through its phytotoxicity as suggested by Graham et al. (2007) for recovering Canadian boreal lakes.

After two decades of lake recovery, however, the zooplankton of Rachelsee have remained rather poor and exceptional. Recently, the zooplankton assemblage have been dominated by *M. clavus*, an acidotolerant rotifer, which was first recorded in 1985 (Fott, unpublished) and likely survived the peak of atmospheric acidification in the lake (Vrba et al. 2003a). Living in submerged peat mosses or among water plants (Koste 1978) it is not regarded as a pelagic rotifer. In Rachelsee, it has taken advantage of an empty niche in open water enabled by both weak competition for resources and the absence of predators. The occurrence of *M. clavus* in the open water of acid-damaged lake is in agreement with the earlier report of Fott et al. (1994). The more recent (since 2008) occurrence of two larger acidotolerant rotifers, *S. tremula* and *Collotheca pelagica*, in Rachelsee may be considered as a response to both increasing food resources and an improving environment.

Unlike Rotifera, a historical record on Crustacea from Rachelsee is available. Frič (1874) and Hellich (1877) recorded three open-water species: *Daphnia* gr. *longispina*, *Cyclops abyssorum*, and *Heterocope saliens* (names according to the current nomenclature), which had disappeared (Vrba et al. 2003a) and are absent yet. On the other hand, we have found *Ceriodaphnia quadrangula*, not recorded by Frič (1874) or Hellich (1877). This species has likely been spread by a bird vector (e.g., by common ducks, which frequently occur at most water bodies in the region, including Rachelsee). Though we can document several more or less remarkable changes in the plankton of Rachelsee during this study, when assessed by a historical benchmark (Gray and Arnott 2009), there has been hardly any zooplankton recovery observed as no indigenous or circum-neutral species have appeared so far. Despite the indisputable amelioration of chemical conditions, the lake still maintains its acidic status. Our study has confirmed the regional tendency of some littoral species, namely *C. quadrangula* and *M. clavus* (Nedbalová et al. 2006), to fill empty niches (Keller and Yan 1998; Gray and Arnott 2009).

Given the obvious positive trend in lake water chemistry until the late 1990s (Schaumburg 2000), the effect of forest die-back caused a considerable delay in both the chemical

and the biological recovery of Rachelsee, on the one hand, and certain gain of its plankton recovery, on the other hand. When the enhanced terrestrial export of nitrate and aluminum suddenly stopped, the in-lake phosphorus inactivation by aluminum (Kopáček et al. 2004) dropped, possibly increasing phosphorus availability (Vrba et al. 2006), and switched over the contrasting plankton composition within a year or two. If the forest die-back did not occur, inter-annual variability in plankton biomass and composition together with gradual changes in pelagic food webs would have probably superimposed such an evident plankton response.

CONCLUSIONS

Many environmental drivers and biological constraints of lake recovery from acid stress have been debated. We examined long-term data on water chemistry of Lake Rachelsee following the world's largest changes in acidic depositions in central Europe since the 1980s. Despite gradual chemical recovery of Rachelsee, however, its biological recovery was delayed. Lake Rachelsee lies in the core zone of the National Park Bavarian Forest, where a strict non-intervention regime has been applied. This excludes any intervention oriented on improvement of the acidic status of the lake. On the contrary, this circumstance provides an opportunity to study the natural process of recovery determined by large-scale operating factors, such as atmospheric depositions or climate change. On the other hand, biological recovery of a lake from acid stress always should be considered in the context of actual deposition rates, other environmental conditions, and vegetation status in its catchment.

Rachelsee remained the most severely acidified lake in the Bohemian Forest in 1999, when both regular sampling of its plankton and bark beetle outbreak in its catchment started. Then, lake recovery was abruptly reversed by a coincident natural forest die-back in its catchment, which resulted in elevated terrestrial export of nitrate and ionic aluminum, lasting ~5 years. This re-acidification episode provided a unique background for the case study, showing prompt plankton recovery in the lake after the rapid decline in nitrate leaching due to natural reforestation of its catchment. There were sudden changes in both lake water chemistry and plankton biomass structure, such as decreased bacterial filaments, increased phytoplankton biomass, and rotifer abundance. The shift from dominance of heterotrophic to autotrophic organisms likely suggested their substantial release from severe phosphorus stress. Yet the three original open-water species of Crustacea, which were extirpated due to acidification, are absent. On the other hand, we proved a tendency of some littoral species,

namely *Ceriodaphnia quadrangula* and *M. clavus*, to fill the empty niches. Such a rapid change in plankton structure in a lake recovering from acidity has previously not been documented.

Acknowledgments This study was supported by several CSF projects (particularly No. 206/07/1200, P504/12/1218). Burkhard Beudert, Claus Bässler, and Heinrich Rall (NPBW) assisted with lake sampling, Ludwig Butz (WWA Passau/Deggendorf) kindly provided data (2000–2002) on lake chemistry, and Keith R. Edwards revised the manuscript.

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