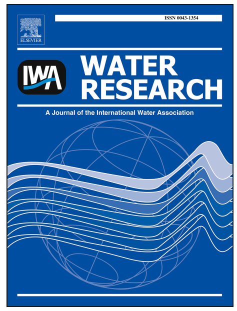
Accepted Manuscript



A meta-analysis of water quality and aquatic macrophyte responses in 18 lakes treated with lanthanum modified bentonite (PHOSLOCK®)

Bryan M. Spears, Eleanor B. Mackay, Said Yasseri, Iain D.M. Gunn, Kate E. Waters, Christopher Andrews, Stephanie Cole, Mitzi de Ville, Andrea Kelly, Sebastian Meis, Alanna L. Moore, Gertrud K. Nürnberg, Frank van Oosterhout, Jo-Anne Pitt, Genevieve Madgwick, Helen J. Woods, Miquel Lürling

PII: S0043-1354(15)30171-8

DOI: 10.1016/j.watres.2015.08.020

Reference: WR 11467

To appear in: *Water Research*

Received Date: 4 April 2015

Revised Date: 1 July 2015

Accepted Date: 10 August 2015

Please cite this article as: Spears, B.M., Mackay, E.B., Yasseri, S., Gunn, I.D.M., Waters, K.E., Andrews, C., Cole, S., de Ville, M., Kelly, A., Meis, S., Moore, A.L., Nürnberg, G.K., van Oosterhout, F., Pitt, J.-A., Madgwick, G., Woods, H.J., Lürling, M., A meta-analysis of water quality and aquatic macrophyte responses in 18 lakes treated with lanthanum modified bentonite (PHOSLOCK®), *Water Research* (2015), doi: 10.1016/j.watres.2015.08.020.

A META-ANALYSIS OF WATER QUALITY AND AQUATIC MACROPHYTE RESPONSES IN 18 LAKES TREATED WITH LANTHANUM MODIFIED BENTONITE (PHOSLOCK®)

Bryan M. Spears[1*], Eleanor B. Mackay[2], Said Yasseri[3], Iain D. M. Gunn[1], Kate E. Waters[1], Christopher Andrews[1], Stephanie Cole[4], Mitzi de Ville[2], Andrea Kelly[5], Sebastian Meis[1,6], Alanna L. Moore[1], Gertrud K. Nürnberg[7], Frank van Oosterhout[8], Jo-Anne Pitt[9], Genevieve Madgwick[10], Helen J. Woods[1], and Miquel Lürling[8,11]

*Corresponding author, email: spear@ceh.ac.uk, tel: +44 (0)131 445 4545, fax: +44 (0)131 445 445 943

1 Centre for Ecology & Hydrology, Penicuik, Midlothian,EH26 0QB, UK

2 Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK

3 Institut Dr. Nowak, Mayenbrook 1, D-28870 Ottersberg, Germany

4 Environment Agency, Red Kite House, Howbery Park, Crowmarsh Gifford, Wallingford, Oxon OX10 8BD, UK

5 Broads Authority, Yare House, 62-64 Thorpe Road, Norwich, NR1 1RY, UK

6 Lanaplan GbR, Lobbericher Str. 5, D-41334, Nettetal, Germany

7 Freshwater Research, 3421 Hwy 117, Baysville, Ontario, P0B 1A0, Canada

8 Aquatic Ecology and Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands

9 Environment Agency, Station Road, Haddiscoe, Great Yarmouth, NR31 9JA, UK

10 Natural England, Eastbrook, Shaftesbury Road, Cambridge CB2 2DF, UK

11 Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB, Wageningen, The Netherlands

Keywords: Geo-engineering, recovery, ecology, water quality, aquatic macrophyte, lake, management, remediation, lake restoration, eutrophication control

ABSTRACT

Lanthanum (La) modified bentonite is being increasingly used as a geo-engineering tool for the control of phosphorus (P) release from lake bed sediments to overlying waters. However, little is known about its effectiveness in controlling P across a wide range of lake conditions or of its potential to promote rapid ecological recovery. We combined data from 18 treated lakes to examine the lake population responses in the 24 months following La-bentonite application (range of La-bentonite loads: 1.4 to 6.7 tonnes ha^{-1}) in concentrations of surface water total phosphorus (TP; data available from 15 lakes), soluble reactive phosphorus (SRP; 14 lakes), and chlorophyll *a* (15 lakes), and in Secchi disk depths (15 lakes), aquatic macrophyte species numbers (6 lakes) and aquatic macrophyte maximum colonisation depths (4 lakes) across the treated lakes. Data availability varied across the lakes and variables, and in general monitoring was more frequent closer to the application dates. Median annual TP concentrations decreased significantly across the lakes, following the La-bentonite applications (from 0.08 mg L^{-1} in the 24 months pre-application to 0.03 mg L^{-1} in the 24 months post-application), particularly in autumn (0.08 mg L^{-1} to 0.03 mg L^{-1}) and winter (0.08 mg L^{-1} to 0.02 mg L^{-1}). Significant decreases in SRP concentrations over annual (0.019 mg L^{-1} to 0.005 mg L^{-1}), summer (0.018 mg L^{-1} to 0.004 mg L^{-1}), autumn (0.019 mg L^{-1} to 0.005 mg L^{-1}) and winter (0.033 mg L^{-1} to 0.005 mg L^{-1}) periods were also reported. P concentrations following La-bentonite application varied across the lakes and were correlated positively with dissolved organic carbon concentrations. Relatively weak, but significant responses were reported for summer chlorophyll *a* concentrations and Secchi disk depths following La-bentonite applications, the 75th percentile values decreasing from 119 µg L^{-1} to 74 µg L^{-1} and increasing from 398 cm to 506 cm, respectively. Aquatic macrophyte species numbers and maximum colonisation depths increased following La-bentonite application from a median of 5.5 species to 7.0 species and a median of 1.8 m to 2.5 m, respectively. The

aquatic macrophyte responses varied significantly between lakes. La-bentonite application resulted in a general improvement in water quality leading to an improvement in the aquatic macrophyte community within 24 months. However, because, the responses were highly site-specific, we stress the need for comprehensive pre- and post-application assessments of processes driving ecological structure and function in candidate lakes to inform future use of this and similar products.

INTRODUCTION

Nutrient (i.e. mainly phosphorus (P) and nitrogen (N)) pollution of freshwater lakes has resulted in widespread degradation of ecological structure and function at a global scale (Smith, 2003). To address this issue, environmental policies have been implemented to reduce nutrient loads to lakes. Such policies often require management actions to improve water quality to support ecological recovery within a given timeframe (e.g. by 2027 in the case of the EU Water Framework Directive; European Commission, 2000). In many catchments large-scale reductions in P loading to fresh waters have been achieved (Withers and Haygarth, 2007). However, after P loading from the catchment is reduced, lake recovery can take several decades (Jeppesen et al., 2005; Sharpley et al., 2013). This is because P, accumulated in lake bed sediments when catchment inputs were high, continues to be released during the recovery process (“internal loading”), maintaining poor water quality conditions (Søndergaard et al., 2012; Spears et al., 2012). The effective control of internal loading may accelerate ecological recovery once external inputs have been reduced (Mehner et al., 2008).

There are few methods available to control internal loading (Hickey and Gibbs, et al., 2009). Sediment dredging has been demonstrated as an internal loading control measure (e.g. Van Wichelen et al., 2007) but may have limited application when habitat destruction, waste disposal and cost are taken into account. In addition, like other restoration measures, sediment dredging has not always been successful (Søndergaard, et al., 2007). P-sorbing materials (e.g. modified clays, industrial by-products, flocculants and physical barriers; Hickey and Gibbs, 2009; Zamparas et al., 2012; Spears et al., 2013a) have also been used to strip P from the water column and, after settling, reduce P release from the sediments (Hickey and Gibbs, 2009; Meis et al., 2012). Lanthanum (La) modified bentonite, is being increasingly used in lakes for P control (Douglas et al., 2000; Robb et al., 2003; Haghseresht et al., 2009). However, there has been limited evaluation of its effectiveness in controlling P across diverse lake conditions, or of its potential to promote rapid ecological recovery.

When considering the effectiveness of any lake management approach it is important to consider responses across multiple lakes (Jeppesen et al., 2005; Spears et al., 2013b). Long-term catchment nutrient load reduction studies indicate that a range of responses characterise the recovery period in lakes. In temperate lakes, following catchment management, a rapid decline in winter P concentration occurs followed by a gradual decline in summer P concentrations as the intensity of internal P loading diminishes with time (Phillips et al., 2005; Søndergaard et al., 2012). Whereas winter P concentrations are generally driven more by catchment inputs, sediment P release is more prominent in the warmer summer months when redox conditions of bed sediments can become reducing (i.e. liberating soluble reactive phosphorus (SRP) from Fe-P sediment complexes) and high temperatures increase sediment-water SRP concentration gradients and diffusive fluxes from the sediment to the water column (Spears et al., 2007). The period over which these responses occur is lake-specific and regulated by various factors including hydraulic residence time, sediment P concentrations and depth (Sas, 1989). Where the phytoplankton community is primarily P limited, reductions in annual average total phosphorus (TP) concentrations should elicit a reduction in phytoplankton biomass (commonly measured as chlorophyll *a* concentration), an increase in water clarity, and an increase in the extent and diversity of aquatic macrophytes (Jeppesen et al., 2000). Where La-bentonite has been successful in controlling internal loading these responses should occur relatively quickly, at least within the recovery time scales known to occur following catchment nutrient load reduction alone (i.e. >5 years; Jeppesen et al., 2005; Sharpley et al., 2013).

We assessed these responses following La-bentonite application (two years post-application), relative to pre-application conditions (i.e. two years pre-application), in 18 lakes and addressed the following specific questions: (1) were the responses in water quality (i.e. concentrations of TP; SRP; and chlorophyll *a* and Secchi disk depth) statistically and ecologically significant and did these responses vary seasonally?; (2) were the responses in water column TP and SRP concentrations regulated by physico-chemical conditions of the receiving lake water?; (3) did aquatic macrophyte diversity and extent increase in treated lakes?; and (4) what are the implications of these results for the use of La-bentonite as an eutrophication management tool in other lakes?

