

## Fighting internal phosphorus loading: An evaluation of the large scale application of gradual Fe-addition to a shallow peat lake



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### ABSTRACT

Abatement of external phosphorus (P) loading and biomanipulation are measures that are often applied with the aim to restore a macrophyte dominated clearwater state in turbid, anthropogenically eutrophied lakes. The recovery of such lakes, however, is often hampered by 'internal eutrophication', as a result of the release of historically accumulated P from the sediment into the water column. One way to combat this internal P loading is by adding iron (Fe) into the lake, which naturally binds to phosphate. Although studied in the laboratory or mesocosms, the effects of iron addition on a whole-lake scale are largely unknown. In this study we therefore compiled lake monitoring data to evaluate the effect of a gradual dose of 33 g Fe m<sup>-2</sup> on the water quality and biotic communities (phytoplankton, zooplankton and macrophytes) of Lake Terra Nova. During and after the Fe-addition, we also carried out assays to evaluate the effect of the Fe-addition on sediment P-release rates. Lake Terra Nova is a eutrophied, shallow peaty lake that has been subjected to biomanipulation measures for 10 years.

The large scale addition of iron during 2010 and 2011 resulted in a substantial reduction of dissolved P, suspended matter (SM), phytoplankton biomass and relative Cyanobacterial biomass, whereas macrophytes reappeared. Fe-addition also resulted in strongly reduced sediment P-mobilisation rates. Nevertheless, reductions of water column TP in response to the Fe-addition were short-lived. This discrepancy between the observed TP-levels in the water column and sediment P-release rates could indicate that sustained loading with external P and interactions of chemical compounds with Fe may jeopardize long term restoration success by depleting the water column reservoir of reactive Fe. Ecological processes can in addition enhance this process by shunting P from the sediment to the water column.

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### 1. Introduction

Eutrophication in fresh water lakes has been a major environmental problem all over the world due to high anthropogenic input of phosphorus (P) and nitrogen (N) from agriculture, industry and waste water during the last century (Smith and Schindler, 2009). This high input of nutrients impacted aquatic ecosystems by enhancing lake productivity, leading to a change in

community structure and a decrease in light availability and biodiversity of these ecosystems (Smith and Schindler, 2009). During the last few decades, however, measures are taken in order to restore aquatic ecosystems. Great efforts have been made ever since, largely by reducing external input of nutrients by either closing off nutrient rich input sources or by pre-treating nutrient rich water before it entered the lakes (Jeppesen et al., 2007). Whereas this has led to considerable improvements of water quality (Jeppesen et al., 2007), a full recovery has not yet been reached in many cases, through internal loading from nutrients that have been building up in the lake sediment (Cooke et al., 1993; Smolders et al., 2006).

Various restoration measures have been applied to lakes in order to tackle this delay in recovery, including managing foodweb dynamics through removal of bioturbating and zooplanktivorous

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fish (e.g., biomanipulation; Meijer et al., 1994; Søndergaard et al., 2007) and by adding one, or various, chemical P binding agents in the lake (Cooke et al., 1993; Burley et al., 2001; Smolders et al., 2006). Biomanipulation has been highly successful in shifting turbid lakes to the clear water state (Meijer et al., 1994; Jeppesen et al., 2012). However, the effect is often short-lived and lakes return to the turbid state when the external or internal nutrient loading is not simultaneously reduced by additional measures (Søndergaard et al., 2007; Jeppesen et al., 2012). Indeed, low P concentrations are a necessity for long-term biomanipulation success (Meijer et al., 1994; Jeppesen et al., 2012). By adding chemical P binding agents to reduce internal P loading, P is chemically precipitated from the water column and P sorption of the lake sediment is enhanced. This is often done through an addition of either iron (Fe) or other chemical P binding agents such as aluminum (Al), calcium (Ca) or lime or lanthanum-enriched bentonite clay (Phoslock<sup>®</sup>) to the water column or sediment (Boers et al., 1994; Burley et al., 2001; Smolders et al., 2006; Lürling and Van Oosterhout, 2013). The long-term effects and potential consequences of these chemical additions have been described for case studies with Al and lime (Cooke et al., 1993; Burley et al., 2001). The restoration success of iron addition has long been debated (see Cooke et al., 1993). Although various mesocosm and field experiments have suggested that addition of Fe has the potential to lower total phosphorus (TP) concentrations in the water column (Boers et al., 1994; Daldorph and Price, 1994; Jaeger, 1994; Van Donk et al., 1994; Burley et al., 2001; Van der Welle et al., 2007; Goldyn et al., 2014), the stability of its bonding capacity to P may strongly depend on specific conditions in the field, such as redox potential, availability of other compounds with high affinity for Fe (such as SO<sub>4</sub>) and the concentrations of Fe relative to P (Cooke et al., 1993; Kleeberg et al., 2013). Evaluation of Fe-application to the scale of entire lakes so far has been limited to the follow-up of very specific variables (e.g., nutrient availability, phytoplankton biomass, macrophyte cover or the occurrence of fish kills) during relatively short time spans (<1 year) (Boers et al., 1994; Daldorph and Price, 1994; Jaeger, 1994). To our knowledge, comprehensive studies consisting of systematic and simultaneous monitoring of multiple abiotic factors and biotic food web components during longer time periods are very scarce or non-existent.

Adding Fe to a lake is not as artificial as it seems, as under natural conditions, Fe can seep in the sediment of lakes via upwelling. However, due to regional changes in hydrological regimes (e.g. through groundwater extraction) such iron-rich seepage has in many places decreased (Van der Welle et al., 2007),

exacerbating the effects of anthropogenic eutrophication. Therefore, addition of Fe may be seen as a way to increase a system's P-binding capacity through a mechanism that is very common in nature. However, it may be expected that the sudden addition of large quantities of iron to a lake may harm aquatic communities. High concentrations of Fe may present a threat to aquatic biota through direct toxicity effects or via abrupt pH reductions (Linton et al., 2007). Accumulation of Fe-precipitates may also reduce availability of light to submerged macrophytes and as such reduce their growth (Immers et al., 2013, 2014) and possibly also survival. Such negative effects may, however, be circumvented if the addition of iron can be supplied at low doses during a prolonged period (months or even years). Technical innovations that permit a gradual supply of Fe may therefore be required to guarantee the safe and sustainable application of FeCl<sub>3</sub> at large scales.

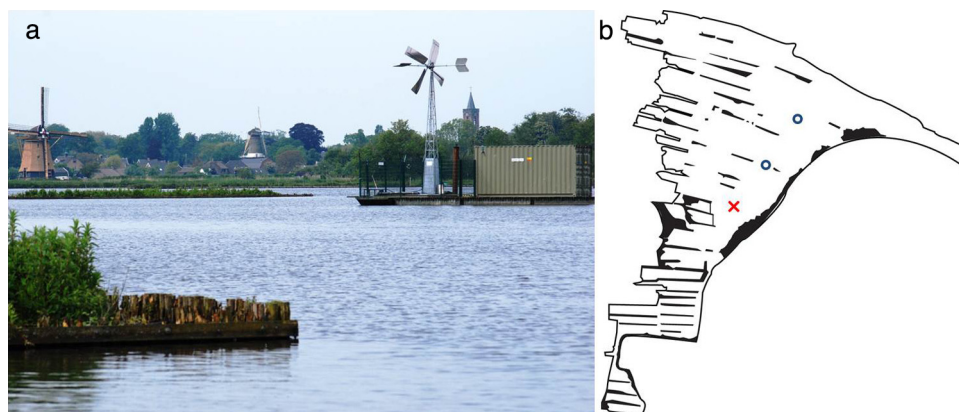
In this study we evaluated the effects of a large scale iron addition project in a eutrophied shallow, peaty lake. A unique aspect of the project is that iron was gradually supplied to the lake in low doses during a period of 1.5 years using an innovative wind-powered method. We simultaneously monitored key physical, chemical and biotic components before, during and after the Fe-addition. These data are complemented with experiments estimating P-mobility in the sediments.

