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Effects of Mechanical Mixing on Lake Water Quality, with Special Emphasis on Under-Ice Phytoplankton

JYVÄSKYLÄN YLIOPISTO

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ABSTRACT

How do phytoplankton develop under changing circumstances in an ice–covered lake? This was the key question that triggered this study and my interest in the neglected transition period from ice-covered to open-water season. Eight years of data from the Enonselkä basin of Vesijärvi, a eutrophic boreal lake, showed that, even though their mid-winter biomasses were low, phytoplankton grew rapidly under the spring ice and often reached biomasses higher than those recorded during the open-water season. Under-ice light and mixing regimes reflected stochastic weather variations and controlled phytoplankton biomass, composition and distribution.

To relieve oxygen depletion in the deepest water of the Enonselkä basin during winter and summer stratification, large-scale mechanical mixing was applied during 2010–2014. Consequently, oxygen conditions in the basin improved, but nutrient levels were not noticeably affected. Because no causality could be shown between oxygen depletion and leaching of nutrients into the deepest water during winter and summer, the main reasons for high nutrient concentrations in the basin should be re-evaluated. The results suggest that the accumulation of nutrients in the deepest water is controlled by the present lake productivity and sedimentation rather than by internal loading due to nutrient leakage from older sediments. It is proposed that the spring phytoplankton maximum is the most significant single phytoplankton event in the basin and that its effect extends to the oxygen and nutrient dynamics during the following summer.

Keywords: Phytoplankton; diatoms; ice; convection; lake restoration; mechanical mixing; Vesijärvi.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–IV.

PS and KS planned all the studies and did the field work together. PS wrote the first drafts for I–III and improved them together with KS. PS counted the phytoplankton samples in II–III and part of the phytoplankton samples in IV. PS analysed data for all the papers. IM helped to gather and analyse data for I. AL counted bacterioplankton samples for II. KS wrote the first draft of IV and JK counted part of the phytoplankton samples related to it. HH assisted in statistical analyses and PS coded the simulation software for IV.

I   Salmi P., Malin I. & Salonen K. 2014. Pumping of epilimnetic water into hypolimnion improves oxygen but not necessarily nutrient conditions in a lake recovering from eutrophication. *Inland Waters* 4: 425–434.

II Salmi P., Lehmijoki A. & Salonen K. 2014. Development of picoplankton during natural and enhanced mixing under late-winter ice. *Journal of Plankton Research* 36: 1501–1511.

III Salmi P. & Salonen K. 2015. Regular build-up of the phytoplankton spring maximum before ice-break in a boreal lake. Submitted manuscript.

IV Salonen K., Salmi P., Högmander H. & Keskitalo J. 2015. An improved approach to phytoplankton counting based on real-time statistics. Manuscript.

1 INTRODUCTION

1.1 Phytoplankton in boreal lakes

The term "boreal" in the context of ecosystems refers to "northern". Based on vegetation, the boreal zone is located to the south from where the treeless tundra begins and gradually changes to temperate where the coniferous forests start to change to deciduous forests (e.g. Ahti *et al*. 1968, Larsen 1980). Northern water bodies are exposed to seasonally changing physical conditions, but, irrespective of light, the contrast between summer and winter is less pronounced than in terrestrial ecosystems. In winter, ice cover is the dominant feature of boreal lakes virtually isolating lakes from the atmosphere. Gas and energy exchange between water and atmosphere are then largely prevented. However, in late winter increasing insolation melts snow and ice so that water temperature increases and under-ice convective mixing begins (Matthews and Heaney 1987, Bengtsson 1996), whereby warmer and heavier water parcels sink to a depth with a similar water density. After the ice-break, when water temperature exceeds 4 °C, the density of the warming surface water decreases until wind cannot mix the water column and summer thermal stratification is established. When surface waters start to cool in autumn, convection again contributes to mixing, but in large lakes wind has the main role, finally leading to overturn of the water column. Along with this temperature-controlled basic framework, other factors, such as lake morphology, chemical properties in the water column, events in the lake's catchment and highly stochastic weather, introduce high variability into the conditions in boreal lakes.

The physical framework, largely defined by temperature, also underlies a succession in the structure and function of plankton. Phytoplankton, which is generally the basis of pelagic food webs, is a very diverse group of photosynthetic micro-organisms living suspended in water. However, many phytoplankton taxa are mixotrophic, which means that they are also capable of heterotrophic growth (Tranvik *et al*. 1989, Jones 2000). Phytoplankton consist of different functional types, and prevailing environmental conditions play key

roles in steering which kind of functionality is favoured at any time (Reynolds 1984, Reynolds *et al.* 2002). Phytoplankton exhibit a wide range of cell size, from a diameter < 2 µm in picophytoplankton to 2–20 µm in nanophytoplankton and > 20 µm in microphytolankton. The phytoplankton production and biomass that the system is able to sustain is closely related to nutrient concentrations (Kalff and Knoechel 1978). Phosphorus is very important although it has no natural biological removal processes, unlike nitrogen, the other important macronutrient for phytoplankton (Wetzel 2001).

1.1.1 Seasonal succession

In boreal and temperate lakes, the first open-water phytoplankton biomass maximum generally occurs in early summer (Sommer *et al.* 2012). After the development of temperature stratification, the epilimnion becomes depleted of limiting dissolved nutrient. Phytoplankton, especially diatoms sedimentate efficiently in the absence of vigorous mixing, which, together with zooplankton grazing, leads to a decline in biomass. In late summer, when temperature stratification starts to erode, nutrients from the hypolimnion become available and another biomass maximum can develop (Lampert *et al.* 1986, Sommer *et al.* 2012).

In large lakes wind can create turbulence which keeps phytoplankton suspended in the water. This is most important for diatoms with heavy silica cell walls. Light availability to phytoplankton depends on the depth of the mixing layer, but diatoms are often able to cope with relatively low light intensity and temperature (Huisman *et al.* 2004, Reynolds *et al.* 2006). Steeply stratified water column may favour motile phytoplankton species which avoid sedimentation and can even migrate to deeper water layers to retrieve nutrients (Salonen *et al.* 1984, Watanabe *et al.* 1991, Salonen and Rosenberg 2000). Buoyant cyanobacteria with gas vesicles often become dominant in late summer (Robarts and Zohary 1987). Therefore, they may shade other photosynthetic organisms dwelling deeper in the water column. Cyanobacteria typically have high temperature optima and tolerance of high pH (Paerl *et al.* 2001, Lopez-Archilla *et al.* 2004) and some species are able to utilise N_2 as a nitrogen source by nitrogen fixation (Mitsui *et al.* 1986, Robarts and Zohary 1987, Paerl *et al.* 2001). These generalized patterns in the seasonal succession of phytoplankton are often largely modified by local conditions and hence other phytoplankton groups may also be prominent.

When conditions are favourable phytoplankton can develop high biomass, which may be harmful in many ways. Cyanobacteria not only cause aesthetic problems, but they may excrete neuro- or hepatotoxins into water (Sivonen *et al.* 1990). Freshwater diatoms do not typically produce toxins, but may develop harmful high biomass during mixing periods. They may also grow attached on surfaces and hamper fishing traps and nets (Lappalainen and Hilden 1993, Vuorio *et al.* 2015). Degrading phytoplankton biomass may cause smell and aesthetic problems and also consumes oxygen, particularly in hypolimnion, which may critically affect the biota and nutrient cycling in lakes (Gächter and

Wehrli 1998, Paerl *et al.* 2001). In addition to cyanobacteria and diatoms, several other groups may also develop mass occurrences.

1.1.2 The role of winter in the development of phytoplankton

For many reasons, studies of lake phytoplankton have mainly focused on the open water season. In addition to the practical problems of sampling in winter, it has been widely assumed that phytoplankton, which are primarily photosynthetic organisms, do not grow significantly in the cold and dark under-ice water. The periods of ice formation and breakage, when changes in the physical and chemical conditions of the water column are rapid and dramatic, belong to the least studied periods of the seasonal cycle in lakes. For phytoplankton many key issues related with overwintering and spring proliferation are not well known. Both qualitative and quantitative aspects of related to winter would serve for better understanding of the complete annual cycle of phytoplankton. This need is more pressing as changing climate affects the duration and extent of ice-cover on lakes (Adrian *et al.* 2009, Salonen *et al.* 2009, McKay *et al.* 2011, Bertilsson *et al.* 2013, Hampton *et al.* 2015).

Solar radiation passes well through clear ice, but snow-ice and especially snow on the top of ice efficiently reflect light so that only little penetrates into water (Bolsenga and Vanderploeg 1992). During winter in boreal lakes, light is the most important factor limiting growth of phytoplankton (Eloranta 1982, Jewson *et al.* 2009). In midwinter under-ice phytoplankton might also be able to grow heterotrophically (Rodhe 1955, Maeda and Ichimura 1973, McKnight *et al.* 2000).

In spite of the lack of wind-forcing driving turbulence and circulations, ice-covered boreal lakes are not totally stagnant. Density gradients created by differences in temperature or salinity and their relation to the bottom topography cause water movements under the ice. In lakes cooled below 4 °C, heat flux from the sediment creates horizontal density currents along lake slopes that also cause ascending compensatory flows over the deepest parts of a lake (Bengtsson 1996). This large scale mixing might be strong enough to keep some phytoplankton, even diatoms, suspended in the water column (Kiili *et al.* 2009).

In late winter, at the onset of under-ice convective mixing, incoming insolation allows photosynthesis. Phytoplankton start to increase already well before the ice-break, but their development under different spring mixing regimes has been described in few lakes (Jewson *et al.* 2009, Kiili *et al.* 2009, Vehmaa and Salonen 2009). Results from oligotrophic Finnish lakes Päijänne (Kiili *et al.* 2009) and Pääjärvi (Vehmaa and Salonen 2009) indicated that development of phytoplankton biomass, distribution and species composition were coupled to development of convective circulations. In spite of deep mixing, biomass of phytoplankton roughly doubled during the last few weeks of ice cover in both lakes. Based on 45 years of data on development of phytoplankton in a more eutrophic Lake Erken, Weyhenmeyer *et al.* (1999)

concluded that the spring phytoplankton maximum usually occurred after ice-break but sometimes developed earlier under ice.

1.2 Approaches to manage water quality to avoid mass occurrences of phytoplankton

Phytoplankton biomass is increased by increasing nutrient concentration which is typically due to loading from the catchment area by human activities. Thus, to avoid phytoplankton mass occurrences, various practices to diminish nutrient load have been suggested. Some of the most important are sewage purification and control of fertilizers leaching from agriculture. Nutrient load can also be controlled by technical arrangements such as artificial wetlands and pools to remove fine particles and dissolved nutrients (e.g. Braskerud *et al.* 2005, Reinhardt *et al.* 2005) or simply by leaving buffer strips between lakes and agriculture runoff. Chemical treatments to precipitate excess nutrients have also been applied at water inlets, artificial constructions or directly into the lake (e.g. Hullebusch *et al.* 2002, Egemose *et al.* 2010). The reduction of external loading of nutrients into lakes is generally considered the primary target, but when it is too expensive or too slow, alternative approaches may also be required. Possibilities of different algicides, such as hydrogen peroxide, has also been studied (e.g. Drábková *et al.* 2007).

1.2.1 Oxygenation and aeration

In limnology, the term "internal loading" is used in the context of diffusion of nutrients from the lake sediment. Although the term is widely used, it is not really comparable with fresh external loading, and rather refers to recycling than to loading (Hupfer and Lewandowski 2008). The early works of Einsele (1938), Ohle (1938) and Mortimer (1942) and numerous subsequent empirical observations and laboratory experiments have led to the wide acceptance of a view that under anoxic conditions phosphorus bound to iron is released from sediments into water (Hupfer and Lewandowski 2008). However, several other mechanisms have also been found to be involved in phosphorus release (Hupfer and Lewandowski 2008).

Large amount of sedimenting degrading organic matter can lead to an extensive oxygen deficit in the deepest water or, in small or shallow lakes, in the whole water column. Even in large lakes, slow horizontal density currents cannot compensate for oxygen consumed in the deepest water. In fact, as such currents sweep along the bottom as a thin layer, anoxic water can accumulate from a large sediment area to limited deep sites in the lake where oxygen consumption becomes accentuated (Pulkkanen and Salonen 2013).

Internal loading typically refers to release and accumulation of phosphorus, but deep water anoxia also leads to accumulation of ammonium-nitrogen. Ammonium is an important growth-stimulating nutrient for

phytoplankton, but high concentration at high pH it is toxic to aquatic organisms (Beutel 2006, Camargo and Alonso 2006).

To prevent oxygen depletion and nutrient accumulation, different aeration and oxygenation methods have been developed. Aeration basically refers to injection of air into the anoxic deep water and oxygenation correspondingly to injection of oxygen. Arrangements may consist of different diffusers adjusted to preserve natural temperature stratification (Beutel and Horne 1999, Singleton and Little 2006). Sometimes simple mixing of oxygen-rich epilimnetic water with oxygen-poor deep water layers (Stephens and Imberger 1993, Lappalainen 1994, Kirke and El Gezawy 1997) has been applied.

1.3 Aims of the study

The main aim of this study was to reveal the poorly known development of phytoplankton, including picophytoplankton, under spring ice in a eutrophic boreal lake. The hypothesis was that the bulk of the phytoplankton spring biomass maximum often develops under the ice cover.

Possibilities to improve water quality and to avoid phytoplankton blooms in a eutrophic lake by pumping oxygen-rich epilimnetic water into the hypolimnion were explored. Effects of mechanical mixing were scrutinized with a special emphasis on the role of winter as a determinant of phytoplankton. It was hypothesized that mechanical mixing would keep the deepest water oxygenated during stagnation periods and thus reduce phosphorus and nitrogen concentrations and consequently also phytoplankton biomass. An alternative hypothesis was that mechanical mixing raises nutrients into the productive layer and further enhances growth of phytoplankton.

Because microscopic counting is a laborious task in phytoplankton research, emphasis was put on making counting as economical and precise as possible. It was hypothesized that real-time application of statistics allows optimization of counting so that results can be more objectively interpreted.

2 MATERIALS AND METHODS

2.1 Lake Vesijärvi

Lake Vesijärvi (Fig. 1) is located in southern Finland, between two glacifluvial eskers called the Salpausselkä ridges. The lake comprises 4 distinct basins, but this study was focused on the southernmost Enonselkä basin (area 26 km², mean depth 6.8 m and maximum depth 33 m) which is connected to the other basins by a relatively shallow strait. The basin residence time is up to 5 years.

Since the beginning of the 20th century, the human impact on Vesijärvi increased tremendously. Due to the nearby city of Lahti, the Enonselkä basin (hereafter Enonselkä) has been the most heavily loaded basin of Vesijärvi. Until 1976, untreated municipal and industrial wastewaters were discharged into Enonselkä. Consequently, phytoplankton was dominated by cyanobacteria, oxygen was depleted in the hypolimnion and the fish community shifted to the dominance of cyprinids. After sewage diversion in 1976, the basin started to recover (Keto and Tallberg 2000), but to support the recovery various additional management efforts were carried out. Oxygenation by mechanical mixing was first tested in 1979–1984, but the subsequent mass occurrence of toxic *Planktothrix agardhii* (Gomont) Anagostidis and Komarék discouraged its continuation. Biomanipulation by removal of cyprinid fish was first implemented in the late 1980s in order to reduce their grazing pressure on zooplankton (Kairesalo *et al.* 1999) and has been continued ever since. During 1989–2012 (years 2007–2008 data missing from the sequence), the annual amount of fish removed has been 30–270 metric tonnes (average 94 metric tonnes). Based on monitoring with echo sounding and fish samples, Malinen *et al.* (2013) estimated that the maximum fish biomass in the areas deeper than 6 m was 73–374 metric tonnes in summers 2009–2010. However, their study did not cover the shallowest littoral areas where large amounts of cyprinids might occur (M.Sc. Tommi Malinen, University of Helsinki, pers. comm.).

To further support the recovery of the lake, Lahti Region Environmental Services together with the Lake Vesijärvi Foundation started an open-ended

project in which deep water oxygen concentration is increased by basin-scale mechanical mixing.
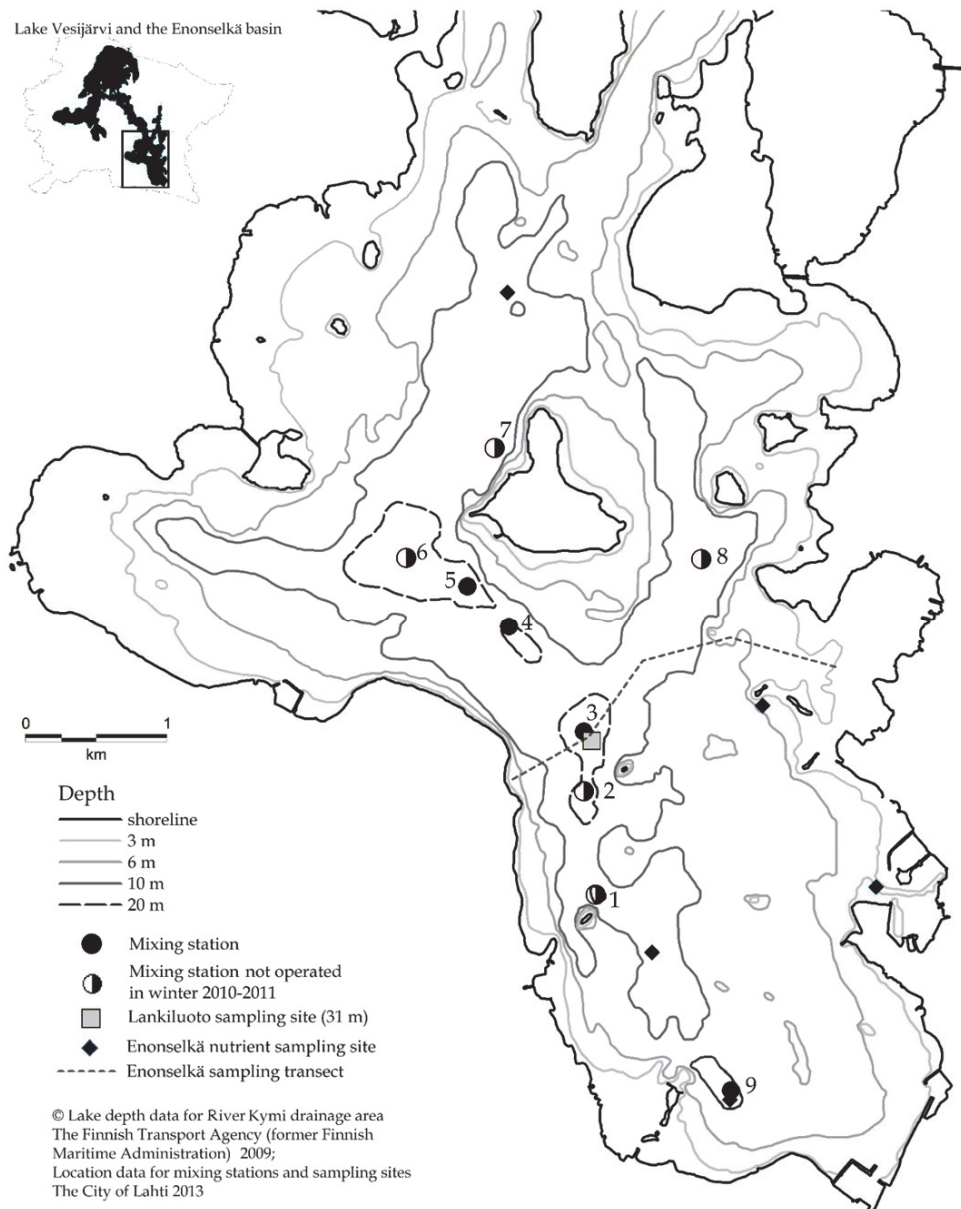

FIGURE 1   Map of the study area with location of mixing stations and sampling sites.