METHODS

Data collation, assessment and processing

The following analyses are based on collated information from 18 lake case studies where La-bentonite has been applied. Information on were compiled for each of the study lakes. Surface water TP, SRP, and chlorophyll *a* concentrations and Secchi disk depth data, in the years preceding and following an application of La-bentonite, were compiled to allow an assessment of general responses across the population of lakes. All available aquatic macrophyte community data, including species lists and maximum colonisation depth estimates, were compiled. The product application procedures for 14 of the study lakes are described by Spears et al., (2013b), with the exception of Mere Mere, Hatchmere, Cromes Broad and Swan Lake to which La-bentonite was added in the absence of a flocculant and as a slurry. In four of the lakes it was reported that repeat applications had been conducted but only data following the first application and prior to the second were included in this study. The number of lakes for which data were available for TP, SRP, chlorophyll *a* concentrations and Secchi disk depth, in the months preceding and following Phoslock application, are reported. Supporting data for location, maximum fetch, mean depth, maximum depth, surface area, alkalinity, dissolved organic carbon (DOC) concentration, La-bentonite dosage and pH were requested for the pre- and post-application periods with which the general chemical and physical conditions of the treated lakes are described.

Determination of TP, SRP, Chlorophyll *a* concentrations and Secchi disk depths

TP was determined following persulfate (or peroxide for German lakes; ISO6878) digestion of unfiltered samples in an autoclave followed by colorimetric analysis to determine concentration. SRP analysis for all lakes was achieved using spectrophotometric methods, as outlined generally by Wetzel and Likens (1991). Chlorophyll *a* concentrations were determined for all lakes using acetone extraction followed by spectrophotometric pigment analysis. Various sized filters were used for SRP and chlorophyll *a* analysis. Water from Clatto Reservoir and Loch Flemington were filtered through Whatman GF/C filters (1.2 µm), and for all other lakes water samples were filtered through 0.45µm pore size filters. Secchi disk depths were measured at the water depth where the disk (25 cm diameter quadrat) was no longer seen from the surface for all lakes with the exception of Lake Rauwbraken, for which the mean of the depths at which the disk was last seen lowering and first seen during rising was recorded.

Assessing water quality responses

All available data for TP, SRP, chlorophyll *a* concentrations and Secchi disk depths were combined across the treated lakes. The sample date was modified to become day relative to the La-bentonite application date (i.e. the last day of the application period) for all data. Responses following La-bentonite application were examined by comparing all data from all lakes for which data were available both 24 months preceding the first application and 24 months following the first application. Pre- and post-application data were available for Secchi disk depth, TP, and chlorophyll *a* concentrations in 15 lakes and SRP concentrations in 14 lakes. Surface water values for TP, SRP and chlorophyll *a* were used in our analysis to reflect the most comparable sampling points across the lakes, each lake having different bottom water depths and therefore environmental conditions. Where more than one surface sample was taken on a particular date in a lake, the values were averaged. This ensured the greatest likelihood that lakes would not be excluded from the analysis on grounds of lack of data and we acknowledge the implications on variance (Helsel and Hirsch, 1992). The number of surface water observations ranged from between 571 to 760 per variable. This analysis of the difference in pre- and post-application values for each variable was carried out using linear mixed effects models, with pre- and post-treatment included as a fixed effect factor and a random intercept term for each lake, respectively. This allows for the calculation of an average effect for all lakes whilst still taking account of between-lake variability and the average value.

Seasonal analyses were performed using lakes where data were available in a particular season both before and after treatment. Seasons were classified as winter (December, January and February), spring (March, April and May), summer (June, July and August) and autumn (September, October and November).

Prior to fitting the linear mixed effects models, the data were standardised through the calculation of Z scores to allow lake responses to be compared on a common scale. As the focus of the study is on the overall response across lakes, the Z score transformation is used to centre each lake's data around its mean, thereby reducing the influence of any individual lake which may have high average raw values of particular variable from unduly affecting the statistical analysis. A strong positive skew in the TP, SRP and chlorophyll *a* concentration data necessitated log transformation of these variables prior to Z score calculations. Z scores were calculated for each observation as the difference between the observed value and the mean value for that variable across the 48 month monitoring period for each lake, divided by the standard deviation of all observations of that variable, giving units of standard deviations (Fowler et al., 1998). To address patterns in the residuals resulting from temporal autocorrelation seen in some models, a lag-1 autocorrelation structure was also added to the model and models with and without this structure compared by using Akaike Information

Criterion (AIC) values. All analyses were carried out in R (Ihaka and Gentleman 1996; R Core team, 2011) using the nlme package (Pinheiro and Bates, 2000).

Assessing the drivers of water quality responses

Principal components analysis (PCA) using correlation was used to produce the two synthetic axes that best captured the variation in log transformed annual mean data from before and after the application including TN, SRP, TP, chlorophyll *a* and DOC concentrations, pH, mean depth and Secchi disk depth (included in both 'before' and 'after' analysis) and La-bentonite dose and the change in TP concentration following application (included only in the 'after' analysis). Mean values of the 24 month pre- and post-application monitoring periods were calculated and log transformed prior to analysis. pH co-varied strongly with conductivity and alkalinity and so pH only was used in this analysis. Similarly, maximum depth co-varied strongly with mean depth and so only mean depth was included in the analysis. Data were available for 9 lakes in the 'before' analysis and for 10 lakes in the 'after' analysis. Person's correlation analyses were conducted to confirm the apparent correlations indicated by PCA between log transformed DOC, TP and chlorophyll *a* concentrations and between La-bentonite dose and TP change (i.e. mean TP before – mean TP after / mean TP before) following the La-bentonite applications. PCA and correlation analyses were carried out using Minitab statistical software, version 16 (Minitab Ltd., Coventry, UK).

Assessing responses in aquatic macrophyte communities

Aquatic macrophyte community compositional data were available for five lakes, pre- and post- application. These were Loch Flemington, Crome's Broad, Hatchmere, MereMere and Lake Rauwbraken (Table 1), although data were separately available for the two basins of

Crome's Broad and were included in the analysis as separate lakes. Aquatic macrophyte maximum colonisation depth estimates were also available for four of these treated lakes.

Annual aquatic macrophyte surveys of Loch Flemington, Hatchmere and Mere Mere were carried out using the standardised approach adopted for assessing the condition of standing waters of conservation importance in the UK (JNCC, 2005). This approach is based on sampling representative sectors of a lake and is designed to be practical and efficient, with the aim of producing quantitative data that is both suitable for characterising the aquatic macrophyte community while being statistically robust enough to detect real changes over time (Gunn *et al.*, 2010). There are three key elements to this aquatic macrophyte survey method: perimeter strandline searches, shore-wader depth transects and boat-based depth transects surveys (JNCC, 2005). Annual pre- and post- application aquatic macrophyte surveys of Cromes Broad (north and south basins) were carried out using a rake-trawl sampling method along previously defined transects, employing the methodology outlined by Kennison et al. (1998). The Lake Rauwbraken aquatic macrophyte surveys were based on the "standard" Dutch approach of using a rake. This involved 6-10 sampling transects located around the lake. Each sampling transect was carried out perpendicular to the shore, from above the water line (to take into account fluctuating water levels) to several metres depth and continued by scuba diving to ensure more accurate determination of aquatic macrophyte coverage, growing depths and species richness.

Annual aquatic macrophyte survey data were collated to produce post-application estimates of the number of species recorded and the maximum colonisation depths for post-application years 1 and 2 in all five lakes. Pre-application replicate year values for each variable were selected from within pre-application years 1 and 2 (i.e. for Lake Rauwbraken, Crome's Broad

North and South basins), where available. Where data were not available for both pre-application years 1 and 2, the missing year (always year 2) was supplemented using data from previous years (i.e. pre-application years 4, 5 and 6 were combined with pre-application year 1 for Mere Mere, Hatchmere and Loch Flemington, respectively). These data were log transformed and two-way Analysis of Variance (ANOVA) was used to test for the effects of lake, La-bentonite treatment, and interactions between the two, on the aquatic macrophyte community structure and extent. Two-way ANOVAs were carried out using Minitab statistical software, version 16 (Minitab Ltd., Coventry, UK).

RESULTS

Description of case study lakes and available data

The 18 lakes varied in physical conditions (Table 1). Alkalinity and DOC in the 24 months preceding application ranged, respectively, from 0.9 meq L^{-1} to 2.7 meq L^{-1} (median alkalinity of 1.82 meq L^{-1}) and 4.15 mg L^{-1} to 20.9 mg L^{-1} (median DOC of 9.95 mg L^{-1}). TN ranged from 0.72 mg L^{-1} to 3.58 mg L^{-1} (median 1.5 mg L^{-1}) and pH from 7.27 to 8.65 (median of pH 7.72). The lakes were generally small (median surface area of 6 ha) and shallow (median of mean depth 2.6 m).

Data availability for TP, SRP chlorophyll *a* concentrations and Secchi disk depth varied with time relative to the La-bentonite application (Figure 1). In general, as time increased from application date, the number of lakes for which data were available, decreased.

Responses in TP, SRP chlorophyll *a* and Secchi disk depth following La-bentonite application

Prior to La-bentonite application, TP and SRP concentrations were high in summer and autumn relative to other seasons apparently confirming the general hypothesis that internal P loading was high relative to catchment loading in these lakes (Figure 2). However, Secchi disk depth values across the lakes did not vary seasonally prior to La-bentonite application. Following the applications, significant decreases in annual (median of 0.08 mg L^{-1} in the 24 month pre-application to median of 0.03 mg L^{-1} in the 24 months post-application), spring (0.05 mg L^{-1} to 0.03 mg L^{-1}), summer (0.09 mg L^{-1} to 0.04 mg L^{-1}), autumn (0.08 mg L^{-1} to 0.03 mg L^{-1}) and winter (0.07 mg L^{-1} to 0.02 mg L^{-1}) TP concentrations were confirmed using linear mixed model analysis on transformed data, as described earlier. The largest relative response as indicated by the difference between pre- and post-application values (standard deviations) of the model (Table 2) occurred in winter for TP. A significant decrease was reported for median SRP concentrations at annual (0.019 mg L^{-1} to 0.005 mg L^{-1}), summer (largest relative response; 0.018 mg L^{-1} to 0.004 mg L^{-1}), autumn (0.019 mg L^{-1} to 0.005 mg L^{-1}) and winter (0.033 mg L^{-1} to 0.005 mg L^{-1}) periods and in chlorophyll *a* concentrations in annual (10.1 µg L^{-1} to 10.0 µg L^{-1}) and spring (largest relative response; 14.0 µg L^{-1} to 6.2 µg L^{-1}) periods, although the reduction in annual chlorophyll *a* concentrations was only apparent in the Z score transformed data.

Although not tested statistically, responses in the 75th percentile of the range of untransformed Secchi disk depth and chlorophyll *a* concentrations were larger in spring and summer. For Secchi disk depth and chlorophyll *a* concentrations, the 75th percentile values increased from 398 cm to 506 cm in summer and decreased from 119 µg L^{-1} to 74 µg L^{-1} in summer, respectively.