## 2. Material and methods

### 2.1. Background and study area

Lake Terra Nova (52°13'N, 5°02'E) is a shallow peaty lake located in the center of The Netherlands, with a lake surface area of 85 ha, a mean depth of 1.4 m and a bottom covered with a 0.9 m organic sediment layer. As the lake is shallow, the water is well-mixed throughout the seasons and stratification only occurs during ice cover. The lake originated due to peat excavation from the 17th to the 18th century, and over time a highly diverse macrophyte community developed (Ter Heerdt and Hootsmans, 2007). During the second half of the 20th century the trophic status of the lake changed from mesotrophic to eutrophic due to the input of nutrient rich river water and runoff from nearby agricultural fields. Consequently, macrophyte abundance decreased considerably and phytoplankton blooms started to occur frequently during summer periods (Ter Heerdt and Hootsmans, 2007).

Early restoration measures in Lake Terra Nova focused on the removal of fish biomass (biomanipulation) rather than on reducing P-loading. The reason for this is that P-load appeared to be low enough to allow successful biomanipulation according to the



**Fig. 1.** Photograph of the wind-powered installation used for the gradual addition of Fe (a) and map of Lake Terra Nova indicating the central sampling site (cross) and the two locations (M-NE and NE) where lake sediment cores were retrieved for the P-release assays (b; blue circles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

standards at that time ( $<0.1 \text{ mg TPL}^{-1}$ , Meijer et al., 1994). During the winters of 2003 and 2004, lake managers carried out a large-scale biomanipulation in the lake, resulting in the removal of  $191 \text{ kg ha}^{-1}$  or 88% of the fish stock (Ter Heerdt and Hootsmans, 2007). These measures have been followed by a yearly standardized fish removal effort, which has been sustained until present day (see Appendix A for more details). The initial biomanipulation efforts were successful, and resulted in increased water clarity, an expansion of macrophyte cover, increased densities of Cladocera and a reduction of Cyanobacteria. This success was short-lived, however, and from 2005 the system shifted to a turbid state again, despite the continued fish stock management. A system analysis (Schip, 2010) showed that high quantities of P reached the system from both internal and external P sources (see Appendix B for more details on external P loading). The study indicated that the total P-loading in Terra Nova strongly approached the critical P-load threshold for a turbid state and also suggested that about half of the loading was attributable to internal loading. This conclusion suggested that a strong reduction of internal P-loading against a background of continued fish removal would have the potential to yield a more sustainable establishment of a macrophyte dominated clear water state in the lake and formed the basis of the current Fe-addition project.

## 2.2. Iron addition

From the start of May 2010 till the end of August 2011, a mobile wind-driven pump (Fig. 1a) gradually supplied 203 t of  $\text{FeCl}_3$  ( $33 \text{ g Fe m}^{-2}$ ) to the lake over 1.5 years. We used a certified  $\text{FeCl}_3$  solution (KIWA, Apeldoorn, The Netherlands) in order to reduce the possible negative effects of heavy metal contamination on the lake biota (Appendix C). The mobile iron pump was driven by a wind mill, which made the quantity of the added iron dependent on prevailing wind speeds. By doing so, local build-up of high  $\text{FeCl}_3$  concentrations on low-wind days could be avoided, preventing acute exposure of biota to high levels of the added chemicals. At the end of the addition period, molar iron:phosphate ratios in the sediments of most areas reached 10, which is shown to be sufficient to reduce P mobilisation from the sediment (Geurts et al., 2008).

## 2.3. Sampling procedures

We compiled already existing data from the Dutch water board Waternet in order to evaluate changes in water chemistry and plankton species composition in response to Fe-addition. The data were collected at a sampling point at the center of the lake (Fig. 1b) and spanned from 2005 to 2013 (two years after iron addition had stopped).

During each sampling day, water measurements were performed, including oxygen concentration (HQ30D flexi with a LDO101 probe, Hach, Tiel, The Netherlands), pH (HQ30D flexi with a WTW SenTix 41 probe, Hach, Tiel, The Netherlands) and temperature (TLC 1598, Ebro, Ingolstadt, Germany). Depth-integrated samples for both chemical and biological analysis were collected using a polyethylene tube of 1 m length and a volume of 2 L. Zooplankton samples were divided in two size fractions by subsequently filtering the lake water through 50 and 30  $\mu\text{m}$  filters. Subsequently, phytoplankton and zooplankton samples were preserved with Lugol solution and stored in the dark room until being counted with an inverted microscope (DMI 4000B, Leica Microsystems b.v., Münster, Germany) and a stereomicroscope (MZ 16, Leica Microsystems b.v., Münster, Germany), respectively. For each genus, 25 body size measurements were performed using a graded ocular mounted on a stereomicroscope (MZ 16, Leica Microsystems b.v., Münster, Germany). These length

measurements were then combined with data on population densities to estimate population biovolumes. In the period after iron addition (2012–2013), only large zooplankton (copepoda and cladocera) were counted in 2013.

Water samples were filtered through a 1.2  $\mu\text{m}$  Whatman GF/C filter (Whatman, Brentford, UK) and the filters and filtrate were stored at  $-20^\circ\text{C}$  until further analysis. Concentrations of  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and  $\text{NO}_2^-$  were colorimetrically determined with a QuAAtro CFA flow analyser (Seal Analytical, Beun de Ronde, Abcoude, The Netherlands). Chloride was measured spectrophotometrically (Aquakem 250, Thermo Fisher Scientific, Waltham, MA, USA) with extinction at 480 nm. Analyses of Fe and  $\text{SO}_4$  were performed using an inductively coupled plasma emission spectrophotometer (ICP; Liberty 2, Varian, Bergen op Zoom, The Netherlands) according to the Dutch NEN-EN-ISO 17294 to estimate dissolved Fe and S in the water column. To determine the organic N fraction, filtered field samples were analysed with a FLASH 2000 Organic Elemental Analyser (Interscience, Breda, The Netherlands). TP concentrations were determined by incinerating filtered field samples for 30 min at  $500^\circ\text{C}$ , followed by digestion in  $\text{H}_2\text{O}_2$ , before analysis with a QuAAtro CFA flow analyser.

Phytoplankton chlorophyll (total) from stored filters was extracted in 80% ethanol (according to the Dutch NEN 6520 protocol) and was measured spectrophotometrically on a UV/vis Thermo spectrophotometer (UV3, Unicam Instruments, Cambridge, England) at 665 nm with a turbidity correction conducted at 750 nm. The concentration of chlorophyll was determined using the calibration equation from Lorenzen (1967). Suspended matter (SM) dry weight was measured by filtering 1 L of field sample through a prewashed and preweighed Whatman GF/A filter (pore size: 1.5  $\mu\text{m}$ ; Whatman, Brentford, UK). Subsequently, filters were dried for 24 h at  $60^\circ\text{C}$  and afterwards weighed to determine the total dry weight.

From 2005 onwards (except for the years 2010 and 2012) the submerged macrophyte vegetation was monitored each summer (July/August) on at least 43 locations following a stratified random design to represent most of the surface area of the lake. The locations were georeferenced and revisited each sampling year. At each of these sites, dominant species and percentage coverage were assessed on a visual basis using a glass-bottomed box. Additional rake sampling was done at turbid sites to complement visual recordings of coverage and species identification. Change in macrophyte coverage over time was expressed as a percentage of sampling sites with submerged macrophytes.

## 2.4. Assessment of internal phosphorus release

We assessed the P-release potential of lake sediments by monitoring the accumulation of orthophosphates and total phosphorus in the water column of experimental intact cores extracted from the lake sediments. Such experiments were performed during three subsequent years, starting just before the start of iron addition (May 2010). In April 2010, 2011 and 2012, 4 intact sediment cores with lake water on top to maintain anaerobic conditions (radius: 60 mm; height sediment cores: 250 mm) were collected with a sediment corer at two sites in lake Terra Nova (M-NE and NE; Fig. 1b). Immediately after collection, the sediment cores were carefully transported to the laboratory and stored in a dark climate-controlled room at  $15^\circ\text{C}$ .