2.1.1 Oxygenation by mechanical mixing

Nine mixing stations (eight 2.5 kW and one 1.5 kW, Water-Eco Ltd., Finland) were installed in the deepest parts of Enonselkä (Fig. 1) in autumn 2009. A mixing station consists of a pump located at 3 m depth. Oxygen-rich surface water is pumped through a canvas tube, whose lower end is 8–10 m above the sediment to minimize its resuspension. The speed of the current inside the tube of a 2.5 kW mixing station is 1 m s^{-1}. Since winter 2009–2010, the mixing stations have been operated year-round, except during the spring and autumn overturn periods, with occasional stoppages due to malfunctions or for experimental purposes (Table 1). Under the ice, when the oxygen-rich and cold surface water emerged from the canvas tube, it starts to rise through the warmer and heavier deep water. This results in mixing so that the ascending water mass gains heat and its density increases and, therefore, it never reaches its original depth (I). Similarly, in summer the flow of warmer and lighter, epilimnetic water mixes with cooler hypolimnetic water and ascends until it meets the water layer with equal density, in practice below the thermocline. Thus in summer, the mixed water volume is much smaller than in winter. The functional principle of the mixing stations is described in more detail by Lappalainen (1994) and Bendtsen *et al.* (2013).

TABLE 1   Dates when the mixing stations in the Enonselkä basin were turned on and off.

Year	Dates and changes in the status of the mixing stations
2009	8 Dec (on)
2010	5 May (off), 3 Jun (on), 27 Sep (off)
2011	15 Jan (on), 19 Apr (off), 6 Jun (on), 26 Jul (off), 2 Aug (on), 9 Sep (off)
2012	13 Jan (on), 27 Mar (off), 4 Jun (on), 13 Aug (off), 20 Aug (on), 7 Sep (off), 28 Dec (on)
2013	19 Apr (off), 29 Jul (on), 20 Sep (off)
2014	21 Feb (on), 16 Apr (off), 4 Jun (on), 25 Sep (off)

2.2   Measurements and sampling

Since the 1960s, the Finnish Environment Institute, its local counterparts and Lahti Region Environmental Services have coordinated a monitoring programme for Lake Vesijärvi, and the results are saved in the database of the Finnish Environment Institute (https://wwwp2.ymparisto.fi/scripts/oiva.asp). When evaluating the effects of mechanical mixing on oxygen and nutrient conditions (I) only results from 2000–2014 were scrutinized to minimize the

effect of any other major factors, such as climate change (Anon. 2014) or the still slowly continuing nutrient reduction following sewage diversion (Keto and Tallberg 2000).

In winters 2007–2014, temperature profiles were measured (hourly or every half an hour) with temperature recorders (Star–Oddi, accuracy 0.05 °C) installed in chains at depth intervals of 5 m (late winter 2007) or 2 m (throughout the other years). During sampling (II and III) in late winter 2009, detailed temperature profiles were measured with a Micro-CTD-3 (Falmouth Scientific, USA, accuracy 0.005 °C). In winters 2011, 2013 and 2014, temperature profiles were measured with a CastAway-CTD (Yellow Springs Instruments, USA, accuracy 0.05 °C). In winters 2013–2014, an XRX-620-CTD (RBR, Canada, accuracy 0.002 °C) was used. Oxygen samples were taken with a Limnos tube sampler (volume 2.1 l) into ca. 100 ml glass bottles with ground-glass stoppers. Oxygen concentration was determined from 50 ml subsamples by Winkler titration. In winters 2010–2012, oxygen profiles were also measured with an optical Pro-ODO-sensor (Yellow Springs Instruments, USA, accuracy 0.1 g m^{-3}) and in winters 2013–2014 with an optical Rinko III (JFE Advantech, Japan, accuracy 2 % of full scale) detector attached to the XRX-620-CTD. In both cases the results were calibrated from Winkler-titrated samples. Ice thickness and snow depth were measured with a gauge and Secchi-depth by using the white upper lid (diameter 11 cm) of the Limnos tube sampler. When the ice cover was weak, a hydrocopter (air boat) was used to reach the sampling sites.

In years 2009–2011, samples of picophytoplankton and bacterioplankton (II) were taken with the Limnos-tube sampler as a vertical series from surface to bottom with 2–10 m depth intervals and poured into 100-ml brown glass bottles. Picophytoplankton and bacteria were sampled once in January–March and roughly weekly in March–April. Samples immediately below the ice were taken with a rectangular 50 x 50 x 750 mm box to avoid disturbance caused by drilling of the ice. The box, equipped with a rolling brush at its front end to detach algae from the bottom of the ice, was pushed horizontally under the ice. Finally its rear end was closed by the palm of a hand and the sample was rapidly transferred into a plastic bucket. Unpreserved picoplankton samples were transported to the laboratory in slush ice. In the laboratory, 5 ml portions of water were filtered onto black polycarbonate membrane filters (pore size 0.2 µm, Millipore, U.S.A.). Filters were dried in air and placed on an objective glass with a drop of non-fluorescent immersion oil on top. The filters were then covered by a cover class and stored at –20 °C. Bacterioplankton samples were treated similarly, except that the filtered sample volume was 1 ml and the filters were stained with acriflavine dye for 1 min (Bergström *et al.* 1986).

In years 2007–2014, phytoplankton samples (III) were taken with the Limnos-tube sampler as a vertical series with 2–10 m depth intervals. Phytoplankton were typically sampled once in January–March and at least weekly in March–April before the ice-break. However, because the development of phytoplankton could not be predicted, sometimes samples were taken with shorter time intervals, and those were later used for counting according to the need seen from the results of already counted samples. Similar

optimization strategy was used in counting of vertical samples. Samples were preserved with 1 ml of Lugol's solution in brown 100-ml glass bottles. Samples immediately below ice were taken as described above for picoplankton.

2.3 Microscopic counting

Picophytoplankton and bacteria (II) were counted using an epifluorescence microscope (Olympus IX50, Olympus Optical Co. Ltd, Japan). For eukaryotic or cyanobacterial picophytoplankton, a blue or green excitation filter set was used, respectively (MacIsaac and Stockner 1993). Bacteria were counted under blue excitation in 10 replicate fields. To reach appropriate precision a computer program was developed which provided real-time statistics. This allowed the number of counted microscopic fields in picophytoplankton counting to be adjusted so that the 95 % confidence interval was ≤ 30 % of the mean total biomass. In sparse samples, however, wider confidence intervals were accepted. Picophytoplankton as well as bacteria were counted in different size classes to allow more reliable total biomasses to be calculated. If difference in a dimension between two individuals were observable utilizing an ocular micrometer, a new size class was established.

Larger phytoplankton (III) were counted with the settling chamber method of Utermöhl (1958) according to Anon. (2006). Inverted microscopes with phase contrast optics (Nikon Diaphot-TMD, Japan, Carl Zeiss, Germany, Olympus IX60, Japan or Wild M40, Switzerland) were used in counting. The volume of settled sample varied from 2.5 ml to 100 ml depending on the abundance of phytoplankton. The goal was to have 5–20 phytoplankton units (cells, filaments or colonies) per counted microscopic field, but in some samples with abundant small diatoms and counted with a wide-field Olympus microscope, a larger number had to be accepted. Two or three different magnifications were used depending on the size distribution of phytoplankton. To estimate the counting effort per sample required to reach adequate precision of total biomass estimate, we used real-time confidence intervals in the same way as when counting picophytoplankton. In practice, 10–50 randomly selected microscopic fields or 3–4 transects were counted. Dimensions of phytoplankton cells were estimated with an ocular micrometer. Biovolumes were calculated from linear dimensions and approximate geometric shapes of cells, and were converted to wet masses by assuming a density of unity.

Phytoplankton wet mass was converted to carbon assuming that ash free dry mass comprises 20 % of phytoplankton wet mass and that 50 % of the ash free dry mass is carbon (Reynolds 2006).

2.4 Statistical methods

Statistical tests were done with SPSS Statistics 20 or 21 (IBM, USA). A paired t-test was used to test differences between two related groups (I, II) and Student's t-test was used to test differences between two independent groups (IV). Prior to all tests, Shapiro–Wilk's test was applied to test normality and Levene's test was used to test equality of variances of the groups involved in tests. If the assumptions of the paired t-test were not fulfilled Wilcoxon's related-samples signed rank test was chosen and if the assumptions of Student's t-test were not met, Mann–Whitney's U test or Kruskal–Wallis test was chosen (IV).

The limits of 95 % confidence intervals (±cfl% of the mean) for mean phytoplankton counts (II–IV) per microscope field (or transect) were calculated as:

$$cfl\% = \frac{100 * t_{0.025} * \sqrt{s^2/n}}{mean}$$

where $t_{0.025}$ is the 97.5 % percentile of the t-distribution with n-1 degrees of freedom, s^2 is sample variance and n is the number of replicate microscope fields or transects. Similarly, the limits of confidence intervals for mean wet mass (II, III) of a magnification were calculated as:

$$cfl\% = \frac{100 * t_{0.025} * \sqrt{\sum s^2/n}}{mean}$$

where $t_{0.025}$ is the 97.5% percentile of the t-distribution with n-1 degrees of freedom, $\sum s^2$ is sum of the variances of biomasses of different size classes and n is the number of replicates.

Confidence intervals for total abundance and biomass obtained at different magnifications were combined after weighing the confidence intervals by their proportions in total abundance or wet mass.

The adequacy of confidence intervals produced in different ways were evaluated (IV) using under-ice samples from Enonselkä as well as summer samples from different lakes in southern Finland (IV).

3 RESULTS AND DISCUSSION

3.1 Effects of mechanical mixing

3.1.1 Temperature and mixing regimes

During 1999–2014 the average ice formation date of Enonselkä was 14 December (SD = 19 d) and average date of ice-break was 26 April (SD = 7 d, Table 2). According to the long time series of ice formation and ice-break dates since 1908 (data from Lahti Region Environmental Services), there was an increase in the frequency of late ice formation and early ice-break since the beginning of 20th century. After 1908 the basin has frozen in 3 years in January and all these years are after 2006. Additionally, 2 of the 3 earliest ice-breaks have also occurred after 2006. It appears that over 106 years the average ice formation date has been delayed by 9 d and the ice-break date advanced by 12 d. These values indicating the changing climate (Adrian *et al.* 2009) are similar to those reported from various lakes in the northern hemisphere (Kirillin *et al.* 2012).

In Enonselkä, the combination of air temperature and wind conditions determines the time of ice formation and also the final water temperature before freezing. Typically the whole basin cools below 1 °C, but the observed minimum in the whole water column was as low as 0.1 ˚C (Fig. 2). In fact in some years the topmost water layers became super-cooled (Fig. 2). After the formation of ice-cover heat flux from the sediment quickly warmed water and in 2009, when the mixing stations were not yet installed, after roughly a month the deep water temperature exceeded 3 °C (Fig. 2). These results suggest that, in the absence of temperature measurements just before ice formation and during the period of weak ice, transient low temperatures have probably been generally missed in medium size lakes which are susceptible to wind mixing. Mechanical mixing during winter distributed heat flux from the sediment more efficiently in the water column (Fig. 2) so that vertical temperature gradient was reduced and the temperature in the deepest water stayed below 3 °C. The water column temperature at the time of ice formation explained most of the variation

in maximum heat content (24–33 MJ m^{-2}) of water in the basin before the beginning of convection (Fig. 3). Although there was only one observation year before mechanical mixing, its heat content was not different from those of the mixing years. It is reasonable, because the release of heat from the sediment is finite and mostly happens in the early part of the ice-covered period (Terzhevik *et al.* 2009). Because the deepest part of Enonselkä is next to a large glacifluvial ridge formation, ground water might theoretically also enter the basin. However, 30 % of the annual accumulation of ground water in Lahti and its neighbourhood is used by the community. In places, direction of flow might rather be out from than into the lake due to the natural properties of the area (data from Lahti Region Environmental Services).

TABLE 2   Ice formation and ice-break dates and lengths of the ice–covered period in Enonselkä in years 1999–2014.

Winter	Ice formation	Ice-break	Duration (days)
1999–2000	9 Dec	25 Apr	137
2000–2001	26 Dec	25 Apr	120
2001–2002	20 Nov	24 Apr	155
2002–2003	13 Nov	9 May	177
2003–2004	17 Dec	27 Apr	131
2004–2005	28 Nov	28 Apr	151
2005–2006	14 Dec	6 May	143
2006–2007	12 Jan 2007	14 Apr	92
2007–2008	14 Dec	21 Apr	128
2008–2009	25 Dec	29 Apr	125
2009–2010	14 Dec	28 Apr	135
2010–2011	26 Nov	1 May	156
2011–2012	8 Jan 2012	30 Apr	112
2012–2013	5 Dec	4 May	150
2013–2014	14 Jan 2014	12 Apr	88
Average	14 Dec	26 Apr	133
Median	14 Dec	28 Apr	135

After the melting of snow, increasing penetration of solar radiation into the water created vertical convective mixing that started 14–41 d before ice-break (III). In winters without mechanical mixing a chemocline developed at 20–25 m depth due to the leaching of electrolytes from the sediment, which delayed deepening of the convective layer. In 2009 the convective layer finally became 0.5 °C warmer than the water below and still the water column did not turn over until ice-break (Fig. 2). Mechanical mixing promoted the progress of vertical convective mixing and in years 2010 and 2014, when the mixing stations were running all the time, the basin was turning over already before ice-break.

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In contrast, in the years when mechanical mixing was stopped 12–34 d before ice-break, a chemocline started to develop at a depth of 20–25 m and the basin did not turn over before ice-break (Fig. 2).
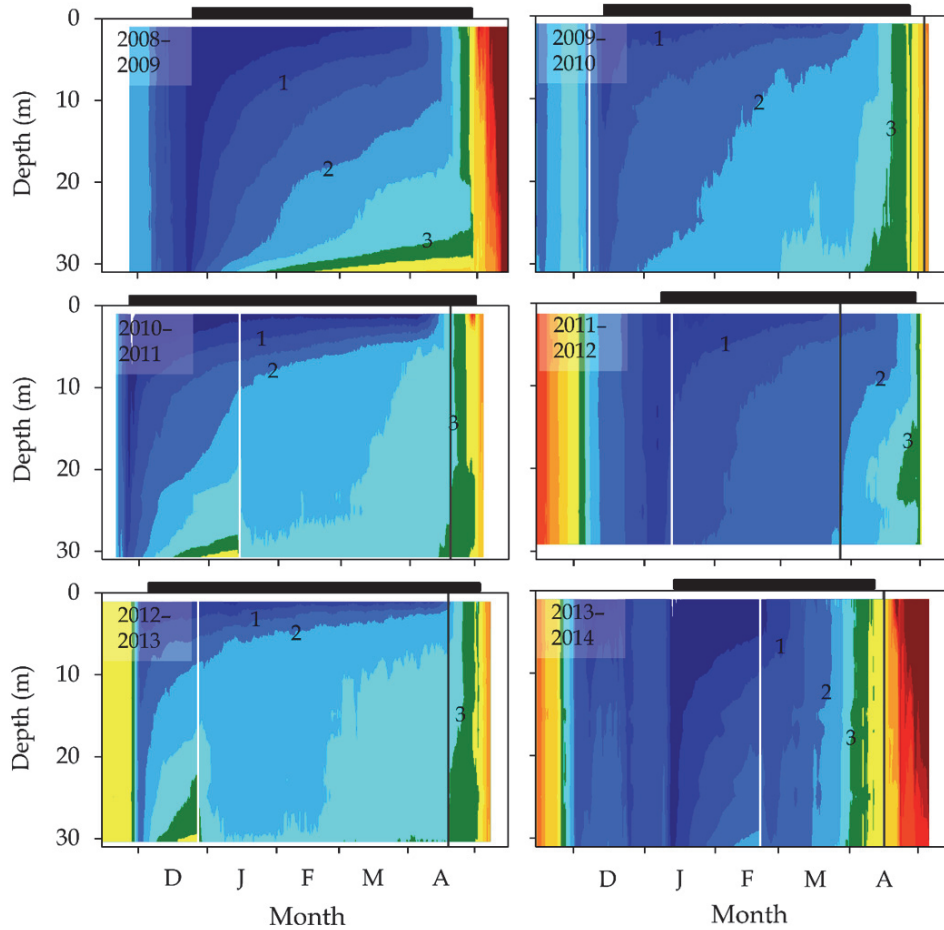

FIGURE 2   Temperature development at the Lankiluoto sampling point in Enonselkä from mid-November to Mid-May in 2008–2014. Black horizontal bars above the panels indicate the presence of ice cover. Vertical lines show the dates when mixing stations were turned on (white) and off (black).

Under the ice, the littoral areas typically warm up faster than the pelagial due to their smaller water volume. Warmer and heavier water from the littoral may flow to deeper areas as horizontal convection (Salonen *et al.* 2014). In Enonselkä, in the absence of mechanical mixing, horizontal convection was observed as an intrusion of warmer water above the chemocline at 15–25 m depth. It was particularly apparent in late winter 2012 after the formation of a weak chemocline (Fig. 2). Due to the elimination of chemical stratification by the mechanical mixing, horizontal convection also extended into the deepest water.

Similar to results from other ice-covered lakes (Kirillin *et al.* 2012, Salonen *et al.* 2014), the under-ice hydrodynamics of Enonselkä include notable interannual variation that is controlled largely by weather conditions particularly before freezing and melting of snow and ice. The under-ice hydrodynamics create a basis for understanding the chemical and biological phenomena of Enonselkä during winter.
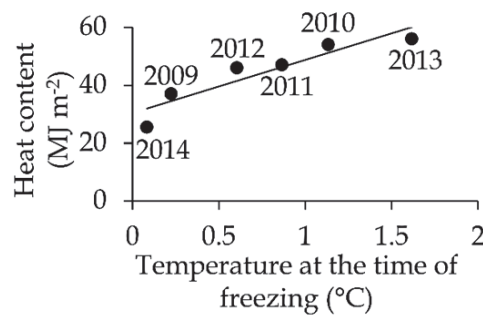

FIGURE 3   Relationship between the maximum heat content of the basin gained between ice formation and March (before the beginning of warming due to solar radiation) and the temperature at 29–31 m depth at the time of ice formation (R^2 = 0.86, p = 0.008).

In winter temperature was inversely distributed, but vertical change was continuous so that no thermocline could be distinguished and thus it is not justified to call it inverse stratification. In contrast, in summer the water column of Enonselkä was thermally stratified, irrespective of whether there was mechanical mixing or not. Results from summer 2008 (without mixing) showed 2 thermoclines in the water column. The first was at around 10 m depth and the second, supported by a distinct chemocline, was at 20–25 m depth (Fig. 4). In the absence of mechanical mixing a chemocline was also found at the same depth during winter, suggesting that its location is due to the topography of the basin.

Establishment of temperature stratification in early summer varied due to prevailing weather conditions. Deep water was isolated from mixing generally at a temperature between 11–13 °C (I). Mechanical mixing by pumping warm surface water downwards prevented the development of chemocline and caused warming of the hypolimnion by over 17 °C in years 2010–2012 and 2014 (Fig. 4). In 2013, the summer temperature stratification was established relatively early leaving the temperature in the deepest water below 9 °C (Fig. 4). Typically the mixing stations were turned on at the beginning of June, but in 2013 the mixing stations were only turned on in late July so that excessive warming of the hypolimnion by mixing could be avoided (Fig. 4). An interesting cooling phase in the hypolimnion was observed in August 2010 and September 2014 (Fig. 4), which was probably due to horizontal convection created by earlier cooling of water in the littoral of the basin and river water.