Assessing the drivers of TP and SRP concentrations following La-bentonite application

Following the application, the PCA results indicate a general positive correlation in product dose and surface water TP, DOC, TN and chlorophyll *a* concentrations with PC 1 (Figure 3). Secchi disk depth, pH, mean depth, and the change in TP concentration following application all varied negatively with PC1. Secchi disk depth and dose varied positively and chlorophyll *a*, TP, SRP, and DOC varied negatively with PC2. Prior to the application, only chlorophyll *a* concentration appeared to correlate (negatively) with TP and SRP concentrations, although this correlation was not significant when tested using Person's correlation analysis of log transformed data. A positive correlation, as indicated by PCA, between DOC, TP and chlorophyll *a* concentration was only observed following the application. These positive correlations were tested using Person's correlation analysis of log transformed data (TP-DOC: correlation coefficient (c.c.) 0.63; p value 0.03; TP-chlorophyll *a*: c.c. 0.68; p <0.01), although no positive correlation was reported between DOC and chlorophyll *a* and/or SRP concentrations. The apparent negative correlations indicated by PCA between Secchi disk depth, chlorophyll *a*, TP, and DOC concentrations were also confirmed (Secchi-chlorophyll *a*: c.c. - 0.67; p <0.01; Secchi-TP: c.c. -0.74; p <0.01; Secchi-DOC: c.c -0.69; p 0.01).

Responses in aquatic macrophyte species numbers and maximum colonisation depths following La-bentonite application

Aquatic macrophyte species numbers generally increased following the La-bentonite application from a median of 5.5 species in the 24 months pre-application to 7.0 species in the 24 months post-application (Figure 4). On average, an increase in species number of 1.6 species (individual lake responses varied between 0 and 4 species) was reported across the six data sets. Aquatic macrophyte maximum colonisation depths also generally increased across the four lakes, for which data were available, from a median of 1.8 m pre-application to a median of 2.5 m post-application (Figure 4). Two-way ANOVA of log transformed data indicated that the responses in aquatic macrophyte species numbers were lake specific and only weakly significant following La-bentonite application with no significant interaction between lake and La-bentonite effects (Table 3). However, in the case of aquatic macrophyte colonisation depths, significant responses to the La-bentonite application were detected and these were also lake specific as indicated by a significant effect of lake and La-bentonite x lake interactions term (Table 3).

DISCUSSION

Responses in water quality following La-bentonite applications

If phytoplankton biomass in our lakes had been strongly P limited and water clarity constrained by phytoplankton we would have expected to see a strong correlation between TP and chlorophyll *a* concentration, and a negative correlation between these two variables with Secchi disk depth, prior to application. This was not the case. Only after the La-bentonite applications did these correlations become significant, indicating a strengthening of the importance of P limitation across these lakes. This is supported by the general reduction in SRP concentrations to very low levels following the application, and across all lakes. The reduction in SRP concentrations was dramatic, with median concentrations in all seasons, except spring, being maintained at or below 0.005 mg L^{-1} following application, conditions which should sustain P limitation of the phytoplankton community in lakes (Reynolds, 2006).

Although significant reductions in SRP and TP concentrations were achieved (i.e. reduced to within ecologically relevant concentration ranges; <50 µg TP L^{-1}; Jeppesen et al., 2000) the expected ecological responses were less pronounced. A reduction in summer and autumn TP and SRP concentrations is expected where internal P loading has been controlled (Nürnberg, 1998; Sondergaard et al. 2012) and this was consistent with our results. However, reductions in winter and spring TP concentrations were also observed across the treated lakes. The mechanisms behind these TP reductions are not obvious. It is unlikely that a significant reduction in catchment P loading will have occurred across all lakes within 24 months of La-bentonite application. It is possible that changes in internal loading caused by the La-bentonite application resulted in a reduction in winter and spring TP concentrations, perhaps through the control of P release during anoxic conditions in winter (Penn et al., 1999) or through the removal of catchment derived SRP to the bed within La-bentonite-P complexes. However, it is also likely that TP concentrations were reduced in general as a result of reduced internal loading. The processes responsible for the winter TP reduction may be lake specific and should be analysed at this scale in detail, elsewhere.

Jeppesen et al. (2000) defined five ecological classes in 71 Danish lakes according to surface water annual mean TP concentrations and indicated that significant decreases in chlorophyll *a* concentrations, and increases in water clarity, aquatic macrophyte community species numbers and maximum growing depths would occur at TP concentrations <50 µg L^{-1}. The reduction in TP concentrations reported here would resemble a shift in the study lakes from class 2 or 3 (i.e. 0.05 -0.10 mg TP L^{-1}) before treatment to class 1 (i.e. < 0.05 mg L^{-1}) after treatment. However, in the case of chlorophyll *a* concentrations, where reductions in median values were ecologically insignificant, pre-application concentrations were already low (corresponding to Jeppesen et al. (2000) lake class 1) in comparison to TP concentrations (i.e. low Chl:TP ratios) in the study lakes, suggesting factors other than P were limiting phytoplankton biomass across the majority of these lakes prior to La-bentonite application. These factors may include nitrogen limitation (May et al., 2010), light limitation from shading caused by suspended inorganic matter in very shallow lakes as a result of bioturbation (Breukelaar et al., 1994) or wind induced bed sediment disturbance (Hilton et al., 1986; Spears and Jones, 2010), and an unbalanced trophic structure leading to an increased grazing pressure of phytoplankton by zooplankton (Horppila et al., 2003).

Our study lakes do not represent the full range of eutrophication conditions that occur across lakes. As such, our results cannot be used to draw conclusions on the capacity for La-bentonite to be used to control TP concentrations in hyper-eutrophic lakes (i.e. reductions from milligrams per litre to micrograms per litre TP). However, for our study lakes and when the summer responses are considered using 75th percentile values (i.e. occurrence of extreme poor conditions for macrophytes) for chlorophyll *a* concentration and Secchi disk depth, it is apparent that water quality has improved and this should support increases in macrophyte species number and colonisation depth.

Explaining the variation in TP and SRP responses following La-bentonite applications

Significant decreases in TP, SRP and chlorophyll *a* concentrations after the La-bentonite application supports the conclusion that the water quality responses reported across the lakes were the direct result of the La-bentonite applications. In addition, changes to catchment nutrient load were not apparent across these treated lakes for the duration of the monitoring periods. Further, these lakes were unlikely to have been affected by common weather events given their application dates varied temporally (i.e. across a 7 year period) and geographically (i.e. across 4 countries and 2 continents).

Although significant reductions in SRP and TP concentrations were observed across the treated lakes, site-specific variation in responses, following La-bentonite application, were apparent. No significant positive correlation was found between La-bentonite dose and the change in TP concentration suggesting that simply adding a higher dose may not result in more effective P control in these lakes. The observed correlation between DOC, chlorophyll *a* and TP concentrations after the applications indicates the importance of DOC as a potential driver of La-bentonite operational performance. However, DOC and TP concentrations are known to correlate across lakes naturally (Nürnberg and Shaw, 1998). That DOC was not correlated with these variables before the application supports the hypothesis that physicochemical interactions between DOC, La-bentonite and SRP can drive post-application responses across lakes. This is in agreement with Lürling et al. (2014) who used laboratory experiments and chemical speciation modelling to explore the relationships between DOC, La and SRP and concluded that the concentration of filterable La in solution increased above DOC concentrations of about 10 mg L^{-1}. The rate of SRP uptake of La-bentonite was lower at DOC concentrations of 10 mg L^{-1} compared to controls with 0 mg L^{-1} DOC. Finally, the end point SRP concentrations in solution following a 42 day incubation in the presence and absence of 10 mg L^{-1} DOC were about 250 µg L^{-1} and 100 µg L^{-1}, respectively, indicating reduced SRP removal by La-bentonite in the presence of DOC. Lürling et al. (2014) hypothesised that extraction of La from the La-bentonite matrix may be one possible mechanism confounding SRP uptake and that humic compounds may act as ligand donors in the complexation of bentonite, forming particles of several micrometers in diameter (Bilanovic et al., 2007). However, the quality (i.e., high molecular weight, high colour, allochthonous versus low molecular weight, low colour, autochthonous DOC compounds; Spears and Lesack, 2006) and quantity of DOC varies between lakes and so the strength and forms of physicochemical interactions between La-bentonite and DOC are also likely to vary, and should be considered further. Similar interactions have been reported for aluminium (De Vicente et al., 2008). It is likely that interactions between La-DOC-P (and other constituents) are important in determining the magnitude of the P decline, and these interactions should be studied experimentally and using chemical modelling approaches.

Interactions between in-lake P, C and Fe species have also been reported where redox conditions in surface sediments can be controlled by reduced water clarity, elevated DOC and phytoplankton biomass. Under these conditions, a feedback loop may establish where DOC and P are continually and rapidly cycled between bed sediments and the water column, resulting in negative effects on water quality (Brothers et al., 2014). This example of DOC as a confounding factor serves to demonstrate that consideration of in-lake management measures designed for P control, alone, is insufficient. Copetti et al. (this issue) reviewed a wide range of factors known to confound the operational performance of La-bentonite including bioturbation of surface sediments by macro-invertebrates and fish, pH, salinity and DOC concentrations. These factors should be comprehensively considered, and preferably their effects quantified, prior to an application of La-bentonite and other similar geo-engineering materials to lakes.

These insights into the importance of DOC as a factor limiting the operational performance of La-bentonite can be placed into the context of future water quality changes likely to occur in a changing climate. An increase in DOC concentrations and changes in DOC quality may occur as a result of changes in atmospheric deposition in lake catchments (Monteith et al., 2007), interactions with redox conditions and iron complexes in inflowing rivers (Kritzberg and Ekström, 2012), and local weather anomalies resulting in wash out of terrestrial DOC into receiving lakes (Brothers et al., 2014).