One week after collection, the original lake water was very carefully replaced by a 0.2 m (volume: 0.57 L) layer of a standard solution containing  $133.2 \text{ mg L}^{-1} \text{ CaCl}_2$ ,  $168 \text{ mg L}^{-1} \text{ NaHCO}_3$  and  $50.8 \text{ mg L}^{-1} \text{ MgCl}_2 \cdot 6\text{H}_2\text{O}$ , which resembles the original lake water without P, Fe and N. Two days later, we started monitoring P-concentrations in the water column by repeatedly sampling it 50 mm above the sediment surface. To keep samples anaerobic, we

used 40 mL vacuum serum bottles connected to Rhizon soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). After each sampling the removed volume was carefully replaced with standard solution. Evaporation losses were regularly compensated with demineralized water.

After sampling, orthophosphate concentrations were measured colorimetrically with an Auto Analyzer 3 system (Bran + Luebbe, Norderstedt, Germany) using ammonium molybdate (Henriksen, 1965). Total phosphorus (TP) concentrations were measured using an ICP Spectrometer (IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA). Prior to this 10 mL subsamples were treated with 100  $\mu$ L  $\text{HNO}_3$  (65%) to prevent metal precipitation.

### 2.5. Data analyses

We distinguished 3 different periods: I, the years after the onset of biomanipulation but preceding the start of iron addition (2005–2009); II, the period during iron addition (2010–2011); and III, the years after iron addition (2012–2013).

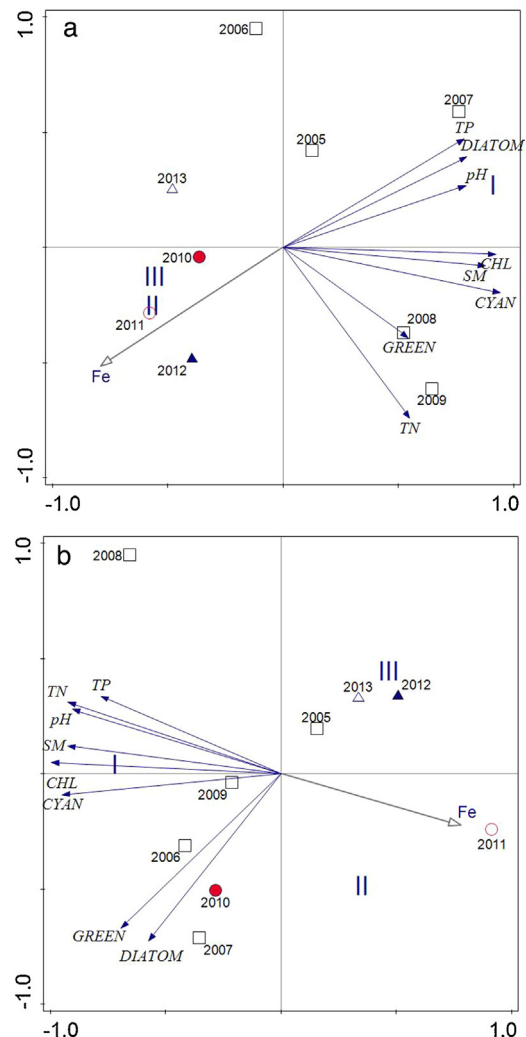
To visualize the responses of chemical and biological variables to the Fe-addition measures, we performed principal components analyses (PCA) using data from 2005 onwards. One analysis was done using spring data (April, May and June), whereas the second analysis was performed to characterise summer conditions (July, August and September). Input data for these analyses were, respectively, spring and summer means of the variables Fe, TP, TN, SM, pH, and total phytoplankton chlorophyll as well as biovolumes of cyanophytes, chlorophytes and diatoms. The PCA was carried out on standardized and log-transformed data (both species and environmental data) using Canoco v. 4.5 (microcomputer Power, Ithaca, USA), with the different periods and Fe as supplementary environmental variables.

To evaluate the effect of Fe addition on the mobility of P in lake sediments, we compared the P-release potential of sediments between years with linear mixed effects models. For this, we used the orthophosphate and TP-concentrations measured in the experiments. The fixed factor component in these models consisted of the year during which the experiment was performed (2010–2012), the period since replacing the original lake water (the first, second and 5th week of the experiments) and their statistical interaction. Random factors in the model were sampling site and individual cores. Significance values for fixed effects were based on F-tests with Kenward–Roger approximation. The analyses were done using the lme4-package (Bates et al., 2014) in R (R Core Team 2014). Posthoc comparisons to evaluate differences between specific years were done using the ‘diffsmeans’ function of the lmerTest package (Kuznetsova et al., 2014). Both variables were log transformed prior to analysis.

## 3. Results

### 3.1. Overall effects of iron addition: a multivariate overview

Water quality variables and major phytoplankton functional groups showed marked responses to iron addition during both spring and summer periods. The first axis of the PCA-analysis on spring data (Fig. 2a), which represents 63% of the total variation, is negatively associated with Fe water column concentrations but positively with suspended matter, phytoplankton chlorophyll, TP, pH and the biovolume of each of the major phytoplankton functional groups. The different periods represent a shift from the right to the left side of the first axis, indicating a shift from high to low levels of phytoplankton biovolume, suspended matter and TP in response to the onset of Fe-addition. The PCA-analysis on summer averages (Fig. 2b), which represents 73% of the variation, reveals patterns very similar to what is observed for the spring. In