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Increase of hypolimnetic temperature by mechanical mixing is undesirable, as it might impair or even be fatal for cold-water fish (Beutel and Horne 1999). In Enonselkä, smelt might have suffered from too warm water, as they disappeared in the first summer (2010) of mechanical mixing, when temperatures in the epi- and metalimnion were highest (Malinen *et al.* 2013). On the other hand, their reappearance in 2012 (Malinen *et al.* 2013) after another warm summer might mean that the fluctuation was caused by some other factor. As also demonstrated in other oxygenation or aeration arrangements (Grochowska and Gawronska 2004, Becker *et al.* 2006), in Enonselkä reduced temperature differences between epi- and hypolimnion advance the beginning of autumnal overturn especially in cool summers which reduces problems of hypolimnetic oxygen consumption.
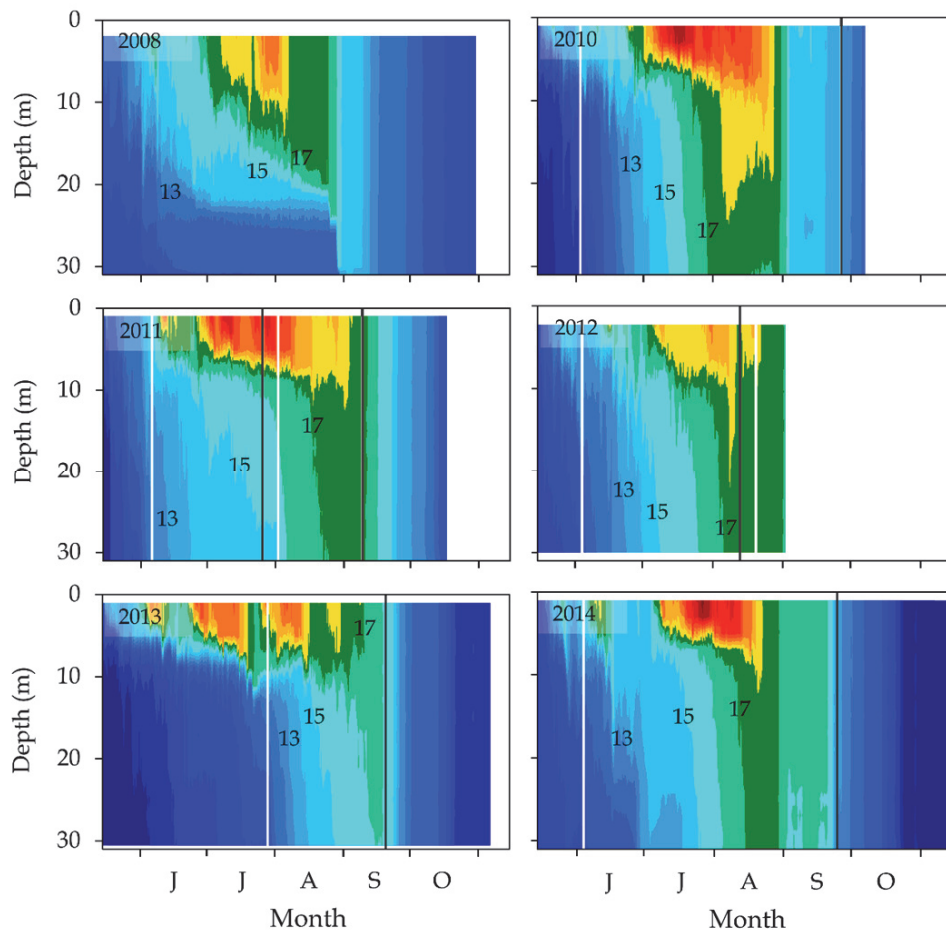

FIGURE 4   Temperature development at the Lankiluoto sampling point of Enonselkä from mid-May to Mid-November in 2008 and 2010–2014. Vertical lines show the dates when mixing stations were turned on (white) and off (black).

3.1.2 Oxygen

In winters without mechanical mixing, hypoxia or anoxia regularly occurred between 25–30 m. In contrast, when the mixing stations were operating, oxygen concentration in the deepest water remained high (I). In the years of mechanical mixing, rather uniform concentrations of oxygen throughout the water column as well as over the whole basin indicated that the mixing stations affected wide area beyond the locations of the mixing stations (I, III). However, it did not reach deeps behind shallow sills, as demonstrated in winter 2010–2011 when only part of the mixing stations were operating (Fig. 5).
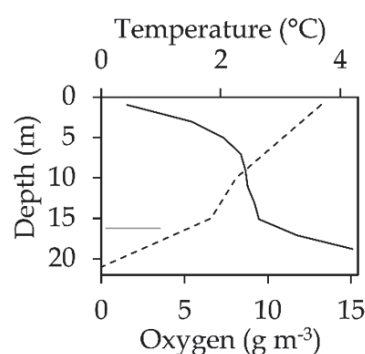

FIGURE 5   Temperature (solid line) and oxygen (dashed line) profiles in close proximity to the unpowered mixing station 1 (see Fig. 1) on 23 March 2011. The nearest operating mixing station was station 3 in the Lankiluoto deep. The grey horizontal line marks the approximate depth of the sill separating the two deeps.

In summers without mechanical mixing, oxygen in the hypolimnion was rapidly consumed after the establishment of temperature stratification. Mixing was not powerful enough to maintain the deepest water well oxygenated, but it did delay the development of hypoxic or anoxic conditions (I). In the latest mixing years (2013–2014), there were measurable concentrations of oxygen in the deepest water in August. Since the mechanical mixing rendered the thermocline sharper (Fig. 4), the volume of hypoxic hypolimnetic water also increased (I). In fact, the hypoxic/anoxic water volume roughly tripled if calculated as enlargement from 15–31 m up to 10–31 m water layers. In spite of continued oxygen depletion, the biomass of benthic fauna increased one order of magnitude in the mixing years 2010–2012 compared to year 2009 without mechanical mixing (Table 3). The benthic community consisted almost entirely of *Chironomus plumosus* (L.) chironomid larvae and *Potamothrix/Tubifex* olichochaetes which are known to tolerate very low oxygen concentration or even anoxia (Hamburger *et al.* 2000). Due to their habit of burrowing into the sediment (Krantzberg 1985), their recovery enhances availability of oxygen and respiration in the sediment (Lagauzere *et al.* 2011). Chironomids are also important food for bream in Enonselkä (Malinen *et al.* 2014).

TABLE 3   Biomass (g m^{-2}) of benthic fauna from four 20–30 m deep sampling stations in Enonselkä in August–September 2009–2013. Data from Lahti Region Environmental Services.

	2009	2010	2011	2012	2013
Median	1.7	9.9	15.8	28.6	8.6
Range	1.5–2.5	8.3–11.0	8.8–25.2	13.2–45.7	4.2–12.7

The inability of the mixing stations to keep the water column fully oxygenated during summer was to a great extent due to the higher temperature which increases oxygen consumption. Bergström *et al.* (2010) showed that in Lake Pääjärvi a 10 °C increase in water temperature increased metabolism of micro-organisms 2.7 fold. According to that, the observed 5 °C increase in hypolimnetic temperature in Enonselkä should have led to 1.64 fold oxygen consumption, which agrees with our 1.3–2.0 fold estimate (Fig. 6).

In addition to temperature, other factors affect oxygen depletion rates. In winter high oxygen concentration could be maintained not only because of lower temperature but particularly of negligible primary production of phytoplankton and consequently low sedimentation of organic matter. Mechanical mixing might increase oxygen consumption by reducing sedimentation rate in the hypolimnion and by facilitating gas exchange between water and sediment.
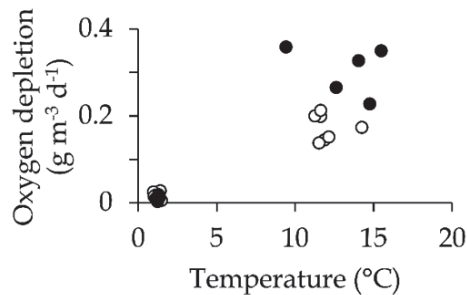

FIGURE 6   Apparent oxygen depletion rate in Enonselkä in January–March (0–31 m) and June–July (12.5–31 m) in relation to average temperature, with (filled circles) and without (open circles) mechanical mixing.

Without mechanical mixing, the gross sedimentation rates in Enonselkä were highly variable (2.8–65 g dry weight m^{-2}) depending on the depth of the measurement station and time of year (Niemistö *et al.* 2012). The highest sedimentation rates were observed at the deepest sites of the basin in late June and autumn.

In Enonselkä, the proportion of organic matter in the topmost 60 mm of sediment, determined as loss of ignition of dry mass, was highest (12.8–13.7 %)

under the 30–32 m deep water column. Deeper in the sediment, at least down to 0.44–0.48 m depth, respective values were rather uniform (average 11.5–12.6 %, data from Lahti Region Environmental Services). According to Niemistö *et al.* (2012) the proportion of organic matter in material collected by sediment traps at different depth zones in Enonselkä was about 17 %. The higher organic proportion in sediment collected in traps than in the surface sediment suggests that significant mineralization had already occurred in the surface sediment.

Matzinger *et al.* (2010) found that in two Swiss lakes with approximately similar trophic state as Enonselkä 61–76 % of hypolimnetic oxygen demand came from mineralization of organic matter in the water column and sediment not more than 2 years old. As the proportion of organic matter in the sediment of Enonselkä is relatively constant below the topmost sediments, one might conclude that deeper sediment contributes little to the total respiration of the sediment. Thus most hypolimnetic oxygen consumption in Enonselkä is probably due to mineralization of settling material and recently sedimentated organic matter rather than to old sediments.

3.1.3 Nutrients

In winters when the deepest water became anoxic, accumulation of total phosphorus (P) as well as phosphate (PO_4-P) and total iron (Fe) at 29 m depth correlated positively with each other (I). Mechanical mixing reduced total P, PO_4-P and Fe concentrations in deep water as predicted by the classical Fe–P complex theory. In summers without mechanical mixing the coupling between oxygen, total P, PO_4-P and Fe in the anoxic hypolimnion was not as clear as in winter (I). The difference might be explained by lower redox potential in the sediment in winter, which is a consequence of the degradation of material settled during the previous summer and horizontal density currents within anoxic bottom water layers towards the deepest parts of the basin which focus oxygen-depleted water from a large sediment area. In summer, respective focusing is unlikely, but sedimentation of organic matter is much higher. Preliminary redox potential measurements have suggested lower values in the surface sediment in winter than in summer (Dr. Juha Niemistö, University of Helsinki, pers. comm.). Unfortunately, no measurements have been done in the absence of mechanical mixing.

In some lakes, increase of total P in the epilimnion has been observed during summer likely due to diminished river inflow (Jeppesen *et al.* 1997) which means lower dilution of total P load from the catchment. On the other hand, in some other lakes total P concentration in the epilimnion decreases due to high nutrient losses in sedimenting material (Kamarainen *et al.* 2009). Epilimnetic Total P concentration of Enonselkä remained stable throughout the summer irrespective of mixing or not (Fig. 7). The median concentrations in the epilimnion in the mixing years were similar to those for the reference years 2000–2009, which implies that the external load was probably also quite stable. In contrast, total P concentrations at 15 m and 29 m in Enonselkä increased during summer (Fig. 7) and there was large variation during the years 2000–

2009 without mechanical mixing. In mixing summers interannual variation of the concentrations increased, but were at the lower end of the range for the reference years or sometimes even lower. Despite the presence of oxygen, or at least nitrate nitrogen (NO_3-N), which is available for nitrate respiration, only a modest decline of hypolimnetic total P concentration occurred (I, Fig. 7). This finding contradicts the theory that P might have been released from reduced sediment. Further, in the absence of mechanical mixing the stable deep water temperatures through summer (I) showed that a steep pycnocline at 20–25 m depth (Fig. 4) makes transport of P from the deepest water layers to the epilimnion unlikely. In this sense, it is not surprising that, despite great interannual variation in deep water total P concentrations in years 2000–2009, the respective epilimnetic variation was low. The transport of P might have been limited to internal water movements such as seiches (Ostrovsky *et al.* 1996, Horppila and Niemistö 2008), but because those should also have affected temperature, our data cannot confirm their importance in Enonselkä.

Due to the scarcity of the measurement depths, the estimates of the volume-weighted mean concentrations of total P in the whole basin are rather crude, but the results indicated no distinct effect of mechanical mixing (I). In agreement with oxygenation and aeration studies made elsewhere (Gächter and Wehrli 1998, Hupfer and Lewandowski 2008) it seems likely that the internal load following the development of anoxia plays less important a role in the phosphorus dynamics of Enonselkä than previously thought. Thus, in Enonselkä internal load of nutrients is not a direct consequence of hypoxia, but both are due to excessive sedimentation.

Tallberg (2000) suggested that silicon (Si) bound in diatoms of the spring bloom in Enonselkä might promote leakage of P from oxic epilimnetic sediments. In the epilimnetic sediments the combined effects of resuspension of shallow sediments (Niemistö *et al.* 2012) and Si may have a major role in the P dynamics of Enonselkä. Because the role of deep water sediments does not seem crucially important in supplying P to the epilimnion in summer, more emphasis should be directed to shallow sediments.

Internal load as a term might best describe a transition situation (highly affected by hydraulic retention time and the flux of nutrients from the sediment) where most of external load has been cut, but the productivity of the lake stays high due to the mobility of P reserves accumulated in the sediment. This kind of situation prevailed in Enonselkä after the sewage diversion in 1976, but it is uncertain to what extent it still continues about 40 y after the sewage diversion. Based on the mean retention time of 5 years, 10 % of the year 1976 water was calculated to be present ten years later. Accordingly, negligible proportion of the 1976 water was present in 2000. Taking into account the widely divergent opinions regarding the role of anoxic sediment in sustaining epilimnetic phytoplankton, *in situ* experiments might provide the most convincing conclusions. Experiments relating to phosphorus flux in both winter and summer might be an interesting possibility to gain further insight into phosphorus dynamics between water and sediment in Enonselkä.

Both in winter and in summer concentrations of ammonium (NH_4-N) in the deepest water decreased and those of NO_3-N increased (I). Compared to the median for the reference years 2000–2009 in the deepest water the concentration of total nitrogen decreased by 12–64 % (Fig. 7). These results indicate enhancement of the natural nitrogen removal processes, which were further emphasized by elevated hypolimnetic temperature in summer.
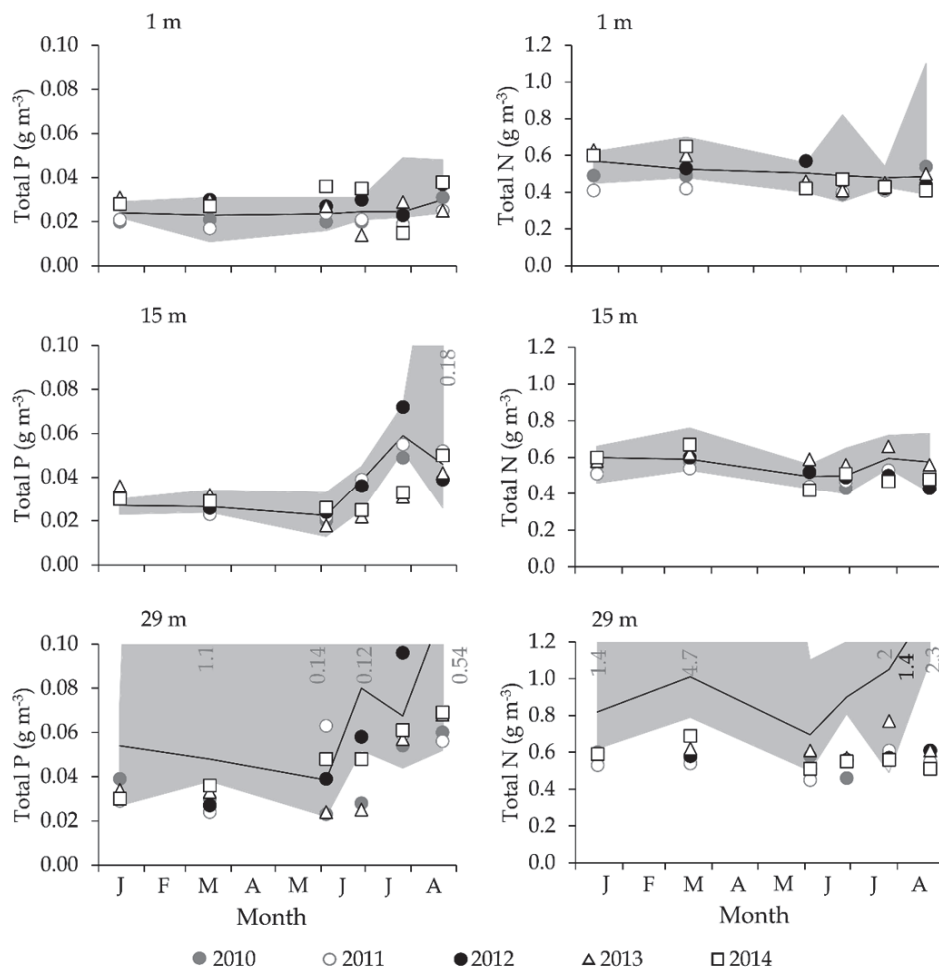

FIGURE 7   Seasonal development of total P (left panel) and N (right panel) concentrations at 1 m, 15 m and 29 m depth in Enonselkä in mixing years 2010–2014 in comparison to the median (black line) and range (grey area) of years 2000–2009 with no mixing. Grey and black numbers in panels indicate maximum and median, respectively, when those exceeded the maximum of the y-axis.

3.2 Development of bacterioplankton and phytoplankton with and without mechanical mixing

3.2.1 Quality control and optimization of phytoplankton counting

The quality of the results of microscopic counting of phytoplankton and bacterioplankton are affected by many sources of variation including sampling, identification, sizing and counting. This complex is controlled by standards and guidelines for sampling and counting practices (Mischke *et al.* 2012). A general idea of the variation between the results of different researchers and laboratories has been obtained from intercalibrations. However, although intercalibrations are necessary, high variability between samples means that standardized counting practices do not necessarily lead to the best results. Further, the vast size range of different species means that obtaining good quality results for biomass is a real challenge which is still under almost no control. These difficulties are hard to overcome and have been called a Gordian knot of phytoplankton counting (Rott *et al.* 2007).

In this study replicate samples were not taken, because it was reasoned that a high effort put to counting would be most profitable if the sampling was done with maximal frequency and depth resolution. In spite of the absence of replicate samples, the smooth course of results through successive depths (II, III) provides a good indication of the reliability of the results for reasonable interpretation, although no numerical value, such as confidence intervals, can be derived. The combination of optimized sampling and counting strategies yielded results which provided adequate answers to the research questions with reasonable effort. Further, the computer program enabled the use of an adequate number of size classes in counting picoplankton, bacteria or larger phytoplankton, which increased the accuracy of the biomass results. Despite very high variation between different samples and species, the computer program allowed the use of an optimal procedure in each case while still under one and the same counting protocol.

The results showed that the traditional practice (Lund *et al.* 1958) of calculating confidence intervals for microscopic counts of phytoplankton assuming a Poisson distribution leads to highly optimistic values (IV). Instead, and in accordance with many previously published results, phytoplankton cells in settling chambers used in counting generally show overdispersion so that a statistical approach based on a Poisson distribution is invalid. Consequently, confidence intervals calculated from the results of individual microscopic fields or transects are often several times higher than those calculated according to Poisson distribution (IV). The use of real time confidence intervals during counting leads to a dynamic and universal counting standard whereby the quality of the results is foremost rather than the more commonly used quantity of counted cells. This approach efficiently controls both the quality of counting results and the allocation of work effort. We believe that dynamic counting

implemented in counting programs will help to resolve the decades old Gordian knot of phytoplankton counting (Rott *et al*. 2007).

3.2.2 Picoplankton in late winter

In midwinter, autotrophic picophytoplankton were present throughout the whole water column (Fig. 8). After the melting of snow, those responded rapidly to increasing light and their abundance and biomass increased just below the ice. When the vertical convective mixing proceeded further, their volume-weighted biomass increased in the mixed layer by 2.4–12.6 times before ice-break. Observed picophytoplankton abundances corresponded to those observed in other boreal meso- and eutrophic lakes in early summer (Ventelä *et al.* 1998, Jasser and Arvola 2003, Peltomaa and Ojala 2012).

During late-winter mixing, the taxonomic composition of picophytoplankton shifted from dominance by picocyanobacteria to dominance by eukaryotic cells (II). These results support the findings of Somogyi *et al.* (2009) who found from photosynthesis to irradiance curves that the optimum light and temperature were notably higher for picocyanobacteria compared to eukaryotic picophytoplankton. Thus eukaryotic picophytoplankton might have better prerequisites for growth under the deep convection circulation regime. Accordingly, Bręk-Laitinen *et al*. (2011) found that in summer picophytoplankton in Enonselkä were solely phycoerythrin-rich picocyanobacteria.