Responses in aquatic macrophyte community composition and maximum colonisation depth following La-bentonite applications

The increase in aquatic macrophyte species numbers and maximum colonisation depths reported here indicate the onset of ecological recovery within 24 months of La-bentonite application. However, it should be noted that macrophyte data were only available from five treated lakes and so these results do not reflect responses across all lakes. The responses (especially in aquatic macrophyte species numbers) were weak relative to reductions in TP and SRP concentrations (Jeppesen et al., 2000) and were lake specific. Secchi disk depth prior to the La-bentonite applications (24 month median of 257 cm) was sufficient to support relatively diverse communities (median of five species), at least in comparison to lakes where macrophyte communities have collapsed. Jeppesen et al. (2000) indicated that an increase in Secchi disk depth from about 1.5 m to about 3.0 m would support a significant increase in species numbers (from about eight to about ten species) and maximum colonisation depth (i.e. from about 2 m to about 6 m water depth). In our study lakes, an increase in species numbers from five to seven species was observed following the applications and colonisation depths increased from 1.8 m to 2.5 m. It is likely that the significant reduction in TP concentrations across the lakes has resulted in improvements in water clarity, especially in summer, and a moderate improvement in the aquatic macrophyte species numbers and colonisation depths. However, the observed responses varied across the lakes with some lakes exhibiting very limited, if any, response.

Responses in the aquatic macrophyte communities of lakes to reductions in TP concentrations can be highly variable, as was seen in our analysis. This may be due to a range of additional factors including habitat type, and disturbance from waves and waterfowl (Jupp and Spence, 1977), isolated seed banks in deeper sediments (Boedeltje et al., 2003), lack of a distribution network to support ingress of new species (Van Geest et al., 2003), the presence of chemical components of the water column shaping species re-colonisation (e.g. humic substances; Steinberg et al., 2008), and insufficient water depth leading to constrained macrophyte growth under conditions of high physical disturbance (Seabloom et al., 2001; maximum depth range of the study lakes: 1.3 m to 16.0 m; Table 1). It is also possible that the aquatic macrophyte community responses have not yet been completed. Mitsch et al. (2005) conducted a comprehensive analysis of aquatic macrophyte community ingress into created wetlands (<1 m water depth) and reported stable community development only after about five years. Where aquatic macrophyte ingress was managed through planting, the rate of colonisation was faster and the end point community more diverse (Mitsch et al., 2005).

We acknowledge the limitations associated with the use of the available data to draw general conclusions on aquatic macrophyte responses across these treated lakes. To substantiate these results we recommend site specific assessments across treated lakes in the context of long-term community variability (e.g. Gunn et al., 2014). However, the responses reported here represent the first examples of aquatic macrophyte community responses following La-bentonite applications in the literature and our analysis raises clear issues that need to be considered when attempting to use La-bentonite to achieve rapid (i.e. within two years of application) ecological recovery of aquatic macrophytes.

Implications of the results for use of La-bentonite as an eutrophication management tool in other lakes

It is clearly important to focus efforts on quantifying responses in water quality, phytoplankton biomass and aquatic macrophyte community structure following changes in P concentrations at the field scale (Schindler, 1998). Field scale observations have been used to identify reasons of successes and failures in water quality improvement programmes following the application of lake restoration measures such as catchment P load reduction (Sondergaard et al., 2007; 2012), biomanipulation (Jeppesen et al., 2007; Gulati et al., 2008), hypolimnetic withdrawal (Nürnberg, 2007) and sediment dredging (Peterson, 1982). In addition, considerations on the use of geo-engineering materials, including La-bentonite, in lakes is also provided by Hickey and Gibbs, (2009) and for aluminium based materials by

Huser et al. (2011) and Huser et al. (this issue). Here, we present the first meta-analysis of water quality responses following La-bentonite application in many lakes.

Collectively, one conclusion can be drawn from this body of evidence: responses to common management measures can be highly variable between lakes and over time, and can be driven by a myriad of interacting and potentially confounding factors. In addition, most reports on lake restoration successes and failures cite a lack of sufficient understanding of the target system and its catchment basin as the main issue leading to perceived failure of a restoration project (Søndergaard et al., 2012). To support this understanding it is important to combine high quality monitoring data, both prior to and following a management intervention, with expertise in lake functioning. In the present study and others (Spears et al., 2013b), monitoring frequency and duration within lakes to which La-bentonite has been applied has been highly variable, with intensive monitoring occurring only after application and for a relatively short period of time (e.g. Figure 1). It is clearly important that estimates of recovery time are considered and, as before (Spears et al., 2013a), we recommend a standard monitoring protocol as have others, previously (Hickey and Gibbs, 2009; Gibbs et al., 2011). Further, site specific recovery analyses are recommended to determine time scales and end points for a range of chemical and ecological components.

Our results indicate variable chemical and ecological responses following La-bentonite application, with some lakes exhibiting very little response in the 24 months following application. Given the economic burden of lake restoration it is important that the cost of proposed management measures be assessed relative to others and in relation to confidence in their effectiveness (Mackay et al., 2014). Given the cost estimates for P control with La-bentonite are around €0.8 million per km^2 lake surface area (Spears et al., 2013c) it is important that information on potential impacts be made available. This evidence should include assessments of both successes and failures, and especially the causes of failure (Søndergaard et al., 2007; Lürling and Van Oosterhout, 2012).

CONCLUSIONS

- General reductions in surface water TP (data available from n=15 lakes) and SRP (n=14 lakes) concentrations were reported following La-bentonite application to the study lakes, within a 24 month monitoring period.
- Chlorophyll *a* concentrations (n=15 lakes) decreased and Secchi disk depth (n=15 lakes) increased and these responses were most pronounced in summer.
- The median values of TP, SRP and chlorophyll *a* concentrations across the lakes in the 24 months following application were correlated positively with DOC concentration, suggesting DOC as a factor potentially confounding the operational performance of La-bentonite.
- Increases in aquatic macrophyte community species numbers (average increase of 1.6 species; n = 6 lakes) and maximum colonisation depths (mean increase of 0.7 m; n = 4 lakes) were reported.
- Available data across 18 lakes varied considerably in relation to monitoring period. Macrophyte data, in particular, were sparse. It is recommended that a standard monitoring protocol be developed to support future cross-lake comparative analyses of responses in water quality and biological communities.
- Our results indicate variable water quality responses across multiple treated lakes, most likely due to multiple and interacting confounding processes operating within the treated lakes and their catchments. We stress the need for comprehensive site-specific understanding to support the application of similar management measures more generally.

ACKNOWLEDGEMENTS

Sebastian Meis was funded jointly by NERC, Phoslock® Europe GmbH, and by the Deutscher Akademischer Austausch Dienst (DAAD Scholarship agreement number D/08/42393). We are grateful to the Loch Flemington Catchment Management Group, Scottish Natural Heritage, the Scottish Environment Protection Agency, Dundee City Council, the Regional Water Authorities Brabantse Delta and De Dommel (The Netherlands), the Province Noord-Brabant (The Netherlands), the NL Agency of the Ministry of Economic Affairs, Agriculture and Innovation (The Netherlands), and Bay of Plenty Regional Council, New Zealand for continued support of our research and access to field sites. Dennis Waasdorp, Fred Bransen, Irene Paredes, and Wendy Beekman (Wageningen University, The Netherlands), and Stephen Thackeray, Mike O'Malley, Amy Anderson and Joe Turner (Centre for Ecology and Hydrology, UK) are thanked for their assistance. We are grateful for the co-operation of the Cheshire Wildlife Trust and The Mere Golf Resort and Spa in granting permission for this study to be conducted on their lakes. Funding for monitoring of Swan Lake and support for data analysis by Gertrud Nürnberg were provided by the City of Markham, Ontario, Canada. Funding for Phoslock application and monitoring of Crome's Broad and support for data analysis by Andrea Kelly and Erica Murray were provided by the Broads Authority, Broads National Park, Natural England and The Environment Agency, UK. Monitoring activities at Hatchmere and The Mere were supported by a CEH/EA collaborative research project (reference number F/1112/1085). This work was also supported by the CEH Ecosystem Resilience Project (project number NEC05106). Kate Waters was supported by a NERC Industrial Case PhD Studentship (grant reference - NE/L00870X/1).

REFERENCES

Bilanovic, D.D., Kroeger, T.J., Spigarelli, S.A., 2007. Behaviour of humic-bentonite aggregates in diluted suspensions. *Water SA.*, 33, 111-116.

Boedeltjie, G., Bakker, J. P., ter Heerdt, G. N. J., 2003. Potential role of propagule banks in the development of aquatic vegetation in backwaters along navigation canals. *Aquat. Bot*., 77, 53-69.

Breukelaar, A.W., Lammens, E.H.R.R., Breteler, J.G.P.K. Tatrai, I. 1994. Effects of benthivorous bream (*Abramis brama*) and carp (*Cyprinus carpio*) on sediment resuspension and concentrations of nutrients and chlorophyll-a. *Freshwater Biol,*. 32, 113-121.

Brothers, S. Köhler, J., Attermeyer, K., Grossart, H.P., Mehner, T., Meyer, N., Scharnweber, K., Hilt, S. 2014. A feedback loop links brownificatoin and anoxia in a temperature, shallow lake. *Limnol. Oceanog.*, 59, 1388-1398.

Copetti, D., Finsterle, K., Marziali, L., Stefani, F., Tartari, G., Douglas, G., Reitzel, K., Spears, B.M., Winfield, I.J., Crosa, G., D'Haese, P., Lürling, M., This issue. Eutrophication management in surface waters using a lanthanum-modified bentonite: a review. *Water Res*.

De Vicente, I., Jensen, H.S., Andersen, F.O., 2008. Factors affecting phosphate adsorption to aluminum in lake water: Implications for lake restoration. *Sci. Tot. Environm.* 389, 29–36.

Douglas, G.D., Adeney, J.A., Zappia, L.R. 2000. Sediment remediation project:1998/9 laboratory trial report CSIRO land and water, Commonwealth Scientific and Industrial Research Organisation.

European Commission, 2000. Council Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

Fowler, J., Cohen, L., Jarvis, P. (1998) Practical statistics for field biology. John Wiley & Sons, Chichester, UK.

Gibbs, M.M., Hickey, C.W., Ozkundakci, D., 2011. Sustainability assessment and comparison of efficacy of four P-inactivation agents for managing internal phosphorus loads in lakes: sediment incubations. *Hydrobiologia*, 658, 253-275.

Gulati, R., Pires, L.M.D., Van Donk, E. 2008. Lake restoration studies: failures, bottlenecks and prospects of new ecotechnological measures. *Limnologica*, 38, 233-247.

Gunn, I.D.M., O'Hare, M., Carvalho, L., Roy, D.B., Rothery, P., Darwell, A.M. 2010. Assessing the condition of lake habitats – a test of methods for surveying aquatic macrophyte communities. *Hydrobiologia*, 658, 87-97.

Gunn, I.D.M., Meis, S., Maberly, S.C., Spears, B.M., 2014. Assessing the responses of aquatic macrophytes to the application of a lanthanum modified bentonite clay, at Loch Flemington, Scotland, UK. *Hydrobiologia* 737, 309-320.