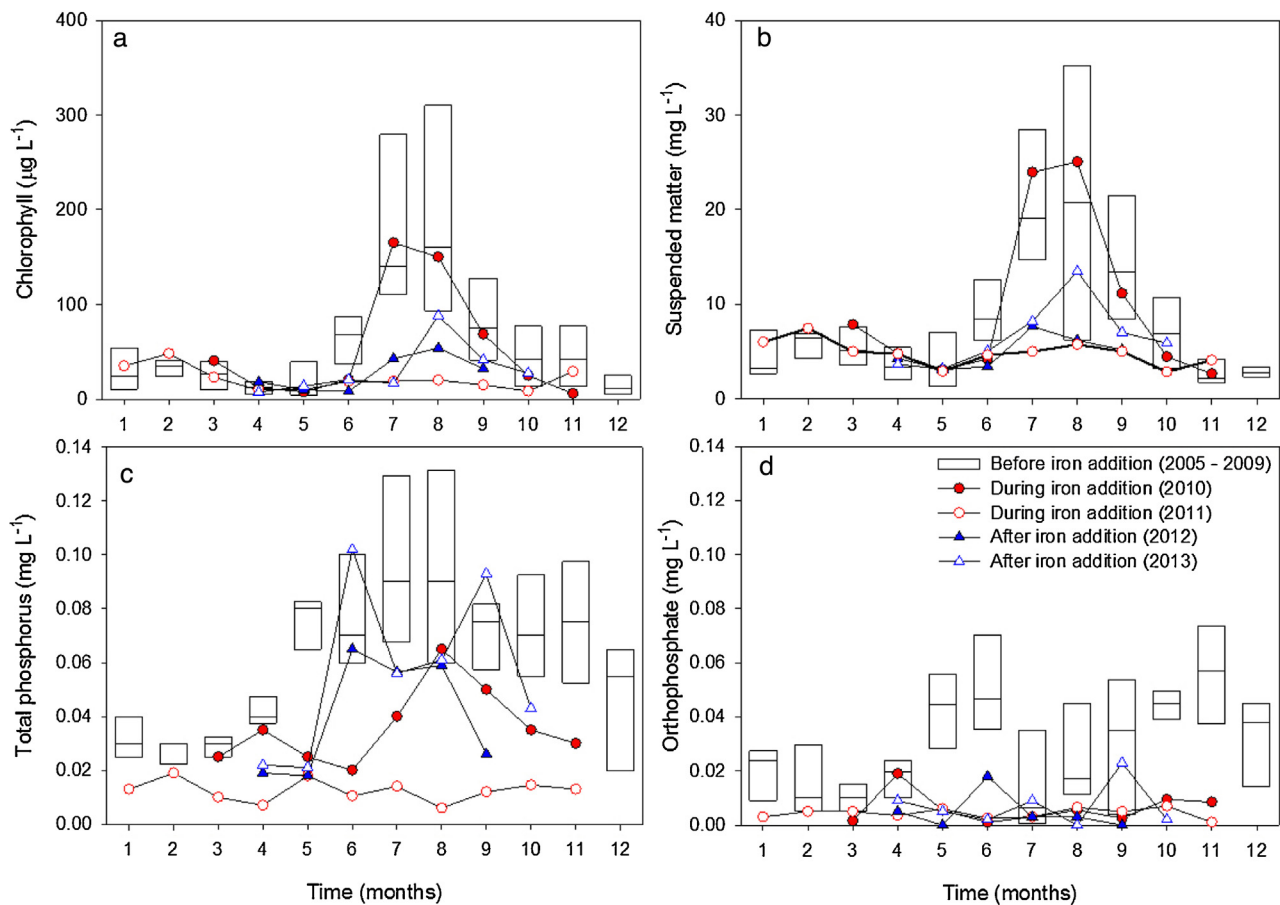


**Fig. 2.** Triplot of PCA ordination on (a) mean spring (April–June) and (b) mean summer (July–September) water chemistry variables and biomass of major phytoplankton groups for the years 2005–2013, with the different restoration periods and Fe water column concentrations projected as supplementary variables. The symbols next to the years correspond to the different restoration periods with squares, circles and triangles representing restoration periods before (I; 2005–2009), during (II; 2010–2011) and after iron addition (III; 2012–2013), respectively.

contrast to the spring data, however, the location of the 2010 centroid indicates a general lack of response of variables to Fe-addition during the first year of Fe-addition.

### 3.2. Effects of iron addition on lake water column chemistry

Iron addition resulted in substantial reductions of phytoplankton chlorophyll, SM, TP and orthophosphate concentrations, especially during the summer months of the second year of the Fe-addition (2011). During that year, summer concentrations of chlorophyll and suspended matter dropped to ca. 20 and 25% of the average of the 5 years preceding Fe-addition, respectively (Fig. 3a and b; Supplementary Fig. 1a). For TP and orthophosphate we observed reductions of 80% and 50% (Fig. 3c and d; Supplementary Fig. 1a), resulting in TP-levels of on average  $0.02 \text{ mg L}^{-1}$  throughout that year. However, as soon as iron addition had stopped, summer TP concentrations increased quickly to pre-restoration concentrations. During 2012 and 2013, we also observed a tendency toward a gradual increase of chlorophyll a and suspended matter (Fig. 3a and b; Supplementary Fig. 1a), whereas orthophosphate



**Fig. 3.** Responses of phytoplankton chlorophyll (a), suspended matter (b), total phosphorus (c) and orthophosphate (d) for each month of the year during each of three time periods. Boxplots represent among-year variability for the period before iron addition (2005–2009), and filled red circles, open red circles, filled blue triangles and open blue triangles represent medians for the years during iron addition (2010 and 2011) and the years after iron addition (2012 and 2013), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

remained low until the end of the study period (Fig. 3d; Supplementary Fig. 1).

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Iron addition resulted in a strong augmentation of water column iron concentrations with peaks of  $0.4 \text{ mg L}^{-1}$  and higher during 2010 and 2011 (Supplementary Fig. 2d). The highest observed concentration of iron during this period reached  $0.74 \text{ mg L}^{-1}$ . Fe-concentrations tended to decline during the years after iron addition had stopped (2012 and 2013), although levels remained higher than in the period preceding the start of iron addition. Throughout the iron addition, surface water pH remained well above 7 (Supplementary Fig. 2a). Moreover, we observed a strong reduction in dissolved organic carbon (DOC) during iron addition, which, in combination with other additional nutrient measurements, is shown in Supplementary Fig. 2.

Supplementary material related to this article found, in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2015.05.034>.

### 3.3. Effects of iron addition on internal phosphorus release

Concentrations of orthophosphate and total phosphorus, measured in the water column above sediment cores, were much lower in the years after (2011 and 2012) than before (2010) iron addition (Fig. 4). Although the concentrations of these nutrients varied with time (significant time effect, see Table 1), differences among years proved to be consistent throughout the duration of

these experiments (significant year effect, see Table 1). In the experiment of 2010, mean values of orthophosphate across replicate cores and sites ranged between  $0.42$  and  $0.71 \text{ mg L}^{-1}$ . In contrast, during 2011 and 2012, orthophosphate ranged between  $0.03$  and  $0.06$  and  $0.01$  and  $0.02 \text{ mg L}^{-1}$ , respectively (Fig. 4a). Similarly, mean TP levels ranged between  $0.23$  and  $0.50 \text{ mg L}^{-1}$  during 2010, whereas ranges were reduced to, respectively  $0.05$  and  $0.11$  and  $0.01$  and  $0.02 \text{ mg L}^{-1}$  in 2011 and 2012 (Fig. 4b). Posthoc comparisons showed very significant differences in orthophosphate concentrations between 2010 and 2011 ( $P=0.004$ ) and between 2010 and 2012 ( $P<0.001$ ), but not between 2011 and 2012. In contrast, posthoc comparisons showed only a marginally significant difference in TP concentrations between 2010 and 2011 ( $P=0.06$ ), but very significant differences for comparisons between 2010 vs. 2012 and 2011 vs. 2012 ( $P$ -values  $< 0.001$ ).

### 3.4. Responses of phytoplankton and zooplankton communities to Fe-addition

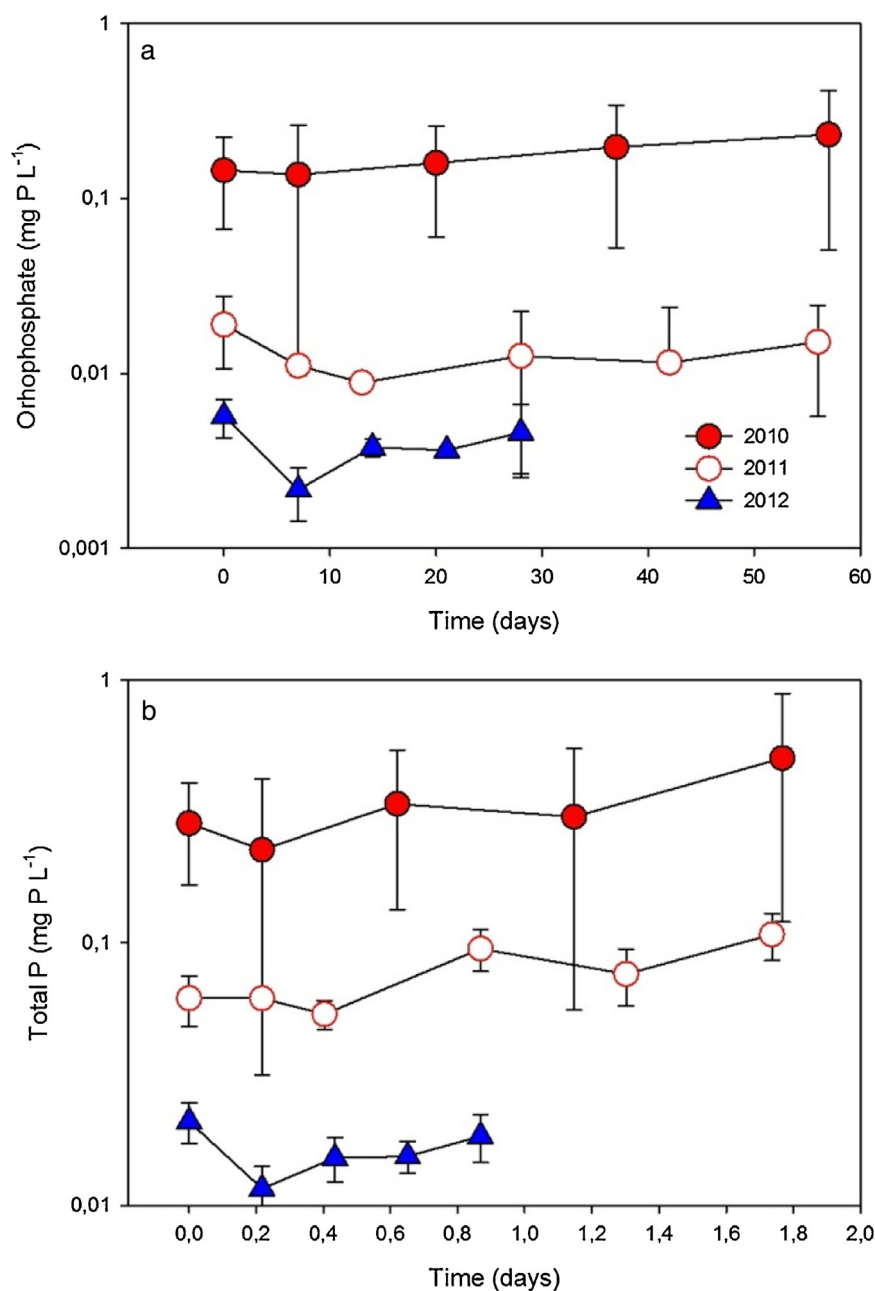
The relative abundance of cyanobacteria remained relatively high during the summer of the first year of iron addition (2010) and contributed ca. 50% to the total phytoplankton biomass. Coinciding with a strong drop in total phytoplankton biomass, absolute and relative biomass of Cyanobacteria was strongly reduced during the second year of iron addition (2011; Fig. 5b) and remained low until the end of the study period (2013; Fig. 5a and b; Supplementary Fig. 3a).