The results showed that picophytoplankton comprised only 0.2–3.3 % of the total phytoplankton biomass under the ice cover (III). Thus organic carbon originating from larger phytoplankton likely fuelled the increase of bacterioplankton biomass. Bręk-Laitinen *et al*. (2011) found the highest production of bacterioplankton in Enonselkä in June after the decline of spring phytoplankton. In contrast to the winter results of this study their results indicated that in summer abundance of picophytoplankton was an order of magnitude higher than under the ice (II) and contributed to most of the chlorophyll *a* concentration in July–August. However, in August, when the biomass of larger phytoplankton increased, biomass and abundance of picophytoplankton decreased (Bręk-Laitinen *et al.* 2011). The combined results from Enonselkä show similar pattern of picophytoplankton development as found in Lake Baikal where picophytoplankton are very important during summer stratification, but during blooms of larger phytoplankton, they comprise only a small part of the biomass (Popovskaya 2000, Belykh and Sorokovikova 2003). Similar negative correlations between biomasses of picophytoplankton and larger phytoplankton have generally been found in the oceans (Morán *et al.* 2010).
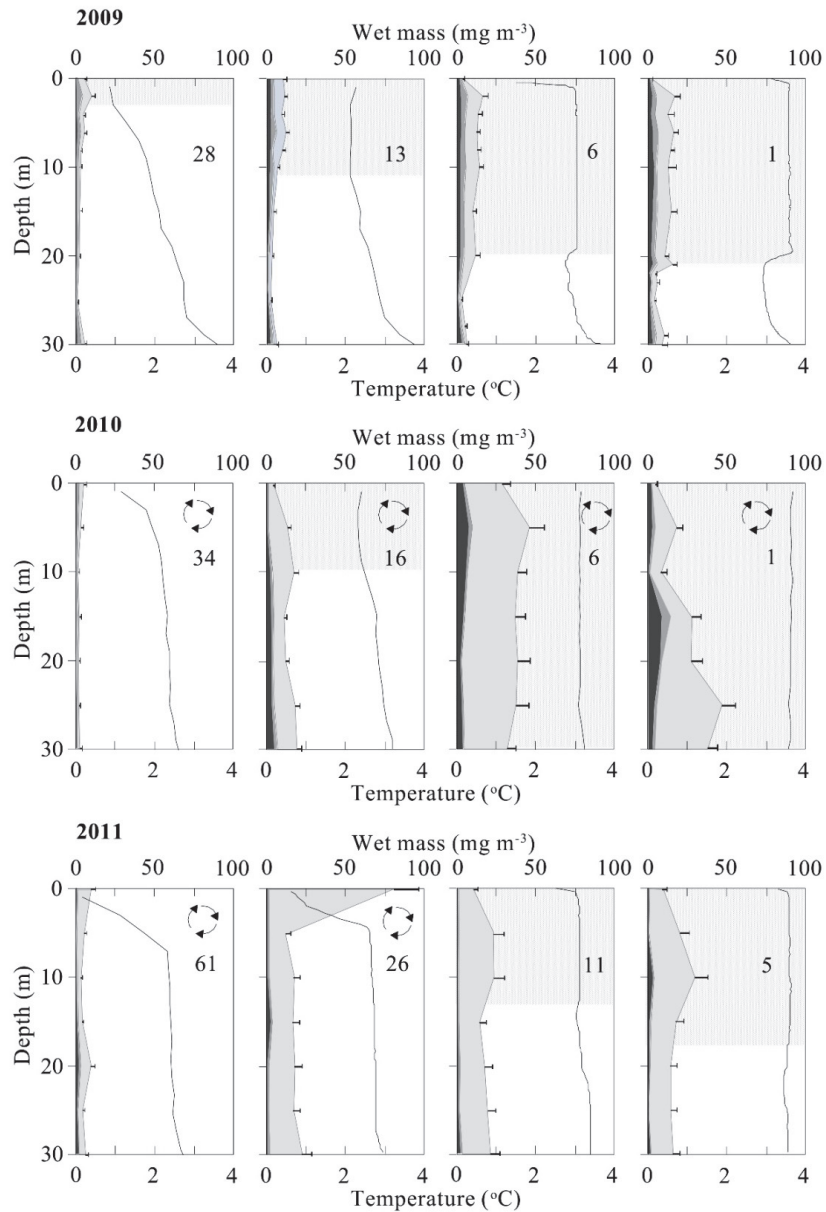
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FIGURE 8   Vertical distribution of picophytoplankton wet mass and temperature (black lines) under the ice cover in Enonselkä in years 2009–2011. The numbers inside the panels denote the number of days before ice-break. Circular arrows in the upper right corner of the panels indicate that mechanical mixing was on. Black area: phycocyanin–rich picocyanobacteria; dark grey area; phycoerythrin–rich picocyanobacteria; light grey area: eukaryotic picophytoplankton. Shaded background highlights the depth of convective layer. Horizontal bars are 95% confidence intervals for the mean biomass counted from parallel microscopic fields. Reprinted with kind permission of Oxford University Press.

During the period of vertical convection, bacterioplankton biomass increased by 100–185 % (Fig 9). Without mechanical mixing, the biomass of heterotrophic bacteria was highest in the anoxic deep water (II), which was due to the higher abundance and larger cell size of bacteria than in oxic water layer. During mechanical mixing, the vertical distribution of bacterioplankton biomass was uniform in the whole water column and there was no cell size differentiation between different water layers. In stratified lakes, bacterioplankton generally appear larger and more abundant in the anoxic hypolimnion compared to their counterparts in the oxic epilimnion (Kuuppo-Leinikki and Salonen 1992, Cole *et al.* 1993, Hernandez-Aviles *et al.* 2012). The larger cell size in the anoxic water might be due to differences in taxonomic composition of bacteria (Shade *et al.* 2007), differences in their metabolic traits, or lower grazing pressure from other micro-organisms and zooplankton (Cole *et al.* 1993).
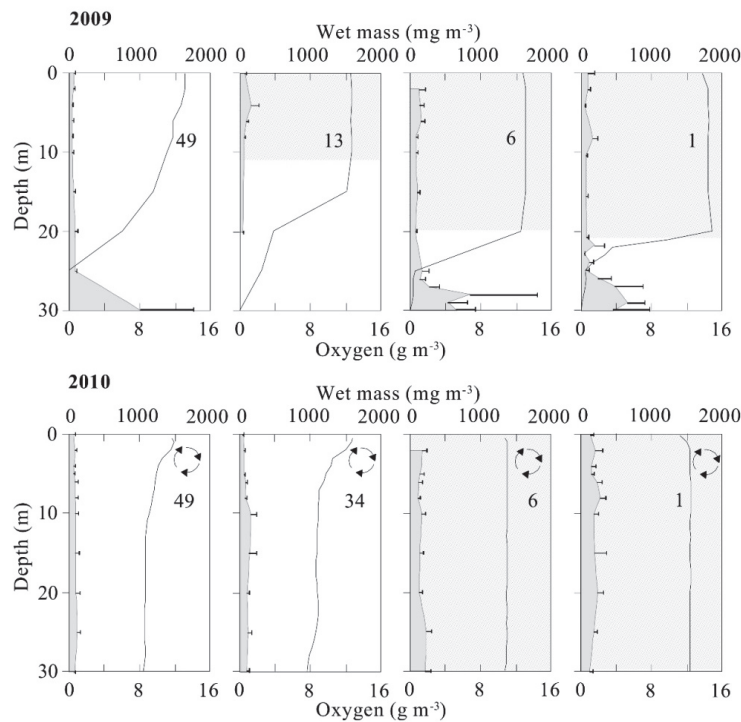

FIGURE 9   Vertical distribution of wet biomass of bacteria (grey areas) and concentration of dissolved oxygen (black lines) in Enonselkä in years 2009–2010. The numbers inside the panels denote the number of days before ice-break. In 2009, data were missing below 20 m depth 13 d before ice-break. Circular arrows in the upper right corner of the panels indicate that mechanical mixing was on. Shaded background highlights the depth of convective layer. Horizontal bars are 95% confidence intervals for the mean biomass counted from parallel microscopic fields. Reprinted with kind permission of Oxford University Press.

3.2.3 Nano- and microphytoplankton in late winter

In mid-winter, phytoplankton biomass was low (volume-weighted wet mass 0.05–0.09 g m^{-3}). Despite very low light, their vertical distribution was uniform throughout the water column also in 2009 without mechanical mixing. This suggests heterotrophic nutrition of phytoplankton or an extreme ability to stay alive at low temperature and with practically no light (Rodhe 1955, McKnight *et al.* 2000). The mid-winter phytoplankton of Enonselkä consisted of many taxonomic groups. A cyanobacterium, *Planktothrix agardhii* (Gomont) Anagnostidis and Komárek, was especially abundant, as well as cryptophytes, dinophytes (such as *Gymnodinium helveticum* Pernard), chrysophytes and diatoms. Some green algae were also found. Their rather uniform vertical distribution is similar to the results of Kiili *et al.* (2009), who suggested that the smooth vertical distribution was due to horizontal density currents which create slow basin wide circulation of lake water.

After the melting of snow, similar to picophytoplankton, the biomass of larger phytoplankton first increased just below the ice (III). A green alga *Chlamydomonas* sp., cryptophytes and a chrysophyte *Uroglena* sp. first became more abundant. These motile species were able to avoid sinking and to utilize the initially shallow zone of adequate illumination. When light increased and convective mixing proceeded, diatoms became dominant in the mixed layer. These were evidently dependent on sufficient mixing of water to maintain themselves in suspension (Kelley 1997) and could tolerate a low and changing light environment (Foy and Gibson 1993).

There were no clear differences in the biomasses of picophytoplankton, bacterioplankton or larger phytoplankton between the years of natural mixing regimes and mechanically enhanced mixing. The length of the convection period, which is related to the input of light energy, was evidently the key factor determining the under-ice biomass of phytoplankton (Fig. 10B, C). The biomass of phytoplankton was lowest in 2011 and 2013, when the ice covered period was longest (Table 2), ice cover was thickest and the increase in heat content was relatively low (Fig. 10A). The highest phytoplankton biomass was observed in late winter 2014, when the ice cover was thinnest. Heat content then also increased more than in the other years (Fig. 10A) indicating the highest input of solar radiation. The maximum of volume weighted under-ice phytoplankton biomasses (median 5.7 g m^{-3} range 1.4–27.1 g m^{-3}) corresponded to those reported by Pettersson (1985) in Lake Erken (wet mass around 5–10 g m^{-3}) where total phosphorus concentration (around 0.02 g m^{-3}) was close to that of Enonselkä. In oligotrophic and very deep lake Pääjärvi, in the same region as Enonselkä, mid–winter phytoplankton biomass was similar to Enonselkä, but in late winter it was two orders of magnitudes lower (Vehmaa and Salonen 2009).
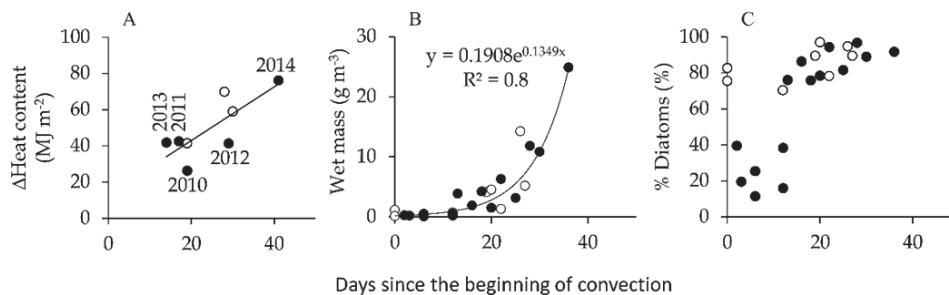
FIGURE 10   Relationship with the length of the under-ice convection period of A) increase in heat content of the basin (R^2 = 0.62), B) diatom wet mass and C) proportion of diatoms in total phytoplankton wet mass during convection in Enonselkä. Open circles represent years without and filled circles years with mechanical mixing.

In late winters 2011 and 2013 the proportion of flagellate phytoplankton stayed relatively high while the appearance of diatoms was delayed until the end of ice cover (III). Pettersson (1985) observed in Lake Erken that, when light intensity stayed low due to prolonged melt of ice cover, flagellate phytoplankton, mainly dinoflagellates, became dominant. He suggested that the motility of dinoflagellates enabled them to exploit favourable light conditions in the upper water column, when diatoms were not yet competitive in the absence of vigorous convection. Weyhenmeyer *et al.* (1999) suggested that diatoms in Lake Erken are unlikely to reach a maximum under the ice cover, but can flourish after ice-break when wind mixes the water column and light conditions are more favourable. Results from Enonselkä do not support that view, because diatoms were able to deplete PO_4-P under the ice cover (III) similar to dinoflagellates in Lake Erken (Pettersson 1985).

After the onset of convection, flagellate species in Enonselkä were soon unable to resist under-ice currents to keep their abundance highest in the upper part of the water column (Fig. 11). This was the case even in 2011 and 2013 when convection was probably the weakest. This is in agreement with observations from Lake Päijänne (Kiili *et al.* 2009), where flagellate species were not able to stay in the upper part of the water column. However, the opposite was found in Lake Pääjärvi (Vehmaa and Salonen 2009), where cryptomonads and chrysophytes at least partly kept their positions despite deep convection. These differences might be explained by variation in the mixing regime. Also, differences in taxa could contribute if they have different abilities of self-propulsion. The development of basin scale and intermittent horizontal convection, which, compared to local and diurnal vertical convection, does not allow phytoplankton to re-establish their distribution at night, was extraordinarily developed in Lake Päijänne (Kiili *et al.* 2009) and also visible in Enonselkä.
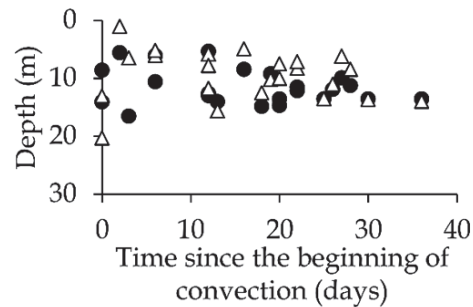
FIGURE 11   Development of the vertical centre of the biomass of *Stephanodiscus* (filled circles) and flagellate phytoplankton (open triangles) in the water column of Enonselkä since the beginning of convection in 2007–2014.

In Enonselkä the small diatom, *Stephanodiscus* cf. *parvus* Stoermer and Håkansson (Fig. 12), was present each year during under-ice convection and dominated the phytoplankton in 2007, 2010, 2012 and 2014 (III). In 2008 the dominant species was *Asterionella formosa* Hassall and in 2009 *Aulacoseira* spp. also had notable biomass (III). However, scarce data before the late-winter mixing hampers finding reasons which led to occasional dominance of these larger species. The low temperature at the end of autumnal overturn in 2009 and consequently high probability of the dominance of horizontal convection over vertical convection (Salonen *et al.* 2014) is one possible candidate. Its associated stronger and more continuous flow regime might have helped larger diatoms to remain suspended. However, because those were never dominant during mechanically enhanced mixing years, this hypothesis would need more studies without mechanical mixing to evaluate it rigorously. *Stephanodiscus parvus* is known to flourish in nutrient-replete conditions (Anderson 1989). Its small cell size and associated low sinking rate give an advantage to remain suspended during convection and to take nutrients efficiently from water. The results of this study agree with the sediment records of Enonselkä (Liukkonen *et al.* 1997) which showed that *S. parvus* typically contributed to the spring phytoplankton maximum while *A. formosa* and *Aulacoseira islandica* were occasionally dominant.

The large biomass of spring diatoms in lakes typically sediments after nutrients have been depleted or the summer thermal stratification is established (Smetacek 1985, Weisse *et al.* 1990). In Enonselkä, it seemed that the bulk of the phytoplankton spring maximum was generally synthesized under the ice cover (III). In late winter 2012, when nutrients were measured in more detail, phytoplankton had evidently already consumed all the available phosphate in the mixed layer under the ice (III). In addition to this gradual depletion of inorganic P, nutrient concentrations in the boundary layer between ice and lake water probably decrease through dilution during melting of snow and ice, although no nutrient measurements were made from the immediate vicinity of the ice to support this view. However, in spring 2011 just before the ice-break the topmost water layers warmed to 4–5 °C even though temperature in the

deeper water was < 4 °C. This was due to the lower concentration of electrolytes in the melt water (Fig. 2, conductivity data not shown) which probably also means lower concentration of nutrients such as P.

The mass of total P calculated from the under-ice phytoplankton maximum biomass (assuming 1 % P in ash free dry mass, Reynolds 2006) was often many times higher than the increase of total P in the hypolimnion during June–August (Table 4). Although such estimate is only directional, its size indicates that phytoplankton biomass developed under the ice may have strong effect on the accumulation of nutrients in the hypolimnion and development of hypolimnetic oxygen conditions during summer stratification. It also corroborates other evidence that hypolimnetic nutrient accumulation is not due to internal loading, but rather due to high sedimentation. The under-ice phytoplankton maximum might thus significantly affect the succession of both pelagic and benthic communities in the lake. However, there was no significant correlation between the spring phytoplankton total P content and total P increase in the deepest water. That is not surprising, because during the open water season many other factors, such as primary and bacterial production, the pelagic microbial food web (Bręk-Laitinen *et al.* 2011), zooplankton (Ketola *et al.* 2013) and sediment resuspension (Niemistö *et al.* 2012), affect sedimentation.
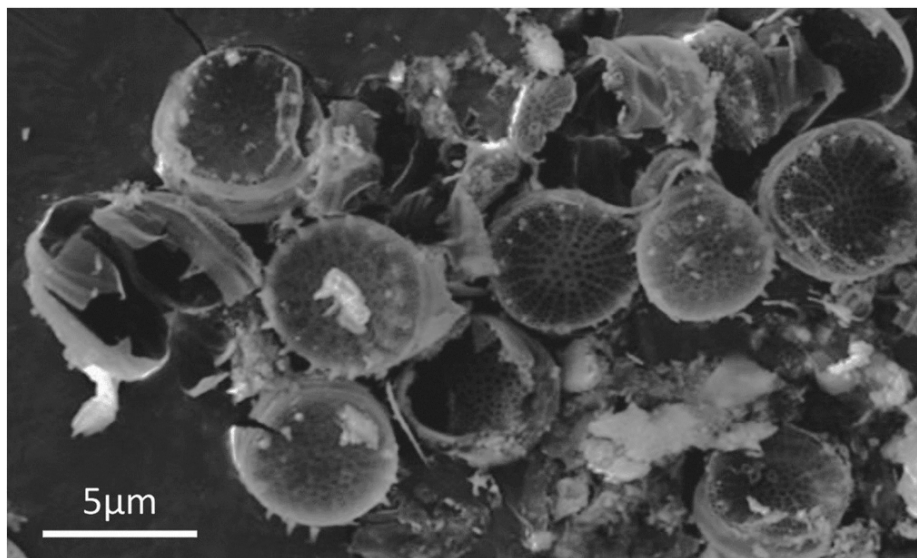

FIGURE 12   Scanning electron microscope image of *Stephanodiscus* cf. *parvus* with an approximate scale.

TABLE 4   Mass of settling phosphorus (P, tonnes) calculated from maximum phytoplankton wet mass under the ice and increase in the amount of phosphorus in the hypolimnion in June–August (tonnes below 12.5 m depth) as well as their ratio in Enonselkä in the years 2007–2014.

Year	Phytoplankton total P	Hypolimnetic total P	Ratio
2007	5.2	0.5	12
2008	1.7	0.4	4
2009	2.1	0.6	4
2010	2.0	0.6	4
2011	0.7	0.4	2
2012	4.5	0.4	11
2013	0.5	0.5	1
2014	9.9	0.4	24

Under the ice cover, the whole basin could be considered as a closed unit for primary production, and as such it offers possibilities to apply bottle-type methods to study lake metabolism under strictly natural conditions. This is a huge potential when set against the background of almost insurmountable problems in applying traditional field methods at a larger scale in winter. When net primary production derived from under-ice oxygen measurements (assuming photosynthetic quotient of 1.2, Kirk 1994) was compared with the increase in phytoplankton biomass (assuming 10 % carbon in wet mass, Reynolds 2006) during convection, the values were similar, except in 2014 (Fig. 13). The only distinct difference in the results from 2014 compared to other years was the high supersaturation of oxygen which is the most likely candidate to explain the divergent results. Loss of oxygen might have happened during transportation of the calibration samples to the laboratory. In Enonselkä under-ice oxygen concentration was horizontally variable in late winter indicating differences in phytoplankton primary production between littoral and pelagial areas (III), which clearly deserves further study. Even though the number of study years was relatively low, measurement of primary production based on increase in oxygen looks promising in the light of recent development of methodology. Field measurements of oxygen profiles using CTD type probes with rapid detector can open a new avenue to approach metabolism of under-ice organisms together with essential knowledge of physical parameters. Unfortunately, reaching adequate horizontal coverage of measurements would also require special vehicles able to provide safe movement over weakening ice. In this respect, and for extended time resolution, automated under-ice profilers and autonomous oxygen recorders can provide important tools to explore phytoplankton biomass and production through the transition period between ice-covered and open-water seasons.
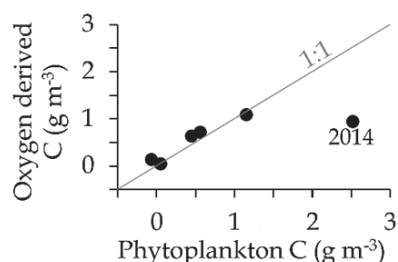
FIGURE 13   Relationship between carbon (C) calculated from the increase in oxygen during the under-ice convection period and volume-weighted increases in phytoplankton carbon in Enonselkä in years 2009–2014.