Haghseresht, F., Wang, S.B., Do, D.D., 2009. A novel lanthanum-modified bentonite, Phoslock, for phosphate removal from wastewaters. *Appl. Clay Sci.*, 46, 369-375.

Helsel, D.R., Hirsch, R, 1992. Statistical methods in water resources. Elsevier Science publishers B.V., Molenwerf 1, Amsterdam, The Netherlands.

Hickey, C.W., Gibbs, M.M., 2009 Lake sediment phosphorus release management-Decision support and risk assessment framework. *New Zeal. J. Mar. Fresh.*, 43, 819-854.

Hilton, J., Lishman, J.P., Allen, P.V., 1986. The dominant processes of sediment distribution and focussing in a small, eutrophic, monomictic lake. *Limnol. Oceanog.,* 31, 125-133.

Horpilla, J., Peltonen, H., Malinen, T., Luokkanen, E., Kairesalo, T. 2003. Top-down or bottom-up effects by fish: issues of concern in biomanipulation of lakes. *Restor. Ecol.,* 6, 20-28.

Huser, B., Brezonik, P., Newman, R., 2011. Effects of alum treatment on water quality and sediment in the Minneapolis Chain of Lakes, Minnesota, USA. *Lake Res. Man.,* 27, 220-228.

Huser, B.J., Egemose, S., Harper, H., Hupfer, M., Jensen, H., Pilgrim, K.M., Reitzel, K., Rydin, E., Futter, M., This issue. Longevity and effectiveness of aluminum addition to reduce sediment phosphorus release and restore lake water quality. *Water Res*.

Ihaka, R., Gentleman, R., 1996. R: a language for data analysis and graphics. *J. Comput. Graph. Stat.*, 5, 299–314.

Jeppesen, E., Meerhoff, M., Jacobsen, B.A., Hansen, R.S., Sondergaard, M., Jensen, J.P., Lauridsen, T.L., Mazzeo, N., Branco, C.W.C., 2007. Restoration of shallow lakes by nutrient control and biomanipulation-the successful strategy varies with lake size and climate. *Hydrobiologia*, 581, 269-285.

Jeppesen, E., Jensen, J.P., Søndergaard, M., Lauridsen, T.L., Landkildehus, F., 2000. Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. *Freshwat. Biol.*, 45, 201-218.

Jeppesen, E., Søndergaard, M., Jensen, J.P., Havens, K., Anneville, O., Carvalho, L., Coveney, M.F., Deneke, R., Dokulil, M.T., Foy, B., Gerdeaux, D., Hampton, S.E., Hilt, S., Kangur, K., Kohler, J., Lammens, E.H.H.R., Lauridsen, T.L., Manca, M., Miracle, M.R., Moss, B., Noges, P., Persson, G., Phillips, G., Portielje, R., Romo, S., Schelske, C.L., Straile, D., Tatrai, I., Willen E., Winder, M., 2005. Lake responses to reduced nutrient loading – an analysis of contemporary long-term data from 35 case studies. *Freshwat. Biol.*, 50, 1747-1771.

JNCC. 2005. Common Standards Monitoring Guidance for Freshwater Habitats and Species. 1st version- March 2005, ISSN 1743-8160. Joint Nature Conservation Committee.

Jupp, B.P., Spence, D.H.N., 1977. Limitations on macrophytes in a eutrophic lake, Loch Leven. *J. Ecol.* 65: 175-186.

Kennison, G. C. B., Dunsford, D. S., Schutten, J. 1998. Stable or changing lakes? A classification of aquatic macrophyte assemblages from a eutrophic shallow lake system in the United Kingdom. *Aquat. Conserv.*, 8, 669-684.

Kritzberg, E.S., Ekström, S.M. 2012. Increasing iron concentrations in surface waters – a factor behind brownification? *Biogeosciences,* 9, 1465-1478.

Lürling, M., Van Oosterhout, F., 2012. Case study on the efficacy of a lanthanum-enriched clay (Phoslock®) in controlling eutrophication in Lake Het Groene Eiland (The Netherlands). *Hydrobiologia*, 710, 253-263.

Lürling, M., Waajen, G., Van Oosterhout, F. 2014. Humic substances interfere with phosphate removal by lanthanum modified clay in controlling eutrophication. *Wat. Res.*, 54, 78-88.

Mackay, E. Maberly, S.C., Pan; G., Reitzel, K., Bruere, A., Corker, N., Douglas, G., Egemose, S., Hamilton, D., Hatton-Ellis, T., Huser, B., Li, W., Meis, S., Moss, B., Lurling, M., Phillips, G., Yasseri, S., Spears, B.M., 2014. Geo-engineering in lakes: welcome attraction or fatal distraction? *Inland Wat.,* 4, 349-356.

May, L., Spears, B.M., Dudley, B.J. and Hatton-Ellis, T.W., 2010. The importance of nitrogen limitation in the restoration of Llangorse Lake, Wales, UK. J. Environ. Monit., 12, 338-346.

Mehner, T., Diekmann, M., Gonsiorczyk, T., Kasprzak, P., Koschel, R., Krienitz, L., Rumpf, M., Schulz, M., Wauer, G., 2008. Rapid recovery from eutrophication of a stratified lake by disruption of internal nutrient load. *Ecosystems*, 11, 1142-1156.

Meis, S., Spears, B.M., Maberly, S.C., O'Malley, M.B., Perkins, R.G., 2012. Sediment amendment with Phoslock (R) in Clatto Reservoir (Dundee, UK): Investigating changes in sediment elemental composition and phosphorus fractionation. *J. Environ. Man.*, 93, 185-193.

Mitsch, W.J., Zhang, L., Anderson, C.J., Altor, A.E., Hernandez, M.E., 2005. Creating riverine wetlands: Ecological succession, nutrient retention and pulsing effects. *Ecol. Eng.*, 25, 510-527.

Monteith, D. T., Stoddard, J.L., Evans, C.D., de Wit, H.A., Forsius, M., Hogasen, T., Wilander, A., Skjelkvale, B.L., Jeffries, D.S., Vuorenmaa, J., Keller, B., Kopacek, J., Vesely, J., 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, 450, 537-541.

Nürnberg, G.K., 1998. Prediction of annual and seasonal phosphorus concentrations in stratified and polymictic lakes. *Limnol. Oceanogr*. 43, 1544–1552.

Nürnberg, G. K., 2007. Lake responses to long-term hypolimnetic withdrawal treatments. *Lake Reserv. Manage*., 23, 388-409.

Nürnberg, G.K., Shaw, M., 1998. Productivity of clear and humic lakes: nutrients, phytoplankton, bacteria. *Hydrobiologia*, 382, 97–112.

Penn, M.R., Auer, M.T., Doerr, S.M., Driscoll, C.T., Brooks, C.M., Effler, S.W., 1999. Seasonality in phosphorus release rates from the sediments of a hypereutrophic lake under a matrix of pH and redox conditions. *Can. J. Fish. Aquat. Sci.*, 57, 1033-1041.

Peterson, S.A., 1982. Lake restoration by sediment removal. *Wat. Resour. Bull.*, 18, 423-435.

Phillips, G., Kelly, A., Pitt, J-.A., Sanderson, R., Taylor E., 2005. The recovery of a very shallow lake, 20 years after the control of effluent derived phosphorus. *Freshwater Biol.*, 50, 1628-1638.

Pinheiro, J.C., Bates, D.M., 2000. Mixed Effects Models in S and S-PLUS. Springer, New York.

R Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Reynolds C.S., 2006. Ecology of Phytoplankton. Cambridge University Press, Cambridge.

Robb, M., Greenop, B., Goss, Z., Douglas, G., Adeney, J. 2003. Application of Phoslock (TM), an innovative phosphorus binding clay, to two Western Australian waterways: preliminary findings. *Hydrobiologia*, 494, 237-243.

Sas, H., 1989. Lake Restoration by Reduction of Nutrient Loading. Academic Verlag Richarz, Germany.

Schindler, D.W., 1998. Replication versus realism: The need for ecosystem-scale experiments. *Ecosystems,* 1, 323-334.

Seabloom, E.W., Moloney, K.A., Van der Valk, A.G., 2001. Constraints on the establishment of plants along a fluctuating water-depth gradient. *Ecology*, 82, 2216-2232.

Sharpley, A., Jarvie, H.P., Buda, A., May, L., Spears, B.M., Kleinman, P., 2013. Phosphorus Legacy: Overcoming the Effects of Past Management Practices to Mitigate Future Water Quality Impairment. *J. Environ. Qual.*, 42, 1308-1326.

Smith, V., 2003. Eutrophication of freshwater and coastal marine ecosystems: a global problem. Environ. *Sci. & Pollut. Res.* 10, 126-139.

Søndergaard, M., Jeppesen, E., Lauridsen, T.L., Skov, C.E., Van Nes, .H., Roijackers, R., Lammens, E., Portielje, R., 2007. Lake restoration: successes, failures and long-term effects. *J. Appl. Ecol.*, 44, 1095-105.

Søndergaard, M., Bjerring, R., Jeppesen, E., 2012. Persistent internal loading during summer in shallow lakes. *Hydrobiologia*, 710, 95-107.

Spears, B.M., Lesack, L.F.W., 2006. Bacterioplankton production, abundance, and nutrient limitation among lakes of the Mackenzie Delta (western Canadian arctic). *Can. J. Fish. Aquat. Sci.,* 63, 845-857.

Spears, B.M., Carvalho, L., Perkins, R., Kirika, A., Paterson, D.M., 2007. Sediment phosphorus cycling in a large shallow lake: spatio-temporal variation in phosphorus pools and release. *Hydrobiologia,* 584, 37-48.

Spears, B., Jones, I., 2010. The long-term (1979–2005) effects of the North Atlantic Oscillation on wind-induced wave mixing in Loch Leven (Scotland). *Hydrobiologia,* 646, 49-59.

Spears, B.M., Carvalho, L., Perkins, R., Kirika, A., Paterson, D.M. 2012. Long-term variation and regulation of internal phosphorus loading in Loch Leven. *Hydrobiologia,* 681, 23-33.

Spears, B.M., S., Meis, A., Anderson, M., Kellou. 2013a. Comparison of phosphorus (P) removal properties of materials proposed for the control of sediment P release in UK lakes. *Sci. Total Environ.,* 442, 103-110.

Spears, B.M., Lurling, M., Yasseri, S., Castro-Castellon, A.T., Gibbs, M., Meis, S., McDonald, C., McIntosh, J., Sleep, D., Van Oosterhout, F., 2013b. Lake responses following lanthanum-modified bentonite clay (Phoslock®) application: an analysis of water column lanthanum data from 16 case study lakes. *Wat. Res.,* 47,5930-42.