**Table 1**

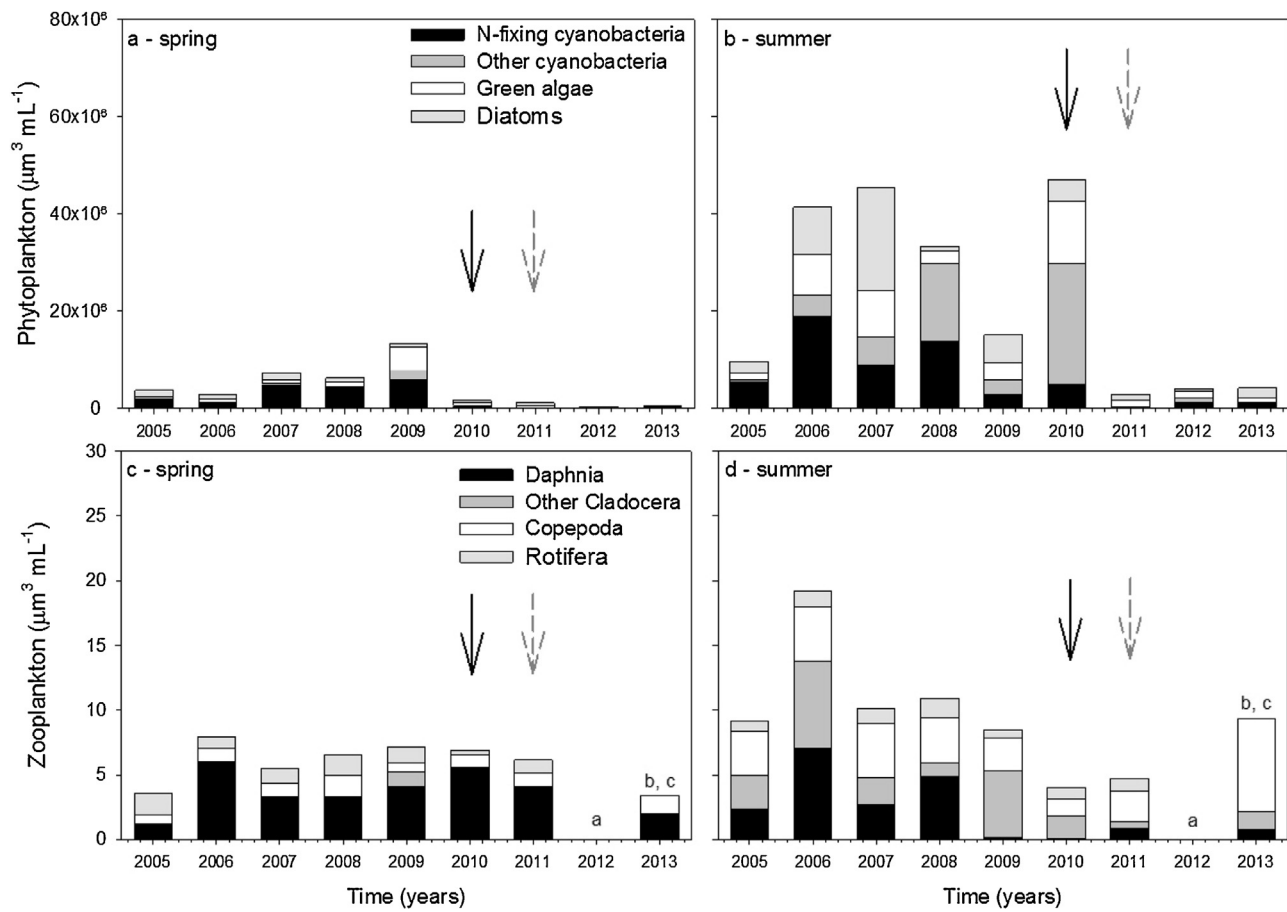
Results of analysis of the effects of iron addition in Terra Nova on sediment orthophosphate and total phosphorus release. Data were analysed with linear mixed effects models with the year of the experiment (2010–2012) and the time period since replacing the original water (the first, second and 5th week of the experiments) as fixed factors.

Factor	SumSq	MeanSq	df	DenDF	F-value	P
<b>Orthophosphate</b>						
Year	3.34	1.67	2	20	12.66	<b>0.000</b>
Time of sampling	2.18	1.09	2	42	8.27	<b>0.001</b>
Year × Time of sampling	0.72	0.18	4	42	1.37	0.261
<b>Total Phosphorus</b>						
Year	1.95	0.97	2	20	22.10	<b>0.000</b>
Time of sampling	0.99	0.50	2	42	11.29	<b>0.000</b>
Year × Time of sampling	0.45	0.11	4	42	2.55	0.053

Bold values indicate  $P \leq 0.05$ .



**Fig. 4.** Concentrations of orthophosphate (a) and total phosphorus (b) measured in the water column above sediment cores in function of the time after the start of the experiments. Filled red circles, open red circles and filled blue triangles represent averages for the years 2010 and 2011 during iron addition and the year 2012 after iron addition, respectively.



**Fig. 5.** Spring (April–June) and summer (July–September) mean phytoplankton (a and b) and zooplankton (c and d) biovolume in  $\mu\text{m}^3 \text{mL}^{-1}$  from 1996 to 2013. Black, dark gray, white and light gray bars represent in (a and b) nitrogen-fixing Cyanobacteria, other Cyanobacteria, green algae and diatoms, respectively and in (c and d) *Daphnia*, other Cladocera, Copepoda and Rotifera, respectively. Solid and dashed gray arrows indicate the start and stop of iron addition, respectively. <sup>a</sup>No measurements performed, <sup>b</sup>no rotifera counted, <sup>c</sup>values are means of 2 months (May–June or July–August).

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Compared with the years before the Fe-treatment, we observed markedly lower cladoceran biomass in the summer months of the years during and after Fe-addition, although spring densities remained relatively constant (Fig. 5c and d; Supplementary Fig. 4a). Whereas *Daphnia* dominated the cladoceran biomass in spring, this group was only found in low numbers during the summer months. Copepods showed no clear response to Fe-addition, but increased to relatively high levels in 2013 (Fig. 5; Supplementary Fig. 4b).

Supplementary material related to this article found, in the online version, at <http://dx.doi.org/10.1016/j.ecoleng.2015.05.034>.