3.2.4 Phytoplankton in summer

Under-ice phytoplankton biomasses were typically higher than those sampled 9–27 d after ice-break. Only in 2011 and 2013 were the under-ice biomasses similar to those in early summer (III). Probably due to the short time interval since ice-break, the dominant species were the same. The massive late-winter/spring blooms are no doubt significant for the whole-basin energy flow. Due to high under-ice growth of phytoplankton during a relatively short time at low temperature, primary production was probably the dominant process, and one with which more slowly growing grazers could not yet cope. Later the situation changes, and in 2009 and 2011 the zooplankton maximum in Enonselkä occurred in June following the decline of the phytoplankton maximum (Ketola *et al.* 2013). Then there is a minimum of phytoplankton often called a clear water phase (Lampert *et al.* 1986, Sommer *et al.* 2012), which is a consequence both of nutrient depletion in the epilimnion by sedimentation after the establishment of summer thermal stratification and of grazing by zooplankton.

After the beginning of autumnal overturn, another phytoplankton maximum developed in August–October. In 2008–2009 without mixing and in 2010 and 2013 with mixing, cyanobacteria were the dominant component of the late summer maximum. In contrast, in 2011–2012 diatoms were dominant. Unlike the spring diatoms, these late summer diatoms, *Asterionella formosa*, *Aulacoseira islandica* (O. Müller) or *Tabellaria fenestrata* (Lyngbye) Kützing, were larger and tiny *Stephanodiscus* was absent (III). Results of this study agree with previous studies that large and heavy diatom cells are more typical of late summer when the turbulence created by wind is stronger than under-ice convection (Sicko-Goad *et al.* 1989, Liukkonen *et al.* 1997).

Phytoplankton biomasses during summer seemed to be lower in the mixing years than in years without mixing (III). The chlorophyll *a* results, which were available with slightly higher temporal resolution, did not show any notable difference (I), but it may also have depended on species composition or other factors which affect chlorophyll a concentration in phytoplankton cells (Reynolds 2006). It was encouraging that the biomass of cyanobacteria stayed low during the mixing summers.

4 CONCLUSIONS

Seasonal development of phytoplankton in Lake Vesijärvi followed the typical bimodal pattern of boreal lakes, but this study showed that important aspects for lake ecosystem occur outside the open water season. Spring phytoplankton maximum evidently starts to develop few weeks before ice-break and probably often reaches its full strength under the ice cover. Thus a common excuse for neglecting winter studies that "nothing important happens under the ice" is not valid. Because key changes in under-ice light and hydrodynamics occur during a short time interval in both autumn and late winter, the conditions for phytoplankton are controlled by highly stochastic weather variations and are hence difficult to predict by models.

Mechanical mixing affected the physical and chemical conditions of the water column and effects highly depend on the season. It has both positive and negative effects on Enonselkä. However, one must bear in mind that the judgements of "positive" or "negative" are from a human perspective and reflect the endeavour to return the lake to its assumed trophic state before the significant growth of human population around its shores. The positive effects are high oxygen level in winter throughout the whole water column, delayed duration of hypoxia and anoxia in summer in the deepest water layers, and increased benthic fauna. Negative effects seem to occur only in summer. The most pronounced one is an increase in hypolimnetic temperature and ascent of the metalimnion, which leads to an increased volume of warm, hypoxic hypolimnetic water. Although no serious bloom of cyanobacteria was found during the 5 years of mixing, it is still too early to evaluate possible effects of mixing on phytoplankton against natural variation. Increased denitrification together with total phosphorus concentrations generally below earlier median values are promising signals that favourable phytoplankton changes may be expected or at least that previous harmful cyanobacteria blooms will be avoided in the future.

In contrast to the still rather common assumption, our results corroborate the concept that anoxia and nutrient accumulation in the deepest water are consequences of the productivity of the lake rather than vice versa. This has

profound ramifications for directing future studies or planning new management activities. Mechanical mixing alone might provide some relief from the symptoms of eutrophication rather than be a solution to the problem of high nutrient loading. In the long term, removal of nutrients or limitation of their loading is needed. In spite of that, even partial relief from the problems of eutrophy may be extremely important. In the light of the results of this study, continuation of the large scale experiment at Enonselkä can yield rarely available information about the management of eutrophic lakes. The cost of mixing is significant, which creates a need for further development of the mixing arrangement. Innovative possibilities might lie in application of wind power or heat pump technologies for more economical mixing. Use of heat pumps for warm water production and heating of nearby apartment houses is particularly attractive, because deep water mixing by convection would be available as a free by-product both in winter and summer. In such case cooling of deep water particularly in summer would also reduce oxygen consumption and improve the environment for cold water fish.

With the help of Enonselkä, a whole-basin scale field laboratory, it was possible to gain new sights into the interactions between seasonal physical and chemical phenomena and the phytoplankton ecology of ice-covered lakes, as well as to evaluate the effects of large-scale oxygenation by mechanical mixing as a restoration method. The importance of the multifaceted monitoring programmes of Lake Vesijärvi for the research and protection of the lake cannot be overstated.

Acknowledgements

YHTEENVETO (RÉSUMÉ IN FINNISH)

Sekoitushapetuksen vaikutukset järven vedenlaatuun ja erityisesti kasviplanktonin jäänalaiseen kehitykseen

Vedessä keijuvat yhteyttävät levät muodostavat järvien kasviplanktonin. Niiden koko vaihtelee alle 2 μm kokoisista pikolevistä 20–200 μm kokoisiin mikroleviin. Veden fysikaaliset ja kemialliset olosuhteet vaikuttavat kasviplanktonin lajistokoostumukseen, sillä erilaiset olot suosivat erikokoisia ja -tyyppisiä lajeja. Yhteyttävä kasviplankton sitoo auringon energiaa biomassaksi, joka siirtyy järvien ravintoverkoissa eteenpäin. Kun valoa on riittävästi, erityisesti veden ravinnepitoisuus säätelee kasviplanktonin maksimibiomassaa. Veden ravinnepitoisuuden runsas kasvu johtaa tyypillisesti haitalliseen kasviplanktonin runsastumiseen.

Mikrobien hajottaessa pohjaan painuvaa kasviplanktonbiomassaa, alusveden happipitoisuus vähenee ja rehevissä järvissä happi jopa loppuu. Useissa tutkimuksissa ympäri maailmaa on havaittu, että happikadon yhteydessä alusveden ravinteiden pitoisuudet kohoavat, minkä on oletettu kiihdyttävän kasviplanktonin kasvua. Tästä syystä järvien alusvettä on erilaisin menetelmin pyritty pitämään hapekkaana.

Lahden Vesijärvi on kirkasvetinen suurehko eteläsuomalainen järvi, jonka eteläisimpään altaaseen, Enonselkään, on 1900-luvun alusta lähtien kohdistunut voimakas ravinnekuormitus. Vuoteen 1976 asti yhdyskunta- ja teollisuusjätevedet laskettiin Enonselälle käytännössä puhdistamattomina. Tuolloin haitalliset leväesiintymät olivat tavallisia ja kalayhteisö oli särkikalavaltainen. Sekoitushapetusta Enonselän kunnostusmenetelmänä kokeiltiin ensimmäisen kerran vuosina 1979–1984, mutta sinilevät runsastuivat entisestään eikä kokeilua jatkettu. Erilaisia ravinnekuormituksen vähentämiseen tähtääviä toimenpiteitä valuma-alueella sekä ravintoverkkokunnostusta särkikaloja poistamalla on tehty 1980-luvulta lähtien. Enonselän ravinnepitoisuudet ovat laskeneet verrattuna 1980-luvun pitoisuuksiin, mutta runsaita leväkukintoja esiintyy edelleen. Vuonna 2009 aloitettiin Enonselän laajamittainen sekoitushapetus. Hapetusta toteutetaan pumppaamalla hapekasta pintavettä syvänteisiin yhteensä yhdeksällä sekoitusasemalla, jotka ovat toiminnassa ympäri vuoden lukuun ottamatta kevään ja syksyn täyskiertoja.

Jotta järveä pystyttäisiin tehokkaasti kunnostamaan ja hoitamaan, on tärkeää hahmottaa sen limnologisten ilmiöiden vuodenaikojen mukainen jatkumo. Tältä osin kasviplanktonin jäänalainen elämä on vielä heikosti tunnettua, sillä suurin osa järvien tilan seurannasta ja tutkimuksesta on keskittynyt avovesikaudelle. Jääkansi ja erityisesti sen päälle satava lumi estävät tehokkaasti auringon valon tunkeutumisen veteen, minkä vuoksi on oletettu, että jään alla kasviplanktonin kasvu on olematonta.

Tässä työssä tutkittiin, miten kasviplankton kehittyy Enonselällä jään alla lopputalvella, kun lumi- ja jääpeite alkavat sulaa ja auringon säteily pääsee enenevässä määrin tunkeutumaan veteen. Tutkimushypoteeseina olivat: 1) kas-

viplanktonin keväinen suuri biomassa kehittyy enimmäkseen jo jään alla ja 2) sekoitushapetus edesauttaa fosforin pysymistä sedimentissä ja vähentää kasviplanktonin biomassaa, tai vaihtoehtoisesti sekoitus edistää ravinteiden kulkeutumista ylöspäin valaistuun kerrokseen ja siten lisää kasviplanktonin biomassaa.

Keskitalvella kasviplanktonin biomassa oli alhainen. Todennäköisesti sedimentin lämpövuon aiheuttamista koko altaan mittakaavassa tapahtuvista virtauksista johtuen sen pystysuuntainen jakauma oli tasainen. Keskitalvella kasviplankton koostui monipuolisesti eri lajeista. Lumen sulaessa liikuntakykyiset kulta-, nielu- ja viherlevät lisääntyivät aivan jään alla. Lisääntynyt auringon säteily lämmitti pintavettä, mikä alle 4 °C vedessä merkitsi veden ominaispainon lisääntymistä. Silloin lämmennyt vesi painui syvemmälle aikaansaaden pystysuuntaista sekoittumista eli konvektiota. Se suosi piileviä, jotka ovat raskaan piikuorensa vuoksi riippuvaisia sekoittumisesta. Yleensä valtalajina esiintynyt pienikokoinen *Stephanodiscus*-piilevä kykenee kasvamaan nopeasti vaihtelevissa valaistusoloissa.

Mitä kauemmin konvektio kesti ennen jäiden lähtöä, sitä suuremman biomassan kasviplankton saavutti jään alla. Konvektiokauden pituutta säätelevät auringon säteilyn määrän lisäksi myös lumi- ja jääpeitteen paksuus. Tyypillisesti koko vuoden suurin kasviplanktonin biomassa esiintyi jo jään alla. Ainoastaan vuosina, jolloin jääpeite oli paksuin ja konvektiokausi lyhin, kasviplanktonin jäänalainen biomassa saattoi jäädä alhaisemmaksi kuin kesällä. Näinä vuosina myös piilevien osuus oli vähäisempi ja lajistossa esiintyi erityisesti siimallisia leviä.

Talvella sekoitushapetus piti syvänteet lähes yhtä hapekkaina kuin ylemmät vesikerrokset. Kesällä talvea korkeamman lämpötilan ja perustuotannon takia hajotustoiminta oli voimakkaampaa, jolloin sekoitushapetus ei tehonnut yhtä hyvin. Se kuitenkin hidasti merkittävästi hapen loppumista alusvedestä. Vaikka sekoitushapetus vähensi alusveden ravinnepitoisuuksia, sillä ei ollut oleellista vaikutusta koko järvialtaan kokonaisravinnepitoisuuksiin eikä kasviplanktonin biomassaan keväällä. Parantuneista happioloista huolimatta syvänteiden kokonaisfosforipitoisuus nousi kesällä lähestulkoon entiseen tapaan. Tutkimuksen tulokset viittaavat siihen, ettei järvialtaan rehevyys ole seurausta syvänteiden happikadosta aiheutuvasta fosforin purkautumisesta sedimentistä, kuten on yleensä totuttu ajattelemaan, vaan että happikato ja fosforin kertyminen syvänteisiin ovat seurausta suuresta alusveteen vajoavan aineksen määrästä.

Tutkimus antoi uutta tietoa talven jäänalaisten tapahtumien vaikutuksesta seuraavan kesän happi- ja ravinneoloihin. Koska järven merkittävin levätuotantojakso näyttää yleensä esiintyvän jään alla, on tärkeää ylläpitää ympärivuotista kasviplanktonin ja muiden vedenlaatumuuttujien seurantaa. Automaattisilla mittalaitteilla voidaan kerätä tietoa myös ajanjaksoilta, jolloin järvellä liikkuminen voi olla mahdotonta, mutta muutokset fysikaalisissa ja kemiallisissa oloissa sekä kasviplanktonissa ovat nopeita ja merkittäviä. Vaikka havaitut sekoitushapetuksen vaikutukset Enonselän vedenlaatuun eivät olleet kovin suuria, ne oli-

vat enimmäkseen toivotun suuntaisia ja tekevät siten sekoitushapetuksen jatkamisen perustelluksi. Koska kyseessä on varsin suuri taloudellinen panostus, on tarpeen selvittää, miten sekoitushapetus voitaisiin toteuttaa nykyistä kustannustehokkaammin. On myös pidettävä mielessä, että sekoitushapetus hoitaa rehevöitymisen oireita ongelman poistamisen sijaan.

REFERENCES

Adrian R., O'Reilly C.M., Zagarese H., Baines S.B., Hessen D.O., Keller W., Livingstone D.M., Sommaruga R., Straile D., Van Donk E., Weyhenmeyer G.A. & Winder M. 2009. Lakes as sentinels of climate change. *Limnol. Oceanogr.* 54: 2283–2297.

Anon. 2006. *SFS-EN standard 15204. Water quality. Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique).* Finnish Standards Association, Helsinki.

Anon. 2014. *Synthesis Report. Contribution of Working Groups I, II and III to the fifth Assessment report of the intergovernmental panel on climate change.* IPCC, Geneva.

Ahti T., Hämet-Ahti L. & Jalas J. 1968. Vegetation zones and their sections in northwestern Europe. *Ann. Bot. Fennici* 5: 169–211.

Anderson N. 1989. A whole-basin diatom accumulation rate for a small eutrophic lake in Northern Ireland and its palaeoecological implications. *J. Ecol.* 77: 926–946.

Becker A., Herschel A. & Wilhelm C. 2006. Biological effects of incomplete destratification of hypertrophic freshwater reservoir. *Hydrobiologia* 559: 85–100.

Belykh O. & Sorokovikova E. 2003. Autotrophic picoplankton in Lake Baikal: abundance, dynamics, and distribution. *Aquat. Ecosyst. Health Manage.* 6: 251–261.

Bendtsen J., Gustafsson K.E., Lehtoranta J., Saarijärvi E., Rasmus K. & Pitkänen H. 2013. Modeling and tracer release experiment on forced buoyant plume convection from coastal oxygenation. *Boreal Env. Res.* 18: 37–52.

Bengtsson L. 1996. Mixing in ice-covered lakes. *Hydrobiologia* 322: 91–97.

Bergström I., Heinänen A. & Salonen K. 1986. Comparison of acridine orange, acriflavine, and bisbenzimide stains for enumeration of bacteria in clear and humic waters. *Appl. Environ. Microbiol.* 51: 664–667.

Bergström I., Kortelainen P., Sarvala J. & Salonen K. 2010. Effects of temperature and sediment properties on benthic CO_2 production in an oligotrophic boreal lake. *Freshwater Biol.* 55: 1747–1757.

Bertilsson S., Burgin A., Carey C.C., Fey S.B., Grossart H., Grubisic L.M., Jones I.D., Kirillin G., Lennon J.T. & Shade A. 2013. The under-ice microbiome of seasonally frozen lakes. *Limnol. Oceanogr.* 58: 1998–2012.

Beutel M.W. 2006. Inhibition of ammonia release from anoxic profundal sediments in lakes using hypolimnetic oxygenation. *Ecol. Eng.* 28: 271–279.

Beutel M.W. & Horne A.J. 1999. A review of the effects of hypolimnetic oxygenation on lake and reservoir water quality. *Lake Reserv. Manage.* 15: 285–297.

Bolsenga S. & Vanderploeg H. 1992. Estimating photosynthetically available radiation into open and ice-covered freshwater lakes from surface characteristics; a high transmittance case study. *Hydrobiologia* 243: 95–104.

Braskerud B., Tonderski K., Wedding B., Bakke R., Blankenberg A., Ulén B. & Koskiaho J. 2005. Can constructed wetlands reduce the diffuse phosphorus loads to eutrophic water in cold temperate regions? *J. Environ. Qual.* 34: 2145–2155.

Bręk-Laitinen G., Bellido J.L. & Ojala A. 2011. Response of a microbial food web to prolonged seasonal hypoxia in a boreal lake. *Aquatic Biol.* 14: 105–120.

Camargo J.A. & Alonso Á. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environ. Int.* 32: 831–849.

Cole J.J., Pace M.L. & Caraco N.F. 1993. More and larger cells in anoxic waters. *Limnol. Oceanogr.* 38: 1627–1632.

Drábková M., Admiraal W. & Maršalek B. 2007. Combined exposure to hydrogen peroxide and light–selective effects on cyanobacteria, Green algae and diatoms. *Environ. Sci. Technol.* 41: 309–314.

Egemose S., Reitzel K., Andersen F.Ø. & Flindt M.R. 2010. Chemical lake restoration products: sediment stability and phosphorus dynamics. *Environ. Sci. Technol.* 44: 985–991.

Einsele W. 1938. Über chemische und kolloidchemische Vorgange in Eisen-Phosphat-Systemen unter limnochemischen und limnogeologischen Gesichtspunkten. *Arch. Hydrobiol.* 33: 361–387.

Eloranta P. 1982. Seasonal succession of phytoplankton in an ice-free pond warmed by a thermal power plant. *Hydrobiologia* 86: 87–91.

Foy R. & Gibson C. 1993. The influence of irradiance, photoperiod and temperature on the growth kinetics of three planktonic diatoms. *Eur. J. Phycol.* 28: 203–212.

Gächter R. & Wehrli B. 1998. Ten years of artificial mixing and oxygenation: no effect on the internal phosphorus loading of two eutrophic lakes. *Environ. Sci. Technol.* 32: 3659–3665.

Grochowska J. & Gawronska H. 2004. Restoration effectiveness of a degraded lake using multi-year artificial aeration. *Pol. J. Environ. Stud.* 13: 671–681.

Hamburger K., Dall P.C., Lindegaard C. & Nilson I.B. 2000. Survival and energy metabolism in an oxygen deficient environment. Field and laboratory studies on the bottom fauna from the profundal zone of Lake Esrom, Denmark. *Hydrobiologia* 432: 173–188.

Hampton S.E., Moore M.V., Ozersky T., Stanley E.H., Polashenski C.M. & Galloway A.W.E. 2015. Heating up a cold subject: prospects for under-ice plankton research in lakes. *J. Plankton Res.* doi:10.1093/plankt/fbv002.

Hernandez-Aviles J.S., Bertoni R., Macek M. & Callieri C. 2012. Why bacteria are smaller in the epilimnion than in the hypolimnion? A hypothesis comparing temperate and tropical lakes. *J. Limnol.* 71: 104–111.

Horppila J. & Niemistö J. 2008. Horizontal and vertical variations in sedimentation and resuspension rates in a stratifying lake – effects of internal seiches. *Sedimentology* 55: 1135–1144.

Huisman J., Sharples J., Stroom J.M., Visser P.M., Kardinaal W.E.A., Verspagen J.M. & Sommeijer B. 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. *Ecology* 85: 2960–2970.

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Hullebusch E.V., Deluchat V., Chazal P.M. & Baudu M. 2002. Environmental impact of two successive chemical treatments in a small shallow eutrophied lake: Part I. Case of aluminium sulphate. *Environ. Pollut.* 120: 617–626.