Spears B.M., Dudley, B., Reitzel, K., Rydin, E., 2013c. Geo-engineering in lakes: a call for consensus. *Environ. Sci. Technol.*, 47, 3953-3954.

Steinberg, C.E.W., Meinelt, T., Timofeyev, M.A., Bitner, M., Menzel, R., 2008. Humic substances (review series). Part 2 – Interactions with organisms. *Env. Sci. Pollut. Res.* 15, 128-135.

Van Geest, G. J., Roozen, F. C. J. M., Coops, H., Roijackers, R. M. M., Buijse, A. D., Peeters, E. T. H. M. and Scheffer, M., 2003. Vegetation abundance in lowland flood plan lakes determined by surface area, age and connectivity. *Freshwater Biol.*, 48: 440–454.

Van Wichelen, J., Declerck, S., Muylaert, K., Hoste, I., Geenens, V., Vandekerkhove, J., Michels, E., De Pauw, N., Hoffmann, M., De Meester, L., Vyverman, W., 2007. The importance of drawdown and sediment removal for the restoration of the eutrophied shallow Lake Kraenepoel (Belgium). *Hydrobiologia,* 584, 291-303.

Wetzel, R. G., Likens, G. E., 1991. Limnological Analyses. Second Edition. Springer-Verlag, New York.

Withers, P.J.A., Haygarth, P.M., 2007. Agriculture, phosphorus and eutrophication: a European perspective. *Soil Use Manage.*, 23, 1-4.

Zamparas, M., Gianni, A., Stathi, P., Deligiannakis, Y., Zacharias, I., 2012. Removal of phosphate from natural waters using innovative modified bentonites. *Appl. Clay Sci.*, 62-63, 101-106.

TABLE LEGENDS

Table 1. Summary of data reported for each of the 18 lakes to which La-bentonite has been applied and for which water quality and macrophyte data were available. UK – United Kingdom, NL – The Netherlands, DE– Germany, CA - Canada. M - indicates lakes for which aquatic macrophyte data were available.

Table 2. Results of linear mixed models run on Z score transformations testing the significance of the responses in the 24 months following La-bentonite applications relative to the 24 months preceding the applications in surface water seasonal and annual total phosphorus (TP) soluble reactive phosphorus (SRP), chlorophyll *a* concentration (Chl*a*) and Secchi disk depth. SE – standard error; DF – degrees of freedom; n – number of observations; t – t statistic; P – *p* value.

Table 3. Results of two-way analysis of variance to test effects of lake, La-bentonite treatment and interactions between the two, on log transformed aquatic macrophyte species numbers and aquatic macrophyte maximum colonisation depths in the two years before and after application. DF- degrees of freedom; SS – Sum Square; MS; Mean Square; F- F value; P - *p* value.

FIGURE LEGENDS

Figure 1. Summary of available data for total phosphorus (TP), soluble reactive phosphorus (SRP), and chlorophyll *a* (Chl*a*) concentrations, and Secchi disk depths in the 24 month periods preceding and following La-bentonite application.

Figure 2. Seasonal and annual ranges of raw data (top panel) and Z score transformed (lower panel) total phosphorus (TP) (a - e) soluble reactive phosphorus (SRP) (f - j), chlorophyll *a* concentration (Chl*a*) (k - o), and Secchi disk depth (p - t) in the 24 months preceding and following an application of La-bentonite. The number of lakes for which data were available is reported in each case. 95^{th} and 5^{th} percentile error bars are shown along with values above or below these values, where appropriate.

Figure 3. Results of principal components analysis for surface water determinands in the 24 months preceding and following the application of lanthanum bentonite showing the weightings and ordination of each environmental variable measured along both principal components. Mean Depth – mean depth of lake; SA – surface area of lake; DOC – mean dissolved organic carbon concentration; Alkalinity - mean alkalinity; TP – median total phosphorus concentration; SRP - median soluble reactive phosphorus concentration; Chla – median chlorophyll a concentration; Secchi – median Secchi disk depth; dose (t ha) – dose of lanthanum bentonite. PC – principal component; EV – eigenvalue; CV – cumulative variance explained.

Figure 4. Ranges of aquatic macrophyte species numbers and aquatic macrophyte maximum colonisation depths in the two years prior, and two years following, La-bentonite applications. The number of lakes for which data were available is reported in each case. 95^{th}

and 5th percentile error bars are shown along with values above or below these values, where appropriate.

Table 1.

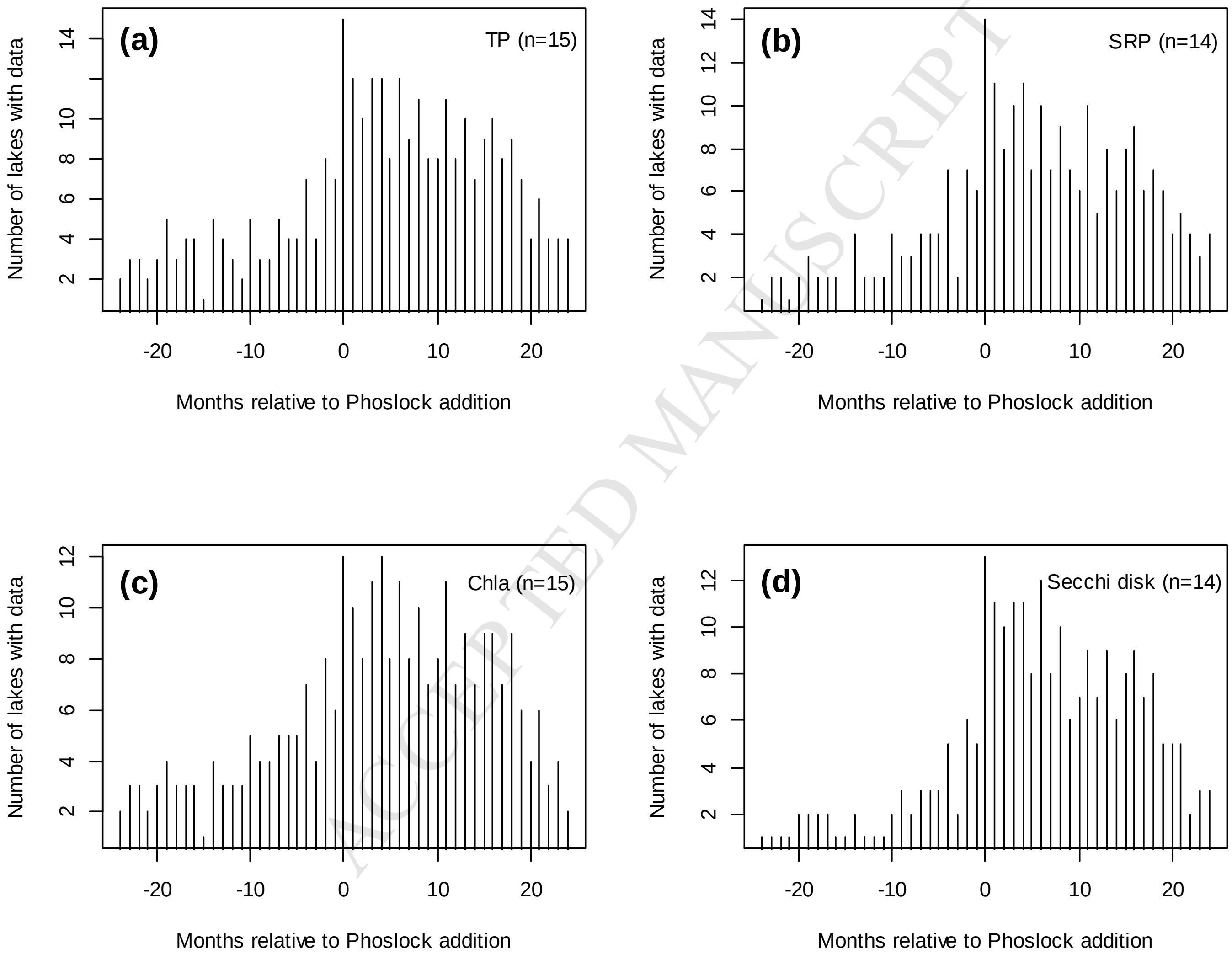
Lake Name	Country	S.A. (ha)	Mean depth (m)	Max depth (m)	Fetch (km)	Date and mass applied (tonnes)	Phoslock® Load (tonnes ha^{-1})
Clatto Reservoir	UK	9.0	2.8	7.0	0.4	04/03/2009 (24.0)	2.7
Loch Flemington [M]	UK	15.7	1.0	2.5	0.7	15/03/2010 (25.0)	1.6
Crome's Broad [M]	UK	3.7	0.8	1.3	0.2	19/03/2013 (9.75)	5.1
Hatchmere [M]	UK	4.7	1.4	3.8	0.3	13/03/2013 (25.2)	5.3
Mere Mere [M]	UK	15.8	2.8	8.1	0.5	09/03/2013 (79.8)	5.1
Lake Rauwbraken [M]	NL	4.0	8.8	16.0	0.2	21/04/2008 (18.0)	4.5
Lake De Kuil	NL	7.0	4.0	10.0	ND	18/05/2009 (41.5)	5.9
Lake Silbersee	DE	7.0	5.0	9.0	0.3	08/11/2006 (21.5)	3.1
Lake Otterstedter See	DE	4.5	5.0	11.0	0.3	30/10/2006 (11.0)	2.4
Lake Behlendorfer See	DE	64.0	6.2	16.0	2.0	02/12/2009 (230.0)	3.6
Lake Blankensee	DE	22.5	1.6	2.5	0.5	16/11/2009 (66.0)	2.9
Lake Baerensee	DE	6.0	2.6	3.8	0.1	11/06/2007 (11.5)	1.9
Lake Kleiner See	DE	0.9	2.0	5.0	0.2	25/05/2010 (6.0)	6.7
Lake Eichbaumsee	DE	23.2	6.5	16.0	0.9	17/11/2010 (148.0)	6.8
Lake Ladillensee	DE	1.0	2.1	5.0	0.1	03/03/2009 (4.7)	4.7
Lake Völlen	DE	2.0	2.5	5.5	0.1	19/03/2008 (10.0)	5.0
Niedersachsen Lake	DE	4.2	2.5	6.0	0.1	19/03/2008 (6.0)	1.4
Swan Lake	CA	5.4	1.9	4.4	0.4	01/05/2013 (25.2)	4.7

Table 2.