### 3.5. Macrophyte coverage

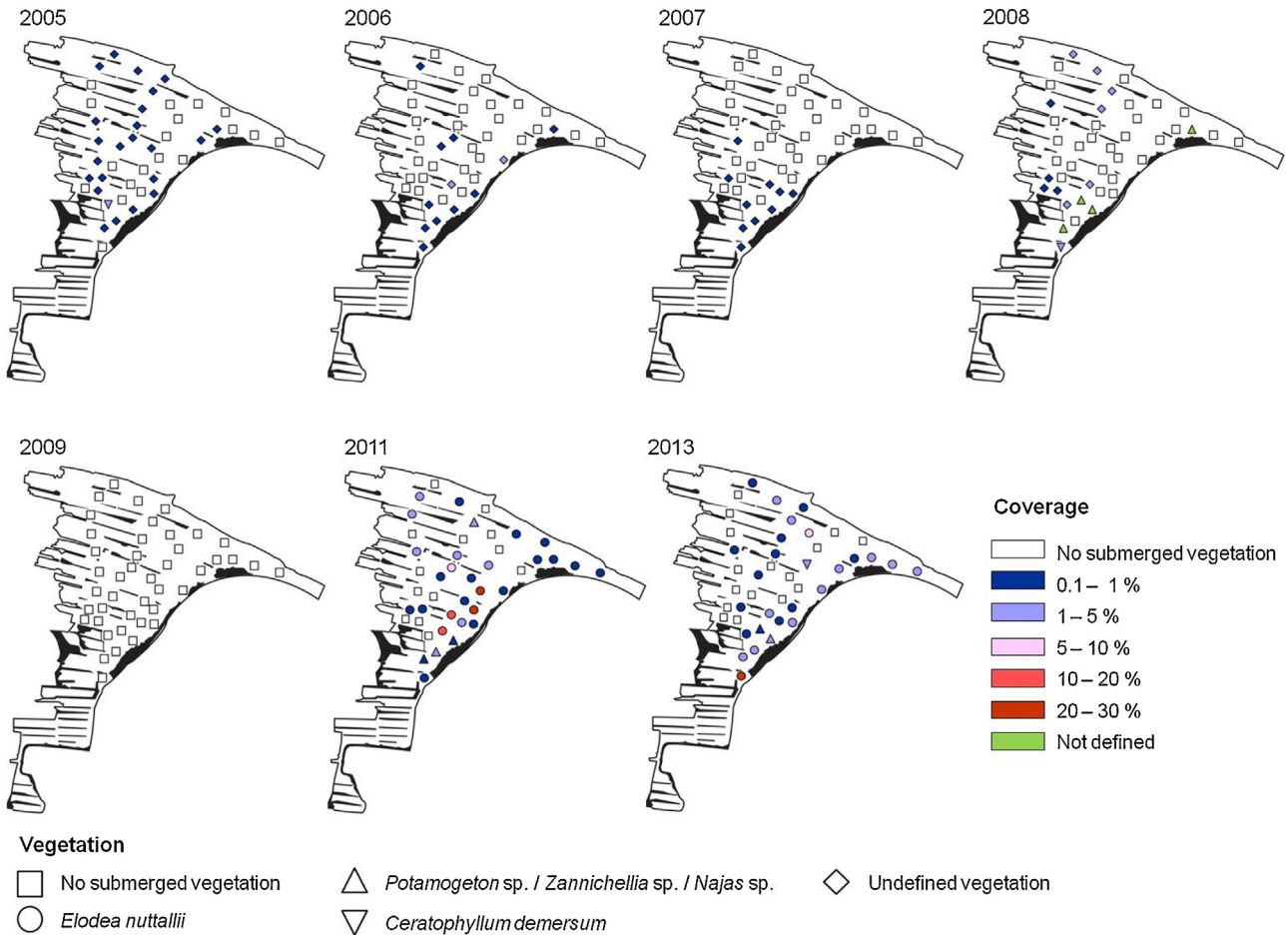
During 2005–2007, the number of sampled locations with submerged macrophytes declined from 56% in 2005 over 28% in 2006 to 23% in 2007 (Fig. 6). Whereas the summer of 2008 showed a slight increase in macrophyte diversity and percentage coverage compared to the previous years, this vegetation had entirely disappeared in all parts of the lake in 2009 (Fig. 6). During the second year of iron addition (2011), however, submerged macrophytes re-appeared in the lake and were found in 70% of the sampling points, with *Elodea nuttallii* being the dominant species (Fig. 6). Moreover, percentage coverage in some areas increased substantially, with several sites having a local macrophyte coverage > 20% (Fig. 6). During the next sampling campaign two

years later (2013), we found an *Elodea* dominated vegetation similar to the one in 2011.

## 4. Discussion

Our results demonstrate strong short-term effects of gradual iron addition on important physical and chemical water quality related variables as well as on key biotic components of the lake food web. Whereas responses of the lake ecosystem were relatively minor during the first year of Fe-addition, we observed a substantial decline in spring and summer concentrations for total and dissolved phosphorus, suspended matter, total phytoplankton biomass and the relative biomass of cyanobacteria during the second year of the Fe-addition. These changes coincided with the reappearance and expansion of a submerged macrophyte vegetation and were also corroborated by the results of our internal P-release experiments which indicated a strongly enhanced P-binding capacity of the sediment after application of Fe to the lake. Our results are in line with other Fe-addition experiments, where additions of 20–500 g Fe  $\text{m}^{-2}$  to either the water column or sediment of lakes or reservoirs resulted in decreased levels of TP, SM and chlorophyll-a (Boers et al., 1994; Daldorph and Price, 1994; Jaeger, 1994; Van Donk et al., 1994; Kleeberg et al., 2013).

Following the termination of the Fe-addition treatment in Lake Terra Nova, the improvement of several important quality related criteria, such as presence of a submerged macrophyte vegetation, low Cyanobacteria biomass and low orthophosphate levels proved robust until the end of the study period. Yet, shortly after the



**Fig. 6.** Estimation of submerged macrophyte coverage on 43 locations in Lake Terra Nova during the summer of the years 2005–2009 before iron addition and the summer of the years 2011 and 2013 during and after iron addition. Colored symbols represent areas with a macrophyte coverage  $\geq 0.1\%$ , with dark blue, light blue, pink, light red and dark red colored symbols representing areas with a macrophyte coverage ranging 0.1–1, 1–5, 5–10, 10–20 and 20–30%, respectively. White squares represent areas with no submerged macrophytes and light green symbols represent areas without a defined coverage. Symbols indicate the dominant group of macrophytes at that location, with circles, downward-facing triangles, upward-facing triangles and diamonds representing *Elodea nuttallii*, *Ceratophyllum demersum*, either alone or a combination of *Potamogeton* sp., *Zannichellia palustris* and *Najas marina*, and undefined vegetation, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table A1**

Overview of the yearly removed fish and crayfish biomass in Lake Terra Nova with estimates of total remaining fish biomass for the years 2003, 2004, 2008 and 2013 ('Total biomass remaining'). Bream, perch, roach, ruffe and red swamp crayfish were caught with fish traps and systematically removed from the lake on a yearly basis. Total fish biomass estimates were made via mark-recapture using a combination of fish traps, seine- and electrofishing.

	Removed fish (kg) per size fraction		Removed crayfish (kg)	Total removed ( $\text{kg ha}^{-1}$ )	Total biomass remaining ( $\text{kg ha}^{-1}$ ) <sup>a</sup>
	0–15 cm	$\geq 15$ cm			
Autumn 2003					215.8
Winter 2003–2004	6564	9643		190.7	47.8
Winter 2004–2005	4585	1072		66.6	
Winter 2005–2006	3563	926		52.8	
Winter 2006–2007	1198	290		18.5	
Autumn 2007	738	24		9.0	
Autumn 2008	733	626	26	16.0	50.4
Autumn 2009	1078	0 <sup>b</sup>	119	12.7	
Autumn 2010	798	0 <sup>b</sup>	188	9.4	
Autumn 2011	625	0 <sup>b</sup>	108	7.4	
Autumn 2012	1002	0 <sup>b</sup>	453	11.8	
Autumn 2013	911	0 <sup>b</sup>	273	10.7	58.7
Total removed (kg)	21795	12581	1167		

<sup>a</sup> Excluding pike (28.0, 11.7 and 8.8  $\text{kg ha}^{-1}$  for 2003, 2008 and 2013, respectively).