Hupfer M. & Lewandowski J. 2008. Oxygen controls the phosphorus release from lake sediments–a long-lasting paradigm in limnology. *Int. Rev. Hydrobiol.* 93: 415–432.

Jasser I. & Arvola L. 2003. Potential effects of abiotic factors on the abundance of autotrophic picoplankton in four boreal lakes. *J. Plankton Res.* 25: 873–883.

Jeppesen E., Jensen J.P., Søndergaard M., Lauridsen T., Pedersen L.J. & Jensen L. 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia* 342: 151–164.

Jewson D.H., Granin N.G., Zhdanov A.A. & Gnatovsky R.Y. 2009. Effect of snow depth on under-ice irradiance and growth of Aulacoseira baicalensis in Lake Baikal. *Aquat. Ecol.* 43: 673–679.

Jones R.I. 2000. Mixotrophy in planktonic protists: an overview. *Freshwat. Biol.* 45: 219–226.

Kairesalo T., Laine S., Luokkanen E., Malinen T. & Keto J. 1999. Direct and indirect mechanisms behind successful biomanipulation. *Hydrobiologia* 395: 99–106.

Kalff J. & Knoechel R. 1978. Phytoplankton and their dynamics in oligotrophic and eutrophic lakes. *Annu. Rev. Ecol. Syst.* 9: 475–495.

Kamarainen A.M., Penczykowski R.M., Van de Bogert M.C., Hanson P.C. & Carpenter S.R. 2009. Phosphorus sources and demand during summer in a eutrophic lake. *Aquat. Sci.* 71: 214–227.

Kelley D.E. 1997. Convection in ice-covered lakes: effects on algal suspension. *J. Plankton Res.* 19: 1859–1880.

Keto J. & Tallberg P. 2000. The recovery of Vesijarvi, a lake in southern Finland: water quality and phytoplankton interpretations. *Boreal Env. Res.* 5: 15–26.

Ketola M., Kuoppamäki K. & Kairesalo T. 2013. *Vesijärven Enonselän ravintoverkon rakenne ja toiminta sekä niissä tapahtuvat muutokset vuosina 2009-2013. Osaraportti (eläinplankton, Vesikemia). Research report to the Lake Vesijärvi Foundation.* http://www.lahti.fi/www/images.nsf/files/FE8A9DB15A750FCAC2257 AED003FCFBD/$file/EPL_raportti2011_Final.pdf (23.4.2015).

Kiili M., Pulkkanen M. & Salonen K. 2009. Distribution and development of under-ice phytoplankton in 90-m deep water column of Lake Päijänne (Finland) during spring convection. *Aquat. Ecol.* 43: 707–713.

Kirillin G., Leppäranta M., Terzhevik A., Granin N., Bernhardt J., Engelhardt C., Efremova T., Golosov S., Palshin N. & Sherstyankin P. 2012. Physics of seasonally ice-covered lakes: a review. *Aquat. Sci.* 74: 659–682.

Kirk J.T.O. 1994. *Light & photosynthesis in aquatic ecosystems.* Cambridge university press, Cambridge.

Kirke B. & El Gezawy A. 1997. Design and model tests for an efficient mechanical circulator/aerator for lakes and reservoirs. *Water Res.* 31: 1283–1290.

Krantzberg G. 1985. The influence of bioturbation on physical, chemical and biological parameters in aquatic environments: a review. *Environ. Pollut. A* 39: 99–122.

Kuuppo-Leinikki P. & Salonen K. 1992. Bacterioplankton in a small polyhumic lake with an anoxic hypolimnion. *Hydrobiologia* 229: 159–168.

Lagauzere S., Moreira S. & Koschorreck M. 2011. Influence of bioturbation on the biogeochemistry of littoral sediments of an acidic post-mining pit lake. *Biogeosciences* 8: 339–352.

Lampert W., Fleckner W., Rai H. & Taylor B.E. 1986. Phytoplankton control by grazing zooplankton: a study on the spring clear-water phase. *Limnol. Oceanogr.* 31: 478–490.

Lappalainen A. & Hilden M. 1993. Recreational fishing and environmental impacts in the archipelago sea and the Finnish part of the Gulf of Bothnia. *Aqua Fenn.* 23: 29–37.

Lappalainen K. 1994. Positive changes in oxygen and nutrient contents in two Finnish lakes induced by Mixox hypolimnetic oxygenation method. *Verh. Internat. Verein. Limnol.* 25: 2510–2513.

Larsen J.A. 1980. The boreal ecosystem. Academic Press Inc., New York.

Liukkonen M., Kairesalo T. & Haworth E.Y. 1997. Changes in the diatom community, including the appearance of Actinocyclus normanii f. subsalsa, during the biomanipulation of Lake Vesijärvi, Finland. *Eur. J. Phycol.* 32: 353–361.

Lopez-Archilla A.I., Moreira D., López-García P. & Guerrero C. 2004. Phytoplankton diversity and cyanobacterial dominance in a hypereutrophic shallow lake with biologically produced alkaline pH. *Extremophiles* 8: 109–115.

Lund J.W.G., Kipling C. & Le Cren E.D. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia* 11: 143–170.

MacIsaac E. & Stockner J.G. 1993. Enumeration of phototrophic picoplankton by autofluorescence microscopy. In: Kemp P., Sherr B., Sherr E. & Cole J. (eds.), *Handbook of methods in aquatic microbial ecology*, Lewis Publishers, Boca Raton, Fla, pp. 187–197.

Maeda O. & Ichimura S. 1973. On the High Density of a Phytoplankton Population Found in a Lake under Ice. *Int. Revue ges. Hydrobiol.* 58: 673–689.

Malinen T., Vinni M. & Ruuhijärvi J. 2014. *Vesijärven lahnan kasvu ja ravinto. Research report to the Lake Vesijärvi Foundation*. http://www.lahti.fi/www/images.nsf/files/68568BF93313A973C2257D2 C00359F9C/$file/2014_Enonsel%C3%A4n_lahnan_kasvututmus_Malinen _ym.pdf. (23.4.2015).

Malinen T., Vinni M., Ruuhijärvi J. & Ala-Opas P. 2013. *Vesijärven Enonselän ravintoverkkotutkimuksen kalatutkimukset vuosina 2009-2012. Research report to the Lake Vesijärvi Foundation*. http://www.lahti.fi/www/images.nsf/files/1AD453D8EF288F6CC2257 AED0023D7F3/$file/kalarapvj2012valmis.pdf. (23.4.2015).

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Matthews P. & Heaney S. 1987. Solar heating and its influence on mixing in ice-covered lakes. *Freshwat. Biol.* 18: 135–149.

Matzinger A., Müller B., Niederhauser P., Schmid M. & Wüest A. 2010. Hypolimnetic oxygen consumption by sediment-based reduced substances in former eutrophic lakes. *Limnol. Oceanogr.* 55: 2073–2084.

McKay R.M.L., Beall B.F.N., Bullerjahn G.S. & Woityra L.W.C. 2011. Winter limnology on the Great Lakes: The role of the U.S. Coast Guard. *J. Great Lakes Res.* 37: 207–210.

McKnight D.M., Howes B., Taylor C. & Goehringer D. 2000. Phytoplankton dynamics in a stably stratified Antarctic lake during winter darkness. *J. Phycol.* 36: 852–861.

Mischke U., Thackeray S., Dunbar M., McDonald C., Carvalho L., de Hoyos C., Järvinen M., Laplace-Treyture C., Morabito G. & Skjelbred B. 2012. *Deliverable D3. 1-4: guidance document on sampling, analysis and counting standards for phytoplankton in lakes. WISER, Seventh framework programme.* http://www.wiser.eu/download/D3.1-4.pdf. (23.4.2015).

Mitsui A., Kumazawa S., Takahashi A., Ikemoto H., Cao S. & Arai T. 1986. Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. *Nature* 323: 720–722.

Morán X.A.G., López-Urrutia Á, Calvo-Díaz A. & Li W.K. 2010. Increasing importance of small phytoplankton in a warmer ocean. *Global Change Biol.* 16: 1137–1144.

Mortimer C.H. 1942. The exchange of dissolved substances between mud and water in lakes. *J. Ecol.* 30: 147–201.

Niemistö J., Tamminen P., Ekholm P. & Horppila J. 2012. Sediment resuspension: rescue or downfall of a thermally stratified eutrophic lake? *Hydrobiologia* 686: 267–276.

Ohle W. 1938. Die Bedeutung der Austauschvorgänge zwischen Schlamm und Wasser für den Stoffkreislauf der Gewässer. *Vom Wasser* 13: 87–97.

Ostrovsky I., Yacobi Y., Walline P. & Kalikhman I. 1996. Seiche-induced mixing: Its impact on lake productivity. *Limnol. Oceanogr.* 41: 323–332.

Paerl H.W., Fulton R.S.,3rd, Moisander P.H. & Dyble J. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *ScientificWorld* 1: 76–113.

Peltomaa E. & Ojala A. 2012. Meteorological drivers of the dynamics of autotrophic picoplankton. *Freshwat. Biol.* 57: 1005–1016.

Pettersson K. 1985. The availability of phosphorus and the species composition of the spring phytoplankton in Lake Erken. *Int. Revue ges. Hydrobiol.* 70: 527–546.

Popovskaya G. 2000. Ecological monitoring of phytoplankton in Lake Baikal. *Aquat. Ecosyst. Health Manage.* 3: 215–225.

Pulkkanen M.J. & Salonen K. 2013. Accumulation of low oxygen water in deep waters of ice-covered lakes cooled below 4 °C. *Inland Waters* 3: 15-24.

Reinhardt M., Gächter R., Wehrli B. & Müller B. 2005. Phosphorus retention in small constructed wetlands treating agricultural drainage water. *J. Environ Qual.* 34: 1251–1259.

Reynolds C.S. 1984. Phytoplankton periodicity: the interactions of form, function and environmental variability. *Freshwat. Biol.* 14: 111–142.

Reynolds C.S. 2006. *Ecology of phytoplankton.* Cambridge University Press, Cambridge.

Reynolds C.S., Huszar V., Kruk C., Naselli-Flores L. & Melo S. 2002. Towards functional classification of the freshwater phytoplankton. *J. Plankton Res.* 24: 417–428.

Robarts R.D. & Zohary T. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *N.Z. J. Mar. Freshwat. Res.* 21: 391–399.

Rodhe W. 1955. Can plankton production proceed during winter darkness in subarctic lakes. *Verh. Int. Verein. Limnol.* 12: 117–119.

Rott E., Salmaso N. & Hoehn E. 2007. Quality control of Utermöhl-based phytoplankton counting and biovolume estimates—an easy task or a Gordian knot? *Hydrobiologia* 578: 141–146.

Salonen K. & Rosenberg M. 2000. Advantages from diel vertical migration can explain the dominance of Gonyostomum semen (Raphidophyceae) in a small, steeply-stratified humic lake. *J. Plankton Res.* 22: 1841–1853.

Salonen K., Jones R. & Arvola L. 1984. Hypolimnetic phosphorus retrieval by diel vertical migrations of lake phytoplankton. *Freshwat. Biol.* 14: 431–438.

Salonen K., Leppäranta M., Viljanen M. & Gulati R. 2009. Perspectives in winter limnology: closing the annual cycle of freezing lakes. *Aquat. Ecol.* 43: 609–616.

Salonen K., Pulkkanen M., Salmi P. & Griffiths R.W. 2014. Interannual variability of circulation under spring ice in a boreal lake. *Limnol. Oceanogr.* 59: 2121–2132.

Shade A., Kent A.D., Jones S.E., Newton R.J., Triplett E.W. & McMahon K.D. 2007. Interannual dynamics and phenology of bacterial communities in a eutrophic lake. *Limnol. Oceanogr.* 52: 487–494.

Sicko-Goad L., Stoermer E. & Kociolek J. 1989. Diatom resting cell rejuvenation and formation: time course, species records and distribution. *J. Plankton Res.* 11: 375–389.

Singleton V.L. & Little J.C. 2006. Designing hypolimnetic aeration and oxygenation systems-a review. *Environ. Sci. Technol.* 40: 7512–7520.

Sivonen K., Niemelä S., Niemi R., Lepistö L., Luoma T. & Räsänen L. 1990. Toxic cyanobacteria (blue-green algae) in Finnish fresh and coastal waters. *Hydrobiologia* 190: 267–275.

Smetacek V. 1985. Role of sinking in diatom life-history cycles: ecological, evolutionary and geological significance. *Mar. Biol.* 84: 239–251.

Sommer U., Adrian R., De Senerpont Domis L., Elser J.J., Gaedke U., Ibelings B., Jeppesen E., Lürling M., Molinero J.C. & Mooij W.M. 2012. Beyond the Plankton Ecology Group (PEG) model: mechanisms driving plankton succession. *Annu. Rev. Ecol. Syst.* 43: 429–448.

Somogyi B., Felföldi T., Vanyovszki J., Ágyi Á, Márialigeti K. & Vörös L. 2009. Winter bloom of picoeukaryotes in Hungarian shallow turbid soda pans and the role of light and temperature. *Aquat. Ecol.* 43: 735–744.

50

Stephens R. & Imberger J. 1993. Reservoir destratification via mechanical mixers. *J. Hydraul. Eng.* 119: 438–457.

Tallberg P. 2000. *Silicon and its impacts on phosphorus in eutrophic freshwater lakes.* Doctoral dissertation. University of Helsinki.

Terzhevik A., Golosov S., Palshin N., Mitrokhov A., Zdorovennov R., Zdorovennova G., Kirillin G., Shipunova E. & Zverev I. 2009. Some features of the thermal and dissolved oxygen structure in boreal, shallow ice-covered Lake Vendyurskoe, Russia. *Aquat. Ecol.* 43: 617–627.

Tranvik L.J., Porter K.G. & Sieburth J.M. 1989. Occurrence of bacterivory in Cryptomonas, a common freshwater phytoplankter. *Oecologia* 78: 473–476.

Utermöhl H. 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik. *Mitt. Int. Ver. Theor. Angew. Limnol.* 9: 1–38.

Vehmaa A. & Salonen K. 2009. Development of phytoplankton in Lake Pääjärvi (Finland) during under-ice convective mixing period. *Aquat. Ecol.* 43: 693–705.

Ventelä A., Saarikari V. & Vuorio K. 1998. Vertical and seasonal distributions of micro-organisms, zooplankton and phytoplankton in a eutrophic lake. *Hydrobiologia* 363: 229–240.

Vuorio K., Järvinen M., Moilanen S., Kotamäki N. & Bilaletdin Ä. 2015. Water level regulation in winter triggers fouling of fishing nets by the diatom *Aulacoseira islandica* in a boreal lake. *Boreal Env. Res.* In press.

Watanabe M., Kohata K. & Kimura T. 1991. Diel vertical migration and nocturnal uptake of nutrients by Chattonella antiqua under stable stratification. *Limnol. Oceanogr.* 36: 593–602.

Weisse T., Müller H., Pinto-Coelho R.A., Schweizer A., Springmann D. & Baldringer G. 1990. Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. *Limnol. Oceanogr.* 35: 781–794.

Wetzel R. 2001. *Limnology: Lake and River Ecosystems*. Academic Press, San Diego.

Weyhenmeyer G.A., Blenckner T. & Petterson K. 1999. Changes of the plankton spring outburst related to the North Atlantic Oscillation. *Limnol. Oceanogr.* 44: 1788–1792.

ORIGINAL PAPERS

I

PUMPING OF EPILIMNETIC WATER INTO HYPOLIMNION IMPROVES OXYGEN BUT NOT NECESSARILY NUTRIENT CONDITIONS IN A LAKE RECOVERING FROM EUTROPHICATION

by

Pauliina Salmi, Ismo Malin & Kalevi Salonen 2014

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Article

Pumping of epilimnetic water into hypolimnion improves oxygen but not necessarily nutrient conditions in a lake recovering from eutrophication

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Abstract

To mitigate deep water oxygen depletion and its consequences, epilimnetic water was pumped into deep water of a eutrophic, 26 km^2 subbasin of Lake Vesijärvi, Finland. In winter, the mechanical mixing largely eliminated vertical differences in temperature, oxygen, and nutrients. Although ice cover prevented oxygen flux from the atmosphere, the high proportion of shallows in the basin with high oxygen concentration facilitated the avoidance of hypoxia by mixing. Despite the disappearance of anoxia in deep water, the volume-weighted mean concentrations of nutrients were not affected. In summer, the introduction of epilimnetic water into the hypolimnion improved oxygen conditions, but anoxia or hypoxia continued to develop. This condition favored the coexistence of nitrification and denitrification and, similar to winter, more than halved total and ammonium-nitrogen (NH_4-N) concentrations in deep water. Despite the presence of nitrate or low concentration of oxygen, phosphorus concentrations continued to be high, and therefore deep water anoxia cannot have been the primary driver maintaining the basin in its eutrophic state. Consequently, in the management of Lake Vesijärvi, a major emphasis should be on external nutrient load and the leakage of nutrients from shallow sediments. A longer time series and attention to biological parameters is needed to determine the final outcome of pumping epilimnetic water into the hypolimnion.

Key words: eutrophic lake, mixing, nutrients, oxygen, temperature

Introduction

Eutrophication in lakes is a common problem connected to anthropogenic activities. High productivity increases sedimentation of organic matter, which can lead to oxygen depletion in deep water. Einsele (1936) and Mortimer (1941) suggested that after the development of anoxia, phosphate is released from sediment by the reduction of an iron–phosphate complex. Phosphate may then accumulate in the deepest water and eventually arrive in the euphotic zone to fuel primary production. Since the formulation of the iron–phosphorus coupling hypothesis, however, evidence has accumulated for alternative or parallel mechanisms of phosphorus (P) release from the

sediment (Golterman 2001, Hupfer and Lewandowski 2008), which should also be considered when interpreting lake P dynamics.

Various techniques have been applied to avoid hypolimnetic oxygen depletion and to accelerate the recovery of lakes from eutrophication (Beutel and Horne 1999, Singleton and Little 2006). Because each lake is unique, however, experiences have been variable (Singleton and Little 2006, Hupfer and Lewandowski 2008, Liboriussen et al. 2009). Due to the short time span and lack of replications, the results of most studies also often suffer from stochastic interannual variations that may override the effects of management efforts. Thus, the interpretation of results is not straightforward.

Management based on simple mechanical mixing, in which oxic epilimnetic water is pumped into deeper water layers, has been attempted in a number of lakes (e.g., Sandman et al. 1990, Matinvesi 1996, Singleton and Little 2006). Compared to many aeration and particularly oxygenation methods, pumping more profoundly affects the stratification of the water column (Singleton and Little 2006). Hence, the associated increase in hypolimnetic temperature from pumping may limit or destroy the habitat of cold-water fish (Beutel and Horne 1999) and enhance respiration, which may counteract the positive effects of mixing.

Mechanical mixing by pumping in winter has been tested in the eutrophic southern basin of Lake Vesijärvi, Southern Finland, but because improved oxygen conditions (Keto 1982) were followed by massive cyanobacteria blooms in summer (Keto and Tallberg 2000), the mixing was discontinued. Cyanobacteria in the less eutrophic and rather isolated (Fig. 1) northern basin of Lake Vesijärvi increased at the same time without any management efforts (Keto and Tallberg 2000); therefore, more regional factors than the mixing at one deep site of another basin was likely involved. Because mechanical mixing was not considered too risky, further experiments were started by local authorities and a foundation committed to long-term operations to improve water quality of the lake.
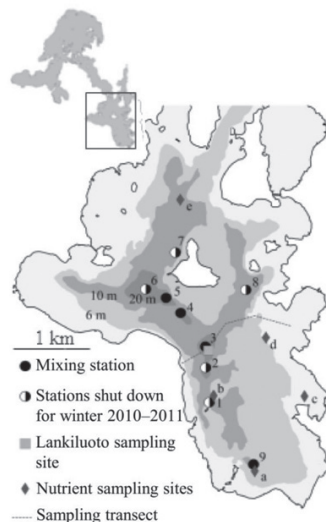

Fig. 1. The Enonselkä basin of Lake Vesijärvi with locations of sampling sites and mixing stations. Sampling depths for nutrient sampling sites were (a) 1, 10, and 12.5–14 m; (b) 0, 10, and 22 m; (c–d) 1 and 3–4 m; and (e) 1, 10, and 17 m.