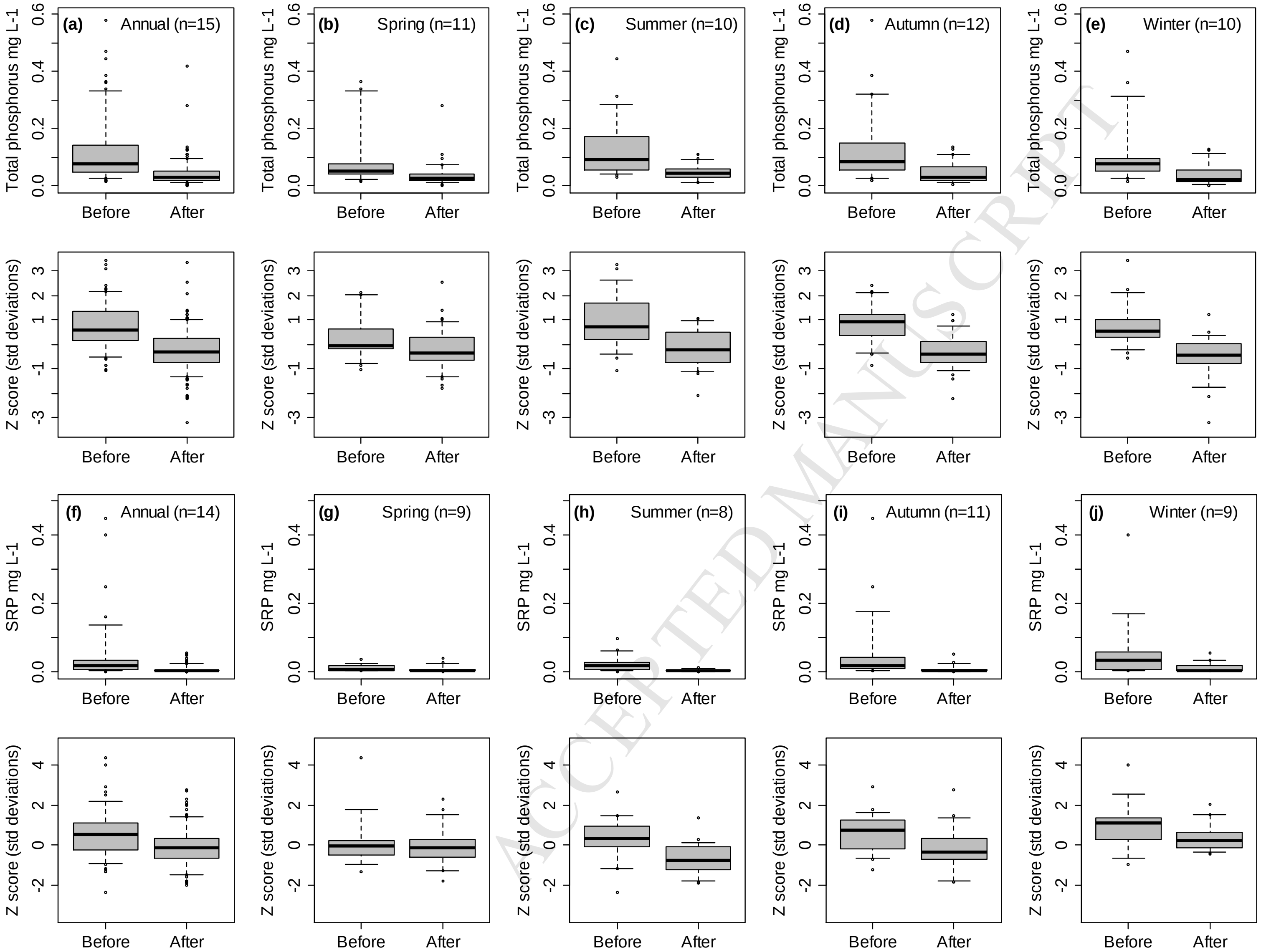
Variable	Season	Difference between pre- and post-application values (standard deviations)	SE	DF	n	P
Total Phosphorus	*Annual*	-0.961	0.113	379	395	<0.001
	Spring	-0.634	0.177	83	95	<0.001
	Summer	-1.057	0.170	76	87	<0.001
	Autumn	-1.142	0.142	81	94	<0.001
	Winter	-1.276	0.216	47	58	<0.001
SRP	*Annual*	-0.794	0.120	285	300	<0.001
	Summer	-1.043	0.207	49	58	<0.001
	Autumn	-0.781	0.214	58	70	<0.001
	Winter	-0.659	0.282	33	43	0.026
Chlorophyll *a*	*Annual*	-0.389	0.107	327	341	<0.001
	Spring	-0.839	0.189	80	90	<0.001
Secchi disk	*Annual*	0.521	0.099	391	406	<0.001
	Summer	0.900	0.265	84	92	0.001
	Winter	0.675	0.261	35	44	0.014

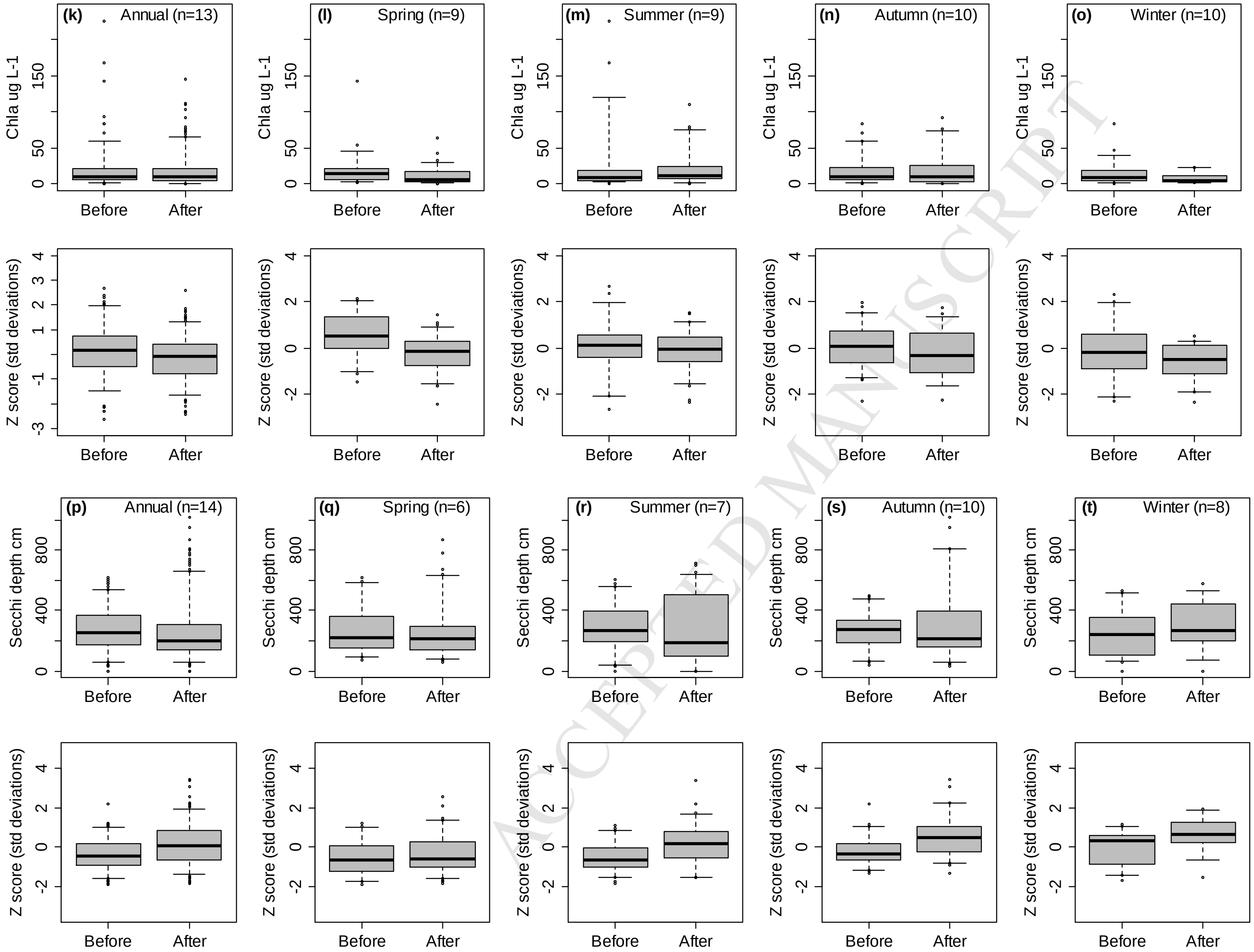
Table 3.

Macrophyte species numbers	DF	SS	MS	F	P
Lake	5	1.46	0.29	54.58	<0.001
La-bentonite	1	0.04	0.04	8.29	0.014
Lake x La-bentonite	5	0.03	<0.01	0.93	0.496
Error	12	0.06	<0.01		
Total	23				
R^2	95.96	**R^2 (adjusted)**	92.26		
Macrophyte maximum growing depths	**DF**	**SS**	**MS**	**F**	**P**
Lake	3	0.27	0.09	135	<0.001
La-bentonite	1	0.12	0.12	183	<0.001
Lake x La-bentonite	3	0.20	0.07	103	<0.001
Error	8	0.01	<0.01		
Total	15	0.60			
R^2	99.11	**R^2 (adjusted)**	98.34		