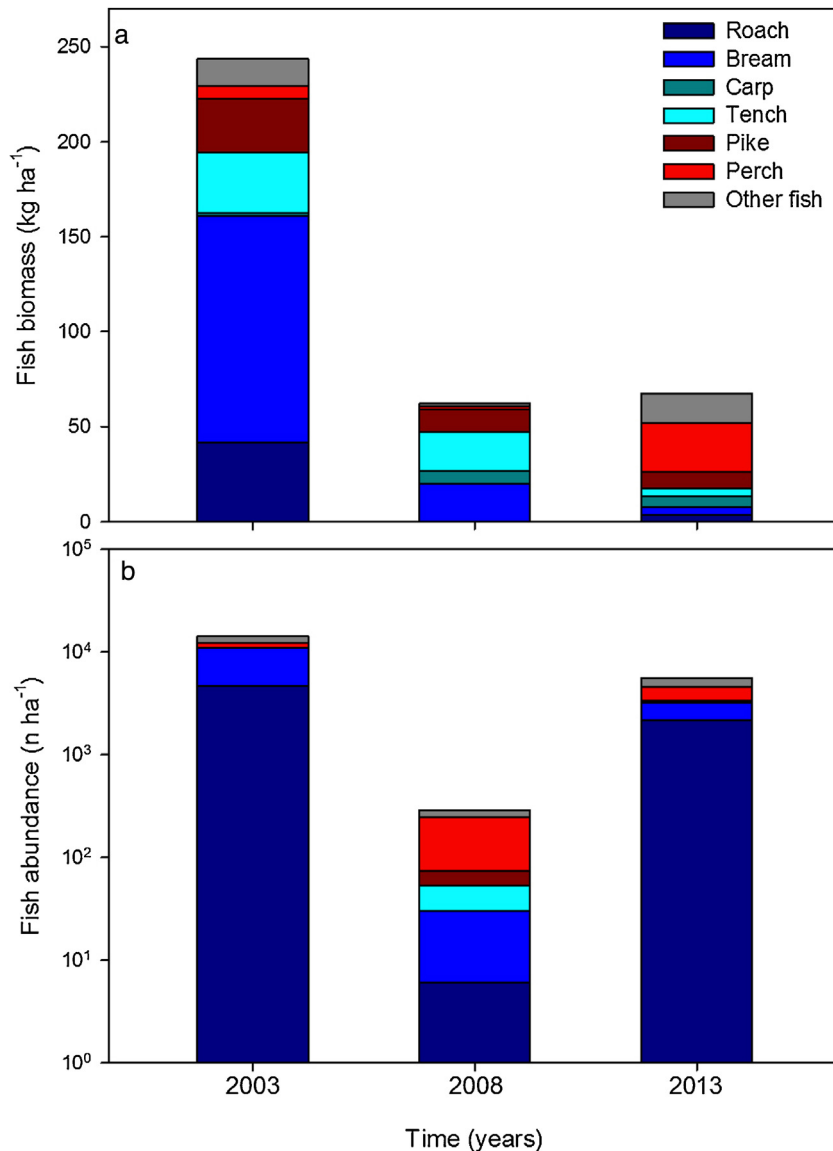
<sup>b</sup> From 2009 onwards fish traps were placed on the winter migration route of young fish only and fish  $\geq 15$  cm were not removed.



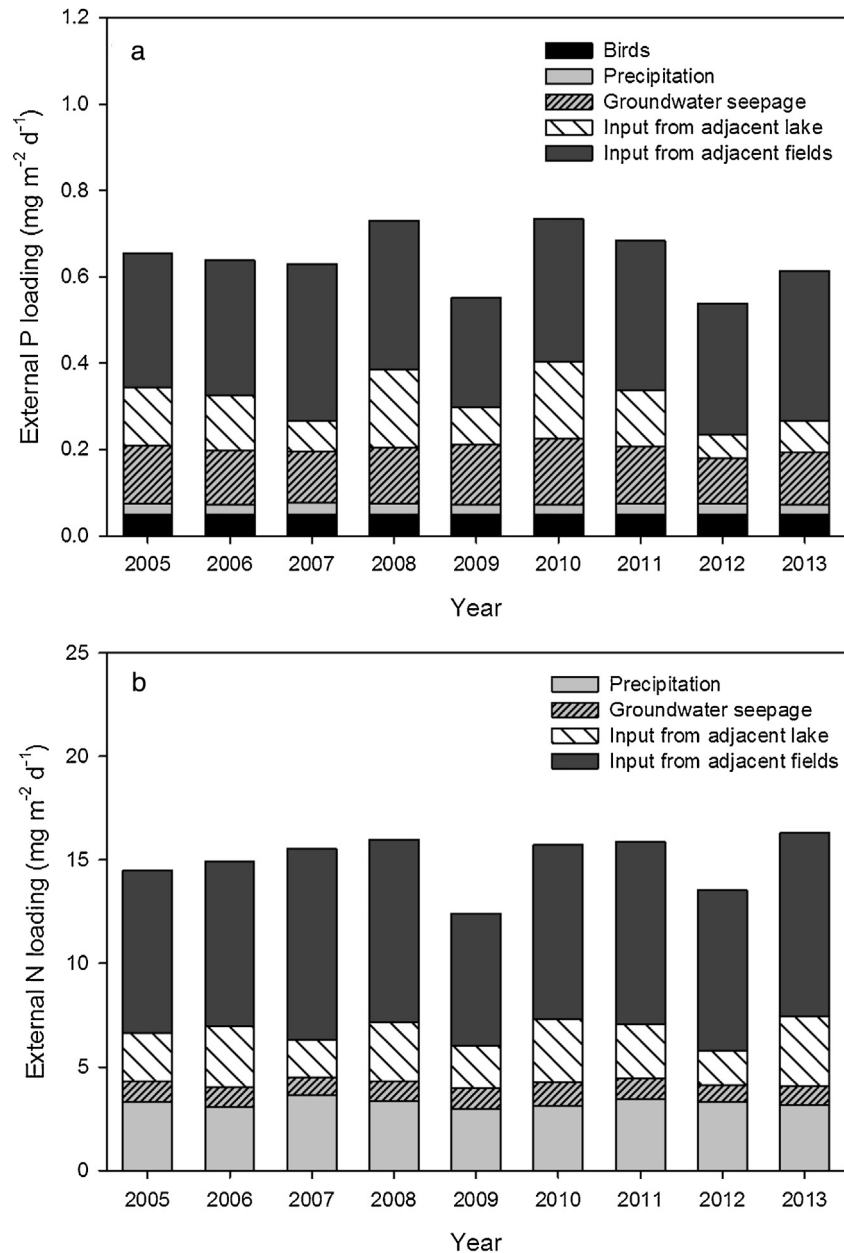
termination of the Fe-addition, water column P-levels increased rapidly to pre-restoration levels again. A similar but weaker trend was also observed for total phytoplankton biomass and suspended matter. The trajectory of the latter variables may indicate that the strong initial water quality improvements may not be sustainable at the longer term and that the lake is gradually shifting back to its original turbid state.