In this study, we investigated the effects of large-scale mechanical mixing in 2010–2013 in the eutrophic southern basin of Vesijärvi. The mixing approach was originally meant to immediately improve water quality, first by eliminating harmful cyanobacteria blooms. We hypothesized that improvement of deep water oxygen conditions by mixing, both in winter and in summer, would reduce the release of P from the sediment and, consequently, reduce problems with frequent algal blooms.

Materials and methods

Lake Vesijärvi is located in Southern Finland between 2 glaciofluvial eskers (the Salpausselkä ridges), and its basic limnological characteristics are impacted by groundwater seepage. The lake's southern Enonselkä basin (area 26 km², volume 176 x 10⁶ m³, mean depth 6.8 m, maximum depth 33 m, and relative depth 0.57% [$Z_r = 50*$max. depth $*\sqrt{(\pi/\text{area})}$]; Fig. 1) is connected to the rest of Vesijärvi by rather narrow straits that prevent significant upstream exchange of water with the other basins. For decades, Enonselkä was heavily loaded with untreated sewage waters from the city of Lahti. After the sewage diversion in 1976, water quality improved (Keto 1982), and thereafter various management attempts were implemented to strengthen the recovery of the lake. In addition to the reduction of external load, since 1989 Vesijärvi has been manipulated by removal of cyprinid fish (Keto and Sammalkorpi 1988, Kairesalo et al. 1999). To increase zooplankton grazing on phytoplankton, during 2000–2012 (2007–2008 data missing) 38–94 tons of fish (equivalent to ~0.23–0.57 tons of P), mainly cyprinids, were annually removed from the Enonselkä basin. Nevertheless, the deepest water has often been depleted of oxygen, and algal blooms have persisted.

In November 2009, an extensive attempt was initiated to relieve the oxygen deficit during ice cover and summer stratification periods. Pumping of oxygen-rich surface water into deep water was deemed the most cost-effective strategy, and it was implemented using 8 Mixox MC-1100 mixing stations (2.5 kW) and one Mixox MC-750 station (1.5 kW; Water-Eco Ltd, Kuopio, Finland) located at the deepest sites of the Enonselkä basin (Fig. 1). Water from about 3 m depth was pumped down through a 1 m diameter canvas tube at a speed of about 1 m s⁻¹ (Fig. 2). The functional principles have been explained in more detail by Lappalainen (1994) and Bendtsen et al. (2013). To limit resuspension of the sediment, the lower ends of the tubes were 8–10 m above the bottom. The stations were generally operated continuously, excluding spring and autumn overturn periods and short operational malfunctions. In winter 2010/2011 only 4 stations were powered (Fig. 1). In summer 2013, mixing stations were activated on 12 July, which was 36–39 days later than in other mixing summers.
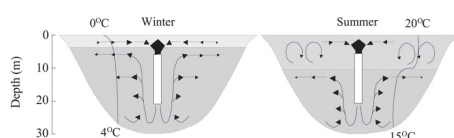
Fig. 2. Flow patterns created by the mixing stations in winter and summer visualized according to temperature and oxygen results of this study. Vertical curves illustrate distributions of temperature with approximately representative values at the surface and at the bottom. Vertical positions of the pumps (3 m depth) and the bottom of the tubes (8–10 m above the bottom) are in scale.

Vertical profiles of dissolved oxygen, nutrient, and iron concentrations were determined in the laboratory from samples collected with a Limnos tube sampler (with inside thermometer) at the 31 m deep site off Lankiluoto islet (Fig. 1), located ~50 m from the mixing station. During ice cover, samples were collected once in January–February and once in March–April. In June–August, samples were collected at roughly monthly intervals. In March–April and in August, samples were also collected from 5 other sampling stations (Fig. 1a–e) for total phosphorus (TP) and total nitrogen (TN). Volume-weighted TP and TN concentrations for the Enonselkä basin were calculated from the depth-integrated product of the mean concentrations in 2.5, 3.5, or 5 m thick water layers and their respective volumes (assuming geometric form of a frustum). Oxygen concentrations were determined by Winkler titration, and nutrient concentrations were determined spectrophotometrically using molybdate-antimony for TP and phosphate-phosphorus (PO_4-P), peroxysulphate or peroxysulphate-sulfanilamide-aromatic amine for TN, and hypochlorite-phenol for ammonium-nitrogen (NH_4-N) methods. Nitrate-nitrogen (NO_3-N) was determined by ion chromatography with conductivity detection. Iron (Fe) concentration was determined by mass spectrophotometry. Epilimnetic pH at the Lankiluoto sampling site was measured from samples taken from 1 m depth with laboratory meters in January–February, March–April, and at 1–5 week intervals during May–August. Chlorophyll a (Chl-a) concentration was determined spectrophotometrically using 75 °C ethanol extraction at 1–5 week intervals during May–August 2000–2012 from integrated samples taken from 0–6 m depth, which was twice the measured Secchi depth. Absorptions were measured at 665 and 750 nm wavelengths by a spectrophotometer, and Chl-a concentration was calculated using an absorption coefficient of 119.

In winter, when oxygen was present throughout the water column, oxygen consumption was calculated from the volume-weighted decrease in oxygen from baseline concentrations measured in January and March at the Lankiluoto sampling site. In summer, oxygen consumption was calculated as described above, but only for the

hypolimnion (depths of 12.5–31 m) and June–July data when some oxygen remained, and extrapolated until the end of August by assuming Q_{10} of 2.7 (Bergström et al. 2010). A conservative estimate of the amount of oxygen pumped into the deep water was made by multiplying pumped water volume by the difference between oxygen concentrations at the inlet depth of the pumps and those at the upper part (12.5–17.5 m) of the hypolimnion. We assumed that water pumped into the hypolimnion spread evenly on a horizontal plane.

Since summer 2008 (excluding summer 2009), more detailed temperature observations have been made at the Lankiluoto sampling site every half hour using temperature recorders (Star-Oddi, Iceland, accuracy ±0.05 °C) attached to a rope at 2 m depth intervals. Water column Schmidt-Idso stabilities (Idso 1973) were calculated from the results of the temperature recorders as:

$$S = \frac{g}{A_0} \int_0^{Zmax} A(z_g - z)(\rho_I - \rho)dz, \qquad (1)$$

where A_0 = lake surface area, A (e.g., km²) = lake area at depth z (m), ρ (kg m⁻³) = water density, ρ_I (kg m⁻³) = water density when completely mixed, g = gravitational acceleration (9.81 kg m⁻¹ s⁻²), and z_g (m) = the depth of the geometric center of gravity of the lake. Heat contents (Θ; Johnson et al. 1978) were calculated as:

$$\Theta = \frac{1}{A_0} \int_0^{Zmax} c\rho ATdz, \qquad (2)$$

where A_0 = lake surface area, c (J g⁻¹ °C⁻¹) = the specific heat capacity of water, ρ (kg m⁻³) = density of the water layer, A (e.g., km²) = lake area at depth z (m), and T = temperature (°C).

Oxygen samples for titration were collected from the Lankiluoto site at 5 m depth intervals. In winters 2010–2012, additional profiles of dissolved oxygen were measured as a transect across the basin (Fig. 1) with an optical sensor (ProODO, Yellow Springs Instruments, USA, accuracy ±0.1 g m⁻³ or ±1% of the reading) calibrated by Winkler titration. Snow depth and ice thickness were measured with a scaled rod. In summers 2008–2013, temperature and oxygen concentrations were also measured hourly at a measurement float with Marvet sensors (Elke Sensor LLC, Estonia, accuracy 0.1 °C and 0.1 g m⁻³ O_2) installed at 10, 20, and 30 m depths.

To minimize the effects of possible directional trends in the time series due to climate change (IPCC 2007) or the reduction of nutrient load in the 1970s (Keto and Tallberg 2000), only the last 10 years before the mixing experiment were used as reference. Confidence limits for the medians of the results of the reference years are given according to tables by Van der Parren (1970). Statistical analyses were conducted with SPSS Statistics version 21 (IBM, USA).

Results

To evaluate the effects of mechanical mixing, we present the results of profoundly different seasons: winter and summer. We took advantage of this contrast, in combination with a mechanical mixing experiment, to evaluate the role of anoxia in the release of nutrients from the sediment.

Winter

During winter of the reference years 2000–2009, deep water temperature under the ice of the Enonselkä basin increased at 29 m with increasing duration of ice cover (r^2 = 0.68, data not shown). In winter 2008/2009 without mixing, this increase was high, 3.7 °C at 30 m between freezing in December and sampling in March. Compared to the reference years, mechanical mixing in the following years kept temperature in the deepest water ~1 °C cooler (Fig. 3), and although the number of observations was too low for statistical testing, the slope of the regression with ice duration seemed similar to the reference years. Mixing largely disrupted the vertical temperature gradient below the depth of the mixing pumps, and the temperature gradient (0.4–0.7 °C) between 5 and 29–30 m depths was greatly reduced compared to the reference years (1.3–2.2 °C). The ascending return flow of pumped water from the deepest layers, however, stayed below 3–5 m depth (Fig. 3), and the thermal stabilities were similar to reference winter 2009 (Table 1). In the period between lake freezing and the end of March, heat content increased 24–32 MJ m^{-2}; thus, mixing probably did not enhance heat loss to ice but rather enhanced heat gain from the sediment. Vertical distributions of temperature, minimal differences in ice thickness over the Enonselkä basin, no detectable delay in freezing, and negligible advance in melting above the pumps beyond a few meters distance also indicated that mixing did not markedly affect the ice cover in the neighborhood of the mixing station.

In late winter of the reference years, Enonselkä frequently suffered from oxygen depletion in the deepest water (Fig. 4), but interannual variation was high due to variable water temperature and duration of ice cover. In the mixing winters, deep water oxygen concentration remained high, mainly due to the dilution effect by mixing; there may also have been a decreasing trend in oxygen consumption of the whole water column (Fig. 5). Because oxygen concentration is more sensitive than temperature for indicating horizontal spread of water from the neighborhood of the mixing stations, even distributions at each depth over the basin (Fig. 6) suggest that the results obtained at ~50 m distance from the mixing station satisfactorily represented the whole basin. Horizontal

mixing, however, was limited only to depths shallower than the shoulders between the isolated deep sites; thus, in winter 2010/2011, anoxic conditions developed in sites where the mixing stations were not powered.

At 1 m depth, the concentrations of TP and PO$_4$-P were similar in March–April, both in the reference and mixing years (Fig. 3). In the deepest water in the reference years, oxygen depletion was regularly accompanied by up to 2 orders of magnitude higher, but interannually variable, concentrations of TP and PO$_4$-P (Fig. 3 and 4). Mixing reduced vertical differences, and deep water concentrations were 50–80% of the median and much lower than the highest concentrations of the reference years. Fe possibly also decreased, but it was at the lower range of the reference years. TN behaved similarly to TP. NH$_4$-N, which was abundant only in deep water of the reference years, was dramatically decreased by mixing. In contrast, the concentrations of NO$_3$-N roughly doubled in relation to the median value (Fig. 3 and 4). The concentrations of TP and TN at different depths of the water column remained relatively stable during January–March, both in the reference and mixing winters (Fig. 7). At 29 m the variation of the results was higher than at shallower
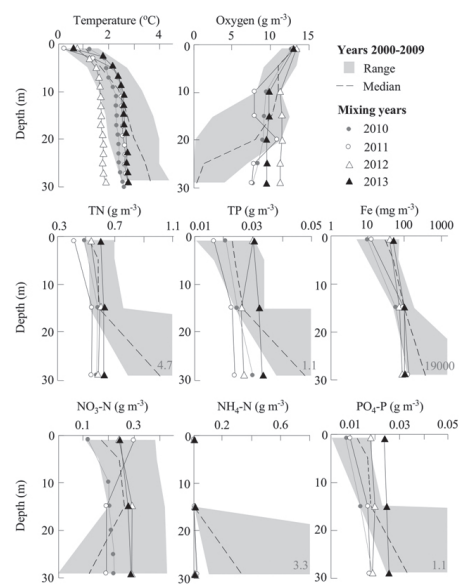

Fig. 3. Vertical distributions of temperature, dissolved oxygen, and nutrient as well as iron concentrations during March–April of the reference and mixing winters. In the graphs of NO$_3$-N and NH$_4$-N, the y-axis begins at the detection limit of 0.01 g m^{-3}, and in the PO$_4$ graph at 0.002 g m^{-3}.

depths, probably due to differences in the distance from the bottom following variation in the precise location of sampling around the deepest point. The small contribution of deep water to the total volume of the basin, however, led to no differences in volume-weighted TP and TN concentrations in March between the reference and mixing years (Fig. 8).
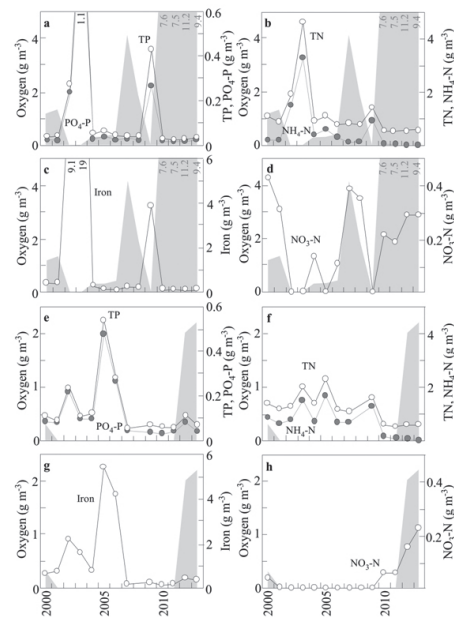

Fig. 4. Deep water (29 m) concentrations of dissolved oxygen, nutrients, and iron during (a–d) March–April and (e–h) August of the reference and mixing years. In the graphs of NO_3-N and NH_4-N, the y-axes begin at the detection limit of 0.01 g m^{-3}. Grey area represents oxygen concentration. Results from August 2008 were excluded due to early beginning of autumn convection.
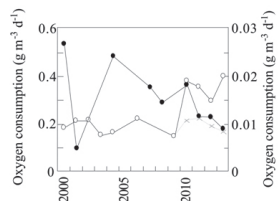

Fig. 5. Oxygen consumption in January–March in the whole water column (dots, right scale) and in June–August in the 12.5–31 m hypolimnion (circles, left scale). The crosses show results where oxygen pumped into deep water have not been taken into account.
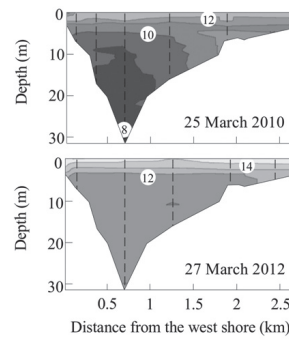

Fig. 6. The extremes of horizontal distributions of dissolved oxygen concentration in March of the mixing winters 2010–2012 through a sampling transect shown in Fig. 1.
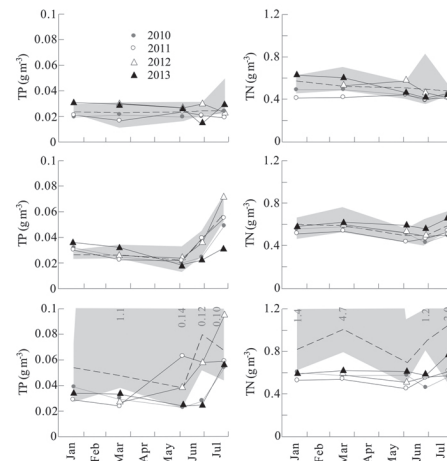

Fig. 7. Seasonal development of the concentrations of TP and TN at 1 m (upper panels), 15 m (middle panels), and 29 m (lower panels). Grey area = range 2000–2009; dashed line = median 2000–2009.
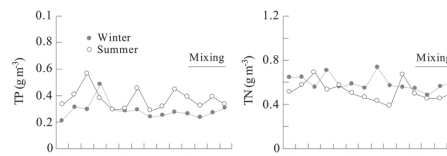

Fig. 8. Volume-weighted TP (left panel) and TN (right panel) in winter (Mar-Apr) and summer (Aug) calculated for the whole Enonselkä basin.

Table 1. Maximum stabilities and heat contents of the water column at 12:00 h in winter and summer in the reference (2008-2009) and mixing (2010-2013) years.

	Year	Stability (J m^{-2})	Heat content (MJ m^{-2})
Freezing - March	2009	8	37
	2010	6	54
	2011	10	47
	2012	5	46
	2013	9	56
June - August	2008	94	559
	2010	212	627
	2011	203	608
	2012	103	562
	2013	226	530

Summer

In August of the reference years, the depth of the epilimnion was generally ~10 m, and below the metalimnion, temperature slowly decreased to 10.8–13.4 °C at 29 m (Fig. 9). Detailed temperature recorder data from July–August 2008 showed little fluctuation or increase of deep water temperatures (Fig. 10), which suggests stable stratification. Mixing in the summers 2010–2012 elevated the deepest water temperature to 17–17.5 °C, and the temperature difference between the 5–6.4 m and 29–30 m depths in August was only 0.9–3.9 °C compared to 4.5–9.9 °C in the reference summers. In summer 2013, when mixing began 36–39 days later than in the other years, deep water temperature remained closer to the reference years, and the temperature difference between 5 and 30 m was 6.2 °C (Fig. 9). Mixing led to a sharp metalimnion (Fig. 9 and 10), which moved upward during the summer stratification; thus, warm epilimnetic water pumped into the hypolimnion was mixed so effectively with cooler deep water that the ascending return flow stopped in the metalimnion 4–8 m below the depth of the pumps. Consequently, the volume of deep water affected by mechanical mixing was much smaller than in winter.

Although mixing theoretically decreases thermal stability of the water column, it was masked by interannual variation in weather conditions. In the mixing summers 2010 and 2011, air temperature (Finnish Meteorological Institute, data not shown), and thus the surface water, were unusually warm and explain the higher thermal stabilities compared to summer 2008 without mixing (Table 1); however, heat contents of water in summers 2010 and 2011 were only slightly higher. The higher stability in 2013 than in the other mixing summers may be explained by the late start of mixing.

In August of the reference summers, anoxia regularly occurred below 10–20 m (Fig. 4 and 9). Only in 2000 was a low concentration of oxygen (0.3 g m^{-3}) found at 29 m. The results of the oxygen sensor installed at 30 m depth showed that, compared to summer 2009, mixing reduced the duration of anoxia from 97 days to 35, 60, and 10 days in 2010, 2011, and 2012, respectively, and to about 1 day in 2013; however, mixing generated hypoxic conditions in a much larger water volume, especially in summers 2010 and 2011 compared to previous years (Fig. 9). In the reference summers, the volume-weighted median oxygen consumption in the 12.5–31 m depth layer in June–August was 0.18 g m^{-3} d^{-1} (95% confidence limits 0.15–0.22 g m^{-3} d^{-1}; Fig. 5), while in the mixing summers, when the pumped water was taken into account, it was almost twice as high (range 0.30–0.40 g m^{-3} d^{-1}). Due to totally different stratifications in winter and summer, their oxygen consumptions in the reference years could be compared only at 29 m depth. The median oxygen consumption rate was 0.28 g m^{-3} d^{-1} (range 0.03–0.45 g m^{-3} d^{-1}) in summer, which is one order of magnitude higher than in winter, 0.06 g m^{-3} d^{-1} (range 0.0–0.14 g m^{-3} d^{-1}).

In the mixing summers, TP and PO$_4$-P at 29 m were 51–100% and 32–87%, respectively, of the reference years' median (Fig. 4 and 9). In contrast to winter, no significant relationship between anoxia and TP or PO$_4$-P in the hypolimnion could be found, but respective relationships of Fe with TP or PO$_4$-P were obvious (Fig. 4). When the mixing years were excluded, there was no linear correlation (r^2 = 0.1) between TP concentration and oxygen consumption at 29 m in June–August (Fig. 5). Mixing reduced concentration of TN in deep water, and at 29 m it was only about 50% of the minimum of the reference summers. Mixing even more strikingly reduced NH$_4$-N, so that its concentration in the deepest water decreased from up to 0.17 g m^{-3} to below detection. At the same time, mixing increased NO$_3$-N concentration at 29 m depth, from undetectable concentrations to 0.07–0.23 g m^{-3} (Fig. 4).