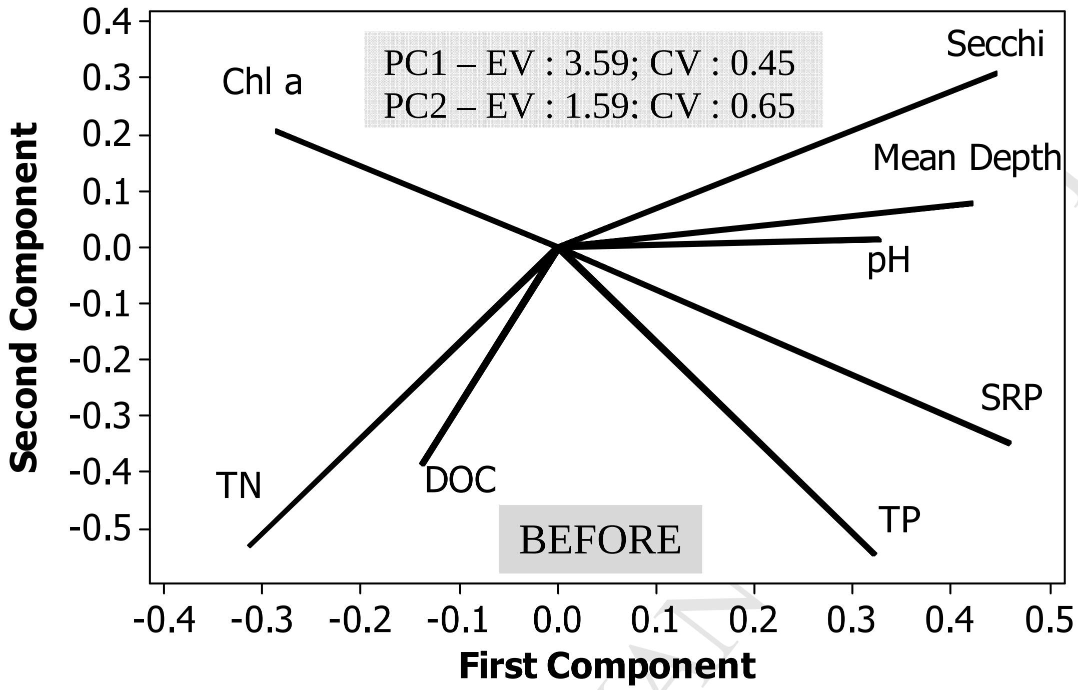


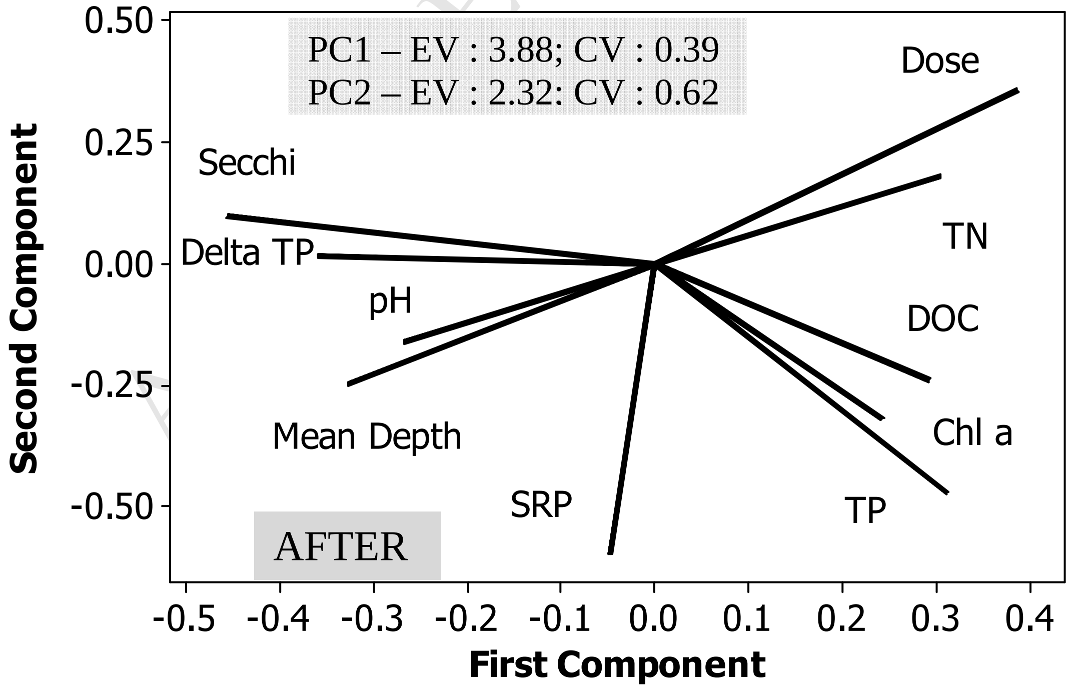
(a)
TP (n=15)
Number of lakes with data
2
4
6
8
10
14
-20
-10
0
10
20
Months relative to Phoslock addition
(b)
SRP (n=14)
Number of lakes with data
2
4
6
8
10
12
14
-20
-10
0
10
20
Months relative to Phoslock addition
(c)
Chla (n=15)
Number of lakes with data
2
4
6
8
10
12
-20
-10
0
10
20
Months relative to Phoslock addition
(d)
Secchi disk (n=14)
Number of lakes with data
2
4
6
8
10
12
-20
-10
0
10
20
Months relative to Phoslock addition

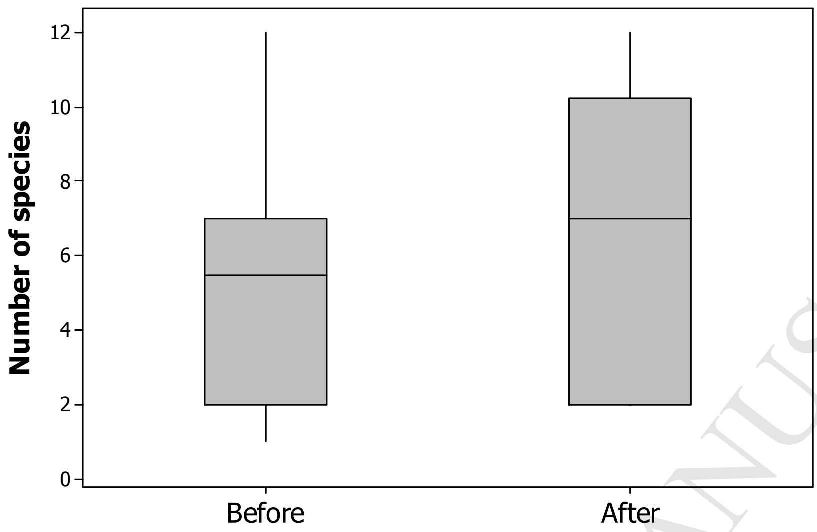

(a) Annual (n=15)
(b) Spring (n=11)
(c) Summer (n=10)
(d) Autumn (n=12)
(e) Winter (n=10)
(f) Annual (n=14)
(g) Spring (n=9)
(h) Summer (n=8)
(i) Autumn (n=11)
(j) Winter (n=9)
Total phosphorus mg L-1
Z score (std deviations)
SRP mg L-1
Before
After

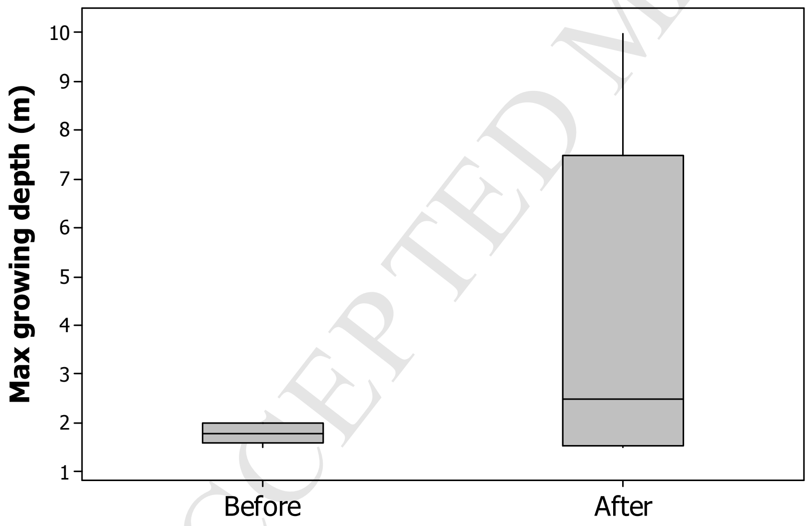


(k) Annual (n=13)
(l) Spring (n=9)
(m) Summer (n=9)
(n) Autumn (n=10)
(o) Winter (n=10)
Chla ug L-1
Z score (std deviations)
(p) Annual (n=14)
(q) Spring (n=6)
(r) Summer (n=7)
(s) Autumn (n=10)
(t) Winter (n=8)
Secchi depth cm
Before
After


PC1 – EV : 3.59; CV : 0.45
PC2 – EV : 1.59: CV : 0.65
BEFORE
Secchi
Chl a
Mean Depth
pH
SRP
TP
DOC
TN
Second Component
First Component
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2 0.3 0.4 0.5


PC1 – EV : 3.88; CV : 0.39
PC2 – EV : 2.32: CV : 0.62
AFTER
Dose
Secchi
TN
Delta TP
pH
DOC
Mean Depth
Chl a
SRP
TP
Second Component
First Component
0.50
0.25
0.00
-0.25
-0.50
-0.5 -0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2 0.3 0.4


Number of species
0
2
4
6
8
10
12
Before
After


Max growing depth (m)
1
2
3
4
5
6
7
8
9
10
Before
After

A META-ANALYSIS OF WATER QUALITY AND AQUATIC MACROPHYTE RESPONSES IN 18 LAKES TREATED WITH LANTHANUM MODIFIED BENTONITE (PHOSLOCK®)

Bryan M. Spears[1*], Eleanor B. Mackay[2], Said Yasseri[3], Iain D. M. Gunn[1], Kate E. Waters[1], Christopher Andrews[1], Stephanie Cole[4], Mitzi de Ville[2], Andrea Kelly[5], Sebastian Meis[1,6], Alanna L. Moore[1], Gertrud K. Nürnberg[7], Frank van Oosterhout[8], Jo-Anne Pitt[9], Genevieve Madgwick[10], Helen J. Woods[1], and Miquel Lürling[8,11]

*Corresponding author, email: spear@ceh.ac.uk, tel: +44 (0)131 445 4545, fax: +44 (0)131 445 445 943

1 Centre for Ecology & Hydrology, Penicuik, Midlothian,EH26 0QB, UK

2 Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK

3 Institut Dr. Nowak, Mayenbrook 1, D-28870 Ottersberg, Germany

4 Environment Agency, Red Kite House, Howbery Park, Crowmarsh Gifford, Wallingford, Oxon OX10 8BD, UK

5 Broads Authority, Yare House, 62-64 Thorpe Road, Norwich, NR1 1RY, UK

6 Lanaplan GbR, Lobbericher Str. 5, D-41334, Nettetal, Germany

7 Freshwater Research, 3421 Hwy 117, Baysville, Ontario, P0B 1A0, Canada

8 Aquatic Ecology and Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands

9 Environment Agency, Station Road, Haddiscoe, Great Yarmouth, NR31 9JA, UK

10 Natural England, Eastbrook, Shaftesbury Road, Cambridge CB2 2DF, UK

11 Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB, Wageningen, The Netherlands

Highlights

1. Water quality and macrophyte community responses were assessed following Phoslock treatments
2. Phosphorus concentration and phytoplankton biomass decreased and water clarity increased.
3. Macrophyte species richness and extent increased.
4. Responses were highly site specific and decreased with increasing DOC concentrations.