The pronounced post-treatment increase in TP, however, shows a remarkable contrast with our experimental P-release data. These experiments demonstrated that a low P-binding capacity of the lake sediments during the first year of the Fe-additions was followed by strongly increasing P-binding capacity of these sediments during the two subsequent years. Whereas water column TP-concentrations in the lake already reached pre-treatment levels in 2012, the binding capacity of sediments for P in that year proved even higher than in 2011. This discrepancy is intriguing and suggests that the long term success of Fe-addition measures may be jeopardized even when P is still tightly bound to the sediments. According to simulations by a hydrological model of Schep (2010; [Appendix B](#)) there are no indications for increased

levels of external P-loading during the period after the Fe-addition measures. However, it cannot be excluded that a continued influx of external P may have contributed to increasing water column P, especially if P entered the lake in the form of organic matter, or when it combined with a gradual depletion of reactive Fe in the water column. With respect to the latter, reactive P can be reduced not only through binding with P but potentially also through the formation of complexes with several other naturally occurring chemical compounds, such as DOC and sulphates ([Smolders et al., 2006](#); [Qualls et al., 2009](#)). Reductions in DOC during iron addition indeed indicate that part of the iron reservoir may have been immobilized by such process. Alternatively, there may also have been other mechanisms at work that realized a shunt of chemically bound P in the sediments to the food web in the water column. For example, macrophytes may have extracted P from the sediments for their growth. This P may have then been released to the water after die off of these macrophytes ([Barko and Smart, 1980](#)). Also other potential mechanisms, such as benthic recruitment of algae ([Barbiero and Welch, 1992](#)), may have played a role.



**Fig. A1.** Overview of the fish community composition evaluated during the estimates of total remaining fish biomass (a) and fish abundance (b) in 2003 (before the start of biomanipulation), 2008 (before iron addition) and 2013 (after iron addition). Please note that while fish biomass remains relatively stable between the years 2008 and 2013 (during and after iron addition), fish abundance increases, indicating an increase in small-sized fish.



**Fig. B1.** Overview of yearly external nutrient input from different sources as estimated via the hydrological model for P (a) and N (b) from 2005 until 2013.

Whereas the addition of iron can improve water quality, high iron concentrations can also have important negative side-effects on lake biota (Linton et al., 2007; Immers, 2014). High concentrations of iron in the water column can impact lake biota directly through the formation of toxic reactive oxygen species (Linton et al., 2007) as well as indirectly through strong reductions in pH or the formation of iron precipitates (Immers, 2014). Our Fe-addition project was unique in that large amounts of  $\text{FeCl}_3$  were added very gradually to the surface water of the lake in an effort to maximally prevent such negative effects. Because dosing of Fe was a function of wind speed, this gradual addition guaranteed good mixing, a stable pH and a rather homogenous distribution of the precipitates over the sediment surface of the lake (J. Geurts, unpublished results). Visual inspections during the yearly fish removals provided no indications for iron precipitates on gills or any other symptoms for iron-related stress (pers. obs. G. ter Heerdt). Direct toxic effects were also avoided: average water column iron concentrations were as low as  $0.23 \text{ mg Fe L}^{-1}$  throughout the

addition process (2010–2011). The highest concentration of  $0.75 \text{ mg Fe L}^{-1}$  was measured in the summer of 2011, which is well below the acute toxicity threshold for many organism groups (Immers, 2014).

During iron addition we observed a summer decline in the densities of cladocerans, which persisted until the end of the study period. It is, however, unlikely that this decline resulted from iron toxicity. Studies have shown that cladocerans are tolerant to surface water iron concentrations up to  $5.9 \text{ mg Fe L}^{-1}$  (Biesinger and Christensen, 1972). More likely, the cladoceran decline resulted from both a strong reduction in algal food and an increased predation pressure by small-sized fish during the period 2008–2013 (Appendix A).

The macrophyte community showed a positive response to iron addition, as it returned in more than half of the sampling points, compared to their absence in 2009. The dominant macrophyte species that emerged during iron addition (*Elodea nuttallii*) is known to be a typical eutrophic species, but various mesotrophic

**Table C1**  
Impurities in the FeCl<sub>3</sub> solution used for dosing in Lake Terra Nova.

	Concentration in FeCl <sub>3</sub> solution (mg kg <sup>-1</sup> ) FeCl <sub>3</sub>	Total amount added (kg)	Concentration in water <sup>a</sup> (μg L <sup>-1</sup> )	Concentration in sediment <sup>b</sup> (mg kg <sup>-1</sup> )
Arsenic	1.5	0.6	1.5	0.022
Cadmium	0.1	0.04	0.1	0.0015
Chrome	23	9.4	23	0.34
Copper	17	6.9	17	0.25
Mercury	0.02	0.008	0.02	0.0003
Nickel	33	13.5	33	0.48
Manganese	364	149	364	5.3
Lead	1	0.4	1	0.015
Antimony	0.3	0.1	0.3	0.0044
Selenium	0.2	0.08	0.2	0.0029
Zinc	2	0.8	2	0.029

<sup>a</sup> Assuming a uniform distribution of the substance over the water column.

<sup>b</sup> Assuming a uniform distribution over the top 10 cm of the sediment.

species also slowly reappeared at several locations, including *Potamogeton obtusifolius* and *Najas marina*. Experiments in Lake Terra Nova have shown that the sediments contain seeds and propagules of eutrophic as well as mesotrophic species, including several charophyte species (Van der Wal et al., 2013; Immers et al., 2014). Charophytes were not encountered during the summer surveys in 2011 and 2013. Given that charophytes are known to be relatively tolerant to effects of iron toxicity (Immers et al., 2013), it is unlikely that the failure of their recovery is caused by the addition of Fe. Recent experiments with sediments from the lake have also shown that the germination of charophyte propagules is not hindered by the addition of iron with concentrations up to 40 g Fe m<sup>-2</sup> (Immers et al., 2014). The absence of a more elaborate and diverse mesotrophic vegetation is therefore most probably related to other factors, such as the presence of invasive crayfish, which both consume macrophytes as well as disturb the sediment by bioturbation. The amount of invasive crayfish removed during the biomanipulation efforts steadily increased from 2008 onwards, which most likely reflects an increase in the size of the crayfish population in the lake. Invasive crayfish, which are increasingly becoming a nuisance in European lakes, are well-known for their ability to alter aquatic ecosystems by decreasing water transparency and destroying macrophyte biomass, particularly *Procambarus clarkii*, the dominant species in Lake Terra Nova (Bakker et al., 2013; Van der Wal et al., 2013). An experiment, for example, showed that transplanted *Chara virgata* grew well in Lake Terra Nova, but survival and growth was reduced in the presence of crayfish (Van der Wal et al., 2013).

## 5. Conclusions

Over a time span of four years and against a background of continued fish removal, the addition of iron contributed substantially to improvements of the ecological state of the peaty Lake Terra Nova. This was manifested by strong reductions in phosphorus availability, phytoplankton biomass, suspended matter, relative Cyanobacterial biomass and by the recovery of a submerged macrophyte vegetation.

Experiments with lake sediments also showed that the Fe-addition measures coincided with strong improvements of sediment P-binding capacity. We, nevertheless, observed a quick recovery of water column TP concentrations along with a trend of increased phytoplankton biomass and suspended matter. A potential explanation for these increased water column TP concentrations is given by the sustained loading with external P, possibly combined with a gradual depletion of the reservoir of reactive Fe in the water column due to binding of Fe to P and other chemical compounds, such as DOC and SO<sub>4</sub>. In addition there may

be ecological processes at work that translocate P from sediments to the water column that may jeopardize the long term success of the Fe-addition measures. Substances interacting with Fe, such as DOC and SO<sub>4</sub>, need therefore to be carefully taken into consideration during the planning of restoration measures through Fe-addition, especially in lakes rich in organic matter.

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## Appendix A.

Species composition of fish biomass during the study period (see Table A1 and Fig. A1).

## Appendix B.

Estimates of external nutrient loading in Terra Nova as estimated via the hydrological model of Schep (2010) (see Fig. B1).

External loading of N and P were estimated using the hydrological model of Schep (2010). This model uses the combination of lake characteristics, meteorological data, nutrient concentrations, estimations of bird densities and water balance to provide reliable predictions on nutrient loading.

## Appendix C.

### Heavy metals in iron(III)chloride solution

In order to reduce heavy metal contamination as much as possible, we used a certified iron(III)chloride solution (KIWA, Apeldoorn, The Netherlands). Table C1 provides specifications of heavy metal concentrations in the solution and their projected concentrations in the water column and sediment after addition to the lake, under the assumption that all particles remain dissolved in the watercolumn (<sup>a</sup>) or precipitate to the sediment (<sup>b</sup>).

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