During summer in the reference years, TP concentration at 1 m depth was stable (Fig. 7). At 15 m and 29 m the concentration increased on average by 76% and 190%, respectively, (paired t-test, $p < 0.05$) in June–July, and the mixing years followed the same trend (up to 200% increase; Fig. 7). TN showed smaller average increases (24% and 71% at 15 m and 29 m depths, respectively, t-test, $p < 0.05$). Compared to the reference years, only at 29 m depth were TN concentrations in the mixing years different (10–50% lower) from the respective median values (Fig. 7). Due to the small volume of deep water, volume-weighted TP and TN concentrations in late summer were not notably affected by the mixing (Fig. 8).

Volume-weighted TP and TN concentrations calculated from samples taken from the 6 sites were on average 1% and 3% lower, respectively, than concentrations calculated only from the samples taken from the Lankiluoto sampling site. In winter, respective concentrations were 7% and 3% higher. Excluding winter TN concentrations (paired t-test, $p = 0.02$), these differences were not significant (paired t-test, $p > 0.05$), indicating that the results obtained from the Lankiluoto sampling site alone well represent the whole basin.
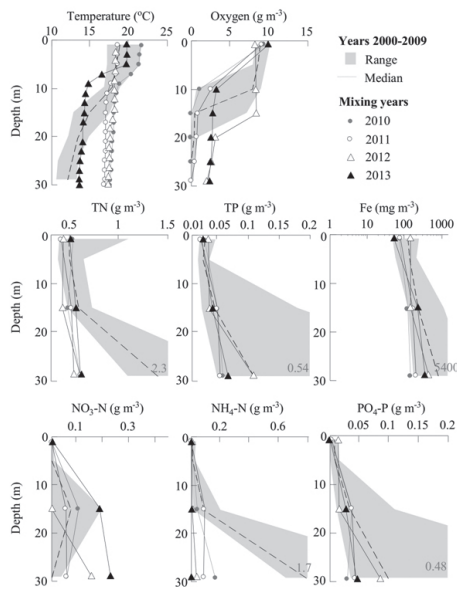

Fig. 9. Vertical distributions of temperature and dissolved oxygen and nutrient and iron concentrations in August of the reference and the mixing summers. In the NO_3-N and NH_4-N graphs, the y-axes start at the detection limit of 0.01 g m^{-3} (0.002 g m^{-3} for PO_4). Results from August 2008 were excluded due to early beginning of autumn convection.
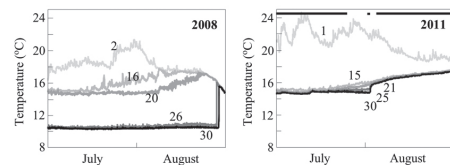

Fig. 10. Temperature at fixed depths (m) in July–August with mixing (right panel) and without mixing (left panel). Shutdowns of mixing station 3 are shown by interruptions in the black horizontal bar on the top of the right panel.

The seasonal development of Chl-*a* concentration in the epilimnion was inter- and intra-annually variable (Fig. 11), but the results of mixing years (summer average 11.0 mg m^{-3}, SD = 8.7) closely corresponded to those of reference years (summer average 10.0 mg m^{-3}, SD = 5.2; Fig. 11). Similarly in both cases, epilimnetic pH typically reached high values (>8) in May during the spring maximum of phytoplankton but decreased in June–July to 7.5–8, and in some years increased again in late summer (Fig. 11).
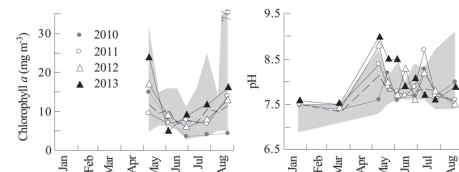

Fig. 11. Seasonal development of chlorophyll *a* at 0–6 m depth (left panel) and pH at 1 m depth (right panel). Grey area = range of 2000–2009; dashed line = median 2000–2009.

Discussion

Technically, the mechanical mixing worked as expected both in winter and summer. In line with earlier studies (e.g., Grochowska and Gawrońska 2004), mixing profoundly affected hypolimnetic temperature and stratification. Due to the dependence of water density on temperature and slightly steeper vertical temperature gradient in summer, density differences between 5 m and 30 m depths were an order of magnitude lower in winter (~0.02 kg m^{-3}) than in summer (~0.6 kg m^{-3}), which greatly facilitated mixing in winter. Despite higher oxygen consumption in the mixing years, the high oxygen binding capacity of cold water and the proportionally very high volume of shallow water maintained the high deep water oxygen concentration. In summer, the power of the mixing stations was not sufficient to avoid hypoxic or anoxic conditions. As indicated by relatively high hypolimnetic temperatures in the reference years, the Enonselkä basin is sensitive to wind and has a tendency to destratify in early autumn (Fig. 10); therefore, at the coolest range of summers, the development of polymictic conditions might be possible, and an increase in mixing power might not be a good alternative. In winter, small changes in temperature due to mixing probably have marginal ecosystem effects, while in summer, a several degrees increase in hypolimnetic temperature affects both organisms and their metabolism. Perhaps the most dramatic ecosystem effect was the destruction of the cool oxygenated metalimnion, an important habitat for cool-water fish.

In the Enonselkä basin, the stability of deep water temperatures before the late summer cooling of the epilimnion in 2008 (Fig. 10) suggests low exchange of water between anoxic and oxic water layers. In addition to temperature, salts (as indicated by 28% higher median conductivity at 29 m compared to 15 m, data not shown) leaching from the sediment significantly contributed to the resistance of the deepest water against mixing. Thus, in the midsummer period of reference years, thermal and chemical stratification together formed a strong barrier against nutrient flux from the anoxic water layers. Despite no difference in thermal stabilities between the reference and mixing years, the conditions within the hypolimnion were different. Small vertical temperature differences throughout the hypolimnion in the mixing summers, compared to those in the reference summers with continuous temperature gradient down to the bottom, should have favored nutrient transport from the hypolimnion to the epilimnion; however, epilimnetic total nutrient concentrations of the mixing summers were not different from 2000 to 2009 (Fig. 7). Because Chl-*a* concentration was not affected (Fig. 11) in relation to the median of the reference years, mechanical mixing did not seem to increase nutrient fluxes from the hypolimnion.

In the mixing summers of the Enonselkä basin, the importance of temperature on respiration was emphasized by the heat flux caused by pumping warmer water into the hypolimnion. Increased oxygen supply and turbulence probably also enhanced the mineralization of organic matter during its sedimentation, but their role could not be quantified. Similarly, Gantzer et al. (2009) found increased hypolimnetic oxygen consumption in 2 drinking water reservoirs in Virginia that were oxygenated in summer by bubble-plume diffusers. Because mixing did not affect epilimnetic Chl-*a* concentration in the Enonselkä basin, phytoplankton biomass likely remained unaffected; therefore, increased temperature and turbulence, and perhaps also availability of oxygen, were mainly responsible for increased hypolimnetic oxygen consumption. Based on Q_{10} of 2.7 (Bergström et al. 2010), the observed 5 °C elevation of hypolimnetic water temperature in late summers 2010–2012 (Fig. 9) by mixing could have increased oxygen consumption by 85%, which agrees with the estimated 62–120% increase in the 12.5–31 m deep water. Consequently, in the Enonselkä basin the observed increase would mean a quantitatively important reduction in the proportion of organic matter accumulating in the sediment, which should lead to a gradual decrease in oxygen consumption by the sediment. In agreement with that, our results suggested the reduction of under-ice oxygen consumption in winter when respiration in the sediment relies on sedimentation in earlier summers; however, due to the low

number of observations this trend is still tentative. The reduction of hypolimnetic oxygen consumption during several years was also observed by Gantzer et al. (2009).

During summer, epilimnetic TP concentration may be decreased by sedimentation and inflow. Kamarainen et al. (2009) found consistently decreasing TP during the summer months in Lake Mendota where epilimnetic concentrations of TP and Chl-*a* are similar to the Enonselkä basin. A gradual increase in epilimnetic TP during summer has been observed in many eutrophic lakes (Jeppesen et al. 1997, Søndergaard et al. 1999, Spears et al. 2008, Solim and Wanganeo 2009), however, possibly due to lower dilution of external load, although it has often also been attributed to leaching from anoxic sediments. Evidence is growing that anoxia is the consequence rather than the cause of elevated nutrient concentrations (Gächter and Wehrli 1998, Hupfer and Lewandowski 2008). This conception is corroborated by our mixing experiment with the contrasting sedimentation conditions between winter and summer.

In the Enonselkä basin, P release from sediment to water was closely coupled with release of Fe (Fig. 4), thus supporting the traditional coupling between Fe and P. Because epilimnetic TP concentration remained stable throughout the summer, external load of P as well as its flux from shallow sediments or deeper water layers were in balance with sedimentation and other losses. Mechanical mixing obviously maintained redox levels so high that nitrate, and in 2012 and 2013 also some oxygen, were left in the hypolimnion at the end of the summer stratification (Fig. 4), conditions that should not favor the release of P from the sediment (Andersen 1982). Because mixing year concentrations of TP at 15 m were similar and at 29 m (Fig. 7) were between the median and the lower range of the reference years, and because the relationship between TP and oxygen concentrations at 29 m depth in late summer (Fig. 4) was negligible, factor(s) other than anoxia must have been more important for the increased hypolimnetic TP concentrations in the Enonselkä basin, as suggested also by Niemistö et al. (2012). Although direct information of P-flux from the sediment is lacking, the improved oxygen conditions, and even absence of anoxia in the water column in mixing summers, provide strong evidence that the reason for the observed increase in deep water TP was high nutrient load rather than oxygen depletion.

When oxygen is depleted in water or sediment, a number of facultatively aerobic microorganisms shift to use NO_3-N (Knowles 1982), which is why NO_3-N was generally depleted in the deep water of the reference years. The presence of NO_3-N in the hypolimnion of the mixing years in the Enonselkä basin down to the sediment surface shows that increased redox potential enabled nitri-

fication, which can occur at rather low oxygen concentrations (>0.3 g m^{-3}; Stenstrom and Poduska 1980). Because of the spatial variability inherent in mixing, virtual coexistence of nitrification and denitrification is possible within a certain range of oxygen concentrations. In winter, such conditions occurred in the sediment, and in summer in hypolimnetic water. The enhancement of nitrification–denitrification due to mixing was clearly observed in deep water, where TN concentration was reduced to half of the reference years' minimum (Fig. 9). This is in agreement with the finding that mixing doubled denitrification rates (S. Hietanen, unpubl. data) from sediment samples taken from 17 m depth at 70 m distance from the mixing station.

Under high pH, P leaching from oxic sediments in the epilimnion may be accelerated (e.g., MacPherson et al. 1958, Christophoridis and Fytianos 2006). During summer stratification, pH values in the epilimnion of the Enonselkä basin were often >8, as also found by Keto and Tallberg (2000). Because pH fluctuates according to the diurnal cycle of primary production, however, our results should be considered only directional. The experiments of Tallberg (2000) and Koski-Vähälä et al. (2001) with sediments taken from 9 m depth in the Enonselkä basin have shown that between pH 7 and 9.5, P mobilization from the sediment is further enhanced by an increase in silicon (Si) concentration. They hypothesized that the sedimentation of diatoms after the spring maximum could increase the concentration of reactive Si so that P mobilization would be amplified. Because the strength and species composition of the high phytoplankton spring maximum may vary (Liukkonen et al. 1997), it would be interesting to relate existing phytoplankton data with deep water TP and PO$_4$-P.

Lake management by mechanical mixing has generally been based on the idea that the consequences of bottom deposits accumulated over the long term need to be controlled, but only a small fraction of organic matter settling out of the epilimnion can be left until next growing season. Matzinger et al. (2010) found that in 2 small (areas 2% and 12% of that of the Enonselkä basin), stratified Swiss lakes, where about half of the water volume was deeper than 10 m, ~80% of oxygen consumption in the hypolimnion was due to the present lake productivity rather than to old organic matter in the sediment. Although management methods other than the reduction of external load might have only limited success, the results of the Enonselkä basin suggest that the mixing approach may still provide small improvements that might be stretched by careful optimization of mechanical mixing practices.

The 4-year mixing experiment of the Enonselkä basin suggested both favorable and unfavorable effects, some of which may only be realized later. In winter, the improvement of oxygen conditions by mixing is indisputable, and due to low water temperature and primary production, improvement was easily attained. Mixing was able to largely homogenize under-ice nutrient concentrations, but as indicated by reduced respiration, it probably also contributed to a slight decrease in nutrient concentrations of water that regulate the important spring phytoplankton maximum. In summer, redox conditions were also clearly improved, particularly indicated by the presence of NO$_3$-N in the hypolimnion, but while the concentrations of N were reduced, those of P were affected only slightly. Although no remarkable reduction in volume-weighted TN was observed (Fig. 8), mixing clearly enhanced the coexistence of nitrification and denitrification. Continued experimentation is needed to evaluate the final importance of the effects of the mixing on the Enonselkä basin and whether they justify its running cost (€97 000   in 2011). It is also too early to judge how mechanical mixing compares with the other management practices of the Enonselkä basin. The mixing approach has an outstanding advantage in that it can be varied, modified, and repeated. Vesijärvi, with its mixing stations, various automatic measurements, and associated monitoring and research activities, provides promising possibilities to test various scientific and management hypotheses with a time span and scale rarely available.

Acknowledgements

References

Andersen JM. 1982. Effect of nitrate concentration in lake water on phosphate release from the sediment. Water Res. 16(7):1119–1126.

Bendtsen J, Gustafsson KE, Lehtoranta J, Saarijärvi E, Rasmus K, Pitkänen H. 2013. Modeling and tracer release experiment on forced buoyant plume convection from coastal oxygenation. Boreal Environ Res. 18:37–52.

Bergström I, Kortelainen P, Sarvala J, Salonen K. 2010. Effects of temperature and sediment properties on benthic CO$_2$ production in an oligotrophic boreal lake. Freshwater Biol. 5(8):1747–1757.

Beutel MW, Horne AJ. 1999. A review of the effects of hypolimnetic oxygenation on lake and reservoir water quality. Lake Reserv Manage. 15(4):285–297.

Christophoridis C, Fytianos K. 2006. Conditions affecting the release of phosphorus from surface lake sediments. J Environ Qual. 35:1181–1192.

Einsele W. 1936. Über die Beziehungen des Eisenkreislaufs zum Phosphatkreislauf im utrophen See. Arch Hydrobiol. 29:664–686.

Gächter R, Wehrli B. 1998. Ten years of artificial mixing and oxygenation: no effect on the internal phosphorus loading of two eutrophic lakes. Environ Sci Technol. 32(23):3659–3665.

Gantzer PA, Bryant LD, Little JC. 2009. Effect of hypolimnetic oxygenation on oxygen depletion rates in two water supply reservoirs. Water Res. 43(6):1700–1710.

Golterman HL. 2001. Phosphate release from anoxic sediments or 'What did Mortimer really write?' Hydrobiologia. 450:99–106.

Grochowska J, Gawrońska H. 2004. Restoration effectiveness of a degraded lake using multi-year artificial aeration. Pol J Environ Stud. 13(6):671–681.

Hupfer M, Lewandowski J. 2008. Oxygen controls phosphorus release from lake sediments – a long lasting paradigm in limnology. Int Rev Hydrobiol. 93(4–5):415–432.

Idso SB. 1973. On the concept of lake stability. Limnol Oceanogr. 18:681–683.

[IPCC] Intergovernmental Panel on Climate Change. 2007. Climate Change 2007: synthesis report. Geneva, Switzerland.

Jeppesen E, Jensen JP, Søndergaard M, Lauridsen T, Pedersen LJ, Jensen L. 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. Hydrobiologia. 342–343:151–164.

Johnson NM, Eaton JS, Richey JE. 1978. Analysis of five North American lake ecosystems. II Thermal energy and mechanical stability. Verh Internat Verein Limnol. 20:562–567.

Kairesalo T, Laine S, Luokkanen E, Malinen T, Keto J. 1999. Direct and indirect mechanisms behind successful biomanipulation. Hydrobiologia. 395–396(0):99–106.

Kamarainen AM, Penczykowski RM, Van de Bogert MC, Hanson PC, Carpenter SR. 2009. Phosphorus sources and demand during summer in a eutrophic lake. Aquat Sci. 71:214–227.

Keto J. 1982. The recovery of L. Vesijärvi following sewage diversion. Hydrobiologia. 86(1–2):195–199.

Keto J, Sammalkorpi I. 1988. Fading recovery: a conceptual model for Lake Vesijärvi management and research. Aqua Fennica. 18:193–204.

Keto J, Tallberg P. 2000. The recovery of Vesijärvi, a lake in southern Finland: water quality and phytoplankton interpretations. Boreal Environ Res. 5(1):15–26.

Knowles R. 1982. Denitrification. Microbiol Rev. 46(1):43–70.

Koski-Vähälä J. Hartikainen H, Tallberg P. 2001. Phosphorus mobilization from various sediment pools in response to increased pH and silicate concentration. J Environ Qual. 30:546–552.

Lappalainen KM. 1994. Positive changes in oxygen and nutrient contents in two Finnish lakes induced by Mixox hypolimnetic oxygenation method. Verh Internat Verein Limnol. 25:2510–2513.

Liboriussen L, Søndergaard M, Jeppesen E, Thorsgaard I, Grünfeld S, Jakobsen TS, Hansen K. 2009. Effects of hypolimnetic oxygenation on water quality: results from five Danish lakes. Hydrobiologia. 625(1):157–172.

Liukkonen M, Kairesalo T, Haworth EY. 1997. Changes in the diatom community, including the appearance of *Actinocyclus normannii* f. *subsalsa*, during the biomanipulation of Lake Vesijärvi, Finland. Eur J Phycol. 32(4):353–361.

MacPherson LB, Sinclair NR, Hayes FR. 1958. Lake water and sediment. III The effect of pH on the partition of inorganic phosphate between water and oxidized mud or its ash. Limnol Oceanogr. 3(3):318–326.

Matinvesi J. 1996. The change of sediment composition during recovery of two Finnish lakes induced by waste water purification and lake oxygenation. Hydrobiologia. 335(3):193–202.

Matzinger A, Müller B, Niederhauser P, Schmid M, Wüest A. 2010. Hypolimnetic oxygen consumption by sediment-based reduced substances in former eutrophic lakes. Limnol Oceanogr. 55(5):2073–2084.

Mortimer CH. 1941. The exchange of dissolved substances between mud and water in lakes. J Ecol. 29(2):280–329.

Niemistö J, Tamminen P, Ekholm P, Horppila J. 2012. Sediment resuspension: rescue or downfall of a thermally stratified eutrophic lake? Hydrobiologia. 686:267–276.

Sandman O, Eskonen K, Liehu A. 1990. The eutrophication history of Lake Särkinen, Finland and the effects of lake aeration. Hydrobiologia. 214(1):191–199.

Singleton VL, Little JC. 2006. Designing hypolimnetic aeration – a review. Environ Sci Technol. 40(24):7512–7520.

Solim SU, Wanganeo A. 2009. Factors influencing release of phosphorus from sediments in a high productive polymictic lake system. Water Sci Technol. 60(4):1013–1023.

Søndergaard M, Jensen JP, Jeppesen E. 1999. Internal phosphorus loading in shallow Danish lakes. Hydrobiologia. 408–409:145–152.

Spears BM, Carvalho L, Perkins R, Paterson DM. 2008. Effects of light on sediment nutrient flux and water column nutrient stoichiometry in a shallow lake. Water Res. 42(4–5):977–986.

Stenstrom MK, Poduska RA. 1980. The effect of dissolved oxygen concentration on nitrification. Water Res. 14(6):643–649.

Tallberg P. 2000. Silicon and its impacts on phosphorus in eutrophic freshwater lakes [dissertation]. [Yliopistopaino (Finland)]: University of Helsinki.

Van der Parren J. 1970. Tables for distribution-free confidence limits for the median. Biometrika. 57(3):613–617.

II

DEVELOPMENT OF PICOPLANKTON DURING NATURAL AND ENHANCED MIXING UNDER LATE-WINTER ICE

by

Pauliina Salmi, Anne Lehmijoki & Kalevi Salonen 2014

III

REGULAR BUILD-UP OF THE PHYTOPLANKTON SPRING MAXIMUM BEFORE ICE-BREAK IN A BOREAL LAKE

by

Pauliina Salmi & Kalevi Salonen 2015

Submitted manuscript

IV

**AN IMPROVED APPROACH TO PHYTOPLANKTON
COUNTING BASED ON REAL-TIME STATISTICS**

by

Kalevi Salonen, Pauliina Salmi, Harri Högmander & Jorma Keskitalo 2015

Manuscript