# Assessing aquatic macrophyte recovery responses following lanthanum-modified bentonite applications

**Kate Elizabeth Waters-Hart**

**December 2019**

Submitted to Biological and Environmental Science

Faculty of Natural Sciences

University of Stirling

Scotland

For the degree of Doctor of Philosophy

Supervisors:

Bryan Spears[1], Nigel Willby[2], Said Yasseri[3], Genevieve Madgwick[4], Iain Sime[5]

[1]UK Centre for Ecology and Hydrology, [2]University of Stirling, [3]Limnological Solutions International Pty Ltd, [4]Natural England, [5]Scottish Natural Heritage

## Statement of originality

I hereby confirm that this PhD thesis is an original piece of work conducted independently by the undersigned and all work contained herein has not been submitted for any other degree.

All research material has been duly acknowledged and cited.

Signature of candidate:

Kate Waters-Hart

Date:

## Acknowledgements

An incredible amount of people has helped me throughout my PhD journey, and I would like to thank you all for your support. First and foremost, I would like to thank my primary supervisor, Dr Bryan Spears for his endless support and encouragement and for continually pushing me in the right direction. I'd also like to thank you for giving me the opportunity to expand my career further through my internship and your personal support and compassion over the last few years.

I extend this thanks to my secondary supervisor Professor Nigel Willby, for his continued support, enthusiasm, motivation and for being submerged for several hours in the cold waters of Airthrey Loch collecting sediment with me. I'm also very grateful for your help getting me through all the admin on time.

I would also like to thank Dr Said Yasseri for his help whilst spending time in Germany surveying lakes and for his continued encouragement and support. I would also like to thank the other members of my supervisory team, Iain Sime and Dr Gen Madgwick for their advice throughout the project.

I thank both Dr Carl Sayer (University College London) and Dr Peter Hunter (University of Stirling) for spending time to read and examine my thesis. Their comments and constructive feedback have improved its quality.

I have been lucky enough to do this PhD in a supportive and encouraging group at the UK Centre for Ecology & Hydrology (CEH) and I wouldn't have been able to complete this PhD without the help of many people there (or now elsewhere). Laboratory and fieldwork work during this PhD was made much easier with the help of Justyna Olszewska, James Watt, Stephen Ives, Alanna Moore, Nick Cowan, Amy Pickard Rosie McDonald and Stella White. I'm incredibly grateful to each of them. I'd also like to thank Iain Gunn for his knowledge and identification on macrophytes, help with fieldwork prep and ongoing support during this project. Thanks to my fellow CEH students from the PhD room for advice, lunchtime chats and walks which has made this PhD very enjoyable.

I would also like to extend my thanks to Tim Epe (Institut Dr Novak) for his help with macrophyte surveys in Germany and to Jan Brückman (Institut Dr Novak) for helping with macrophyte identification. Thanks to John Pottie for help with conducting the Loch Flemington macrophyte survey with me. Thanks also to Rupert Perkins (Cardiff University) for his advice on fluorometry and to Seb Meis (lanaplan GbR) and Valerie

James on guidance on trying to source and grow *Najas flexilis*. Many thanks to Nigel Traill (Phoslock Environmental Technologies) who has provided much support and encouragement throughout the PhD. Thank you to Dr Nicola Cayzer at Edinburgh University for testing some of my macrophyte samples using SEM for macro and elemental analysis.

Thank you to the Natural Environment Research Council, Phoslock Water Solutions and Scottish Natural Heritage (SNH) for funding the project and for SNH for providing the Schedule 4 licence (Number: 123404) for the collection of *Najas flexilis*.

To my Dad, you have never stopped encouraging me since I decided to study science and I am grateful for all the love and support you and Mum have given me. Thank you to my brother Andrew, family and friends that have shown endless support, particularly during the last few years of my PhD when I've needed it most.

Finally, an enormous thank you to my wonderful Husband Ben. You have been the most encouraging, supportive and hilarious person to have been by my side through this PhD journey and I couldn't have done it without your love and encouragement.

I would like to dedicate this thesis to Grandad Jim (1930 – 2018) for his admirable resilience, to my Grandad Tom (1924 – 2018) for his never-ending faith in me and to my incredible Mum, Jane (1957 – 2018) who inspired my love and passion for nature and shown me the absolute definition of strength and determination; all have had a profound influence on my life and I am incredibly lucky to have known such inspirational people.

## General Abstract

Lanthanum – modified bentonite (LMB) has been used successfully in lake restoration projects globally to control phosphorus (P) release from sediments to overlying waters. However, desirable aquatic macrophyte (macrophyte) species recovery following LMB applications, where reported, has been slow or non-existent, despite improvements in water quality. The reasons behind this lack of recovery are unknown. This study is the first comprehensive assessment of macrophyte recovery following sediment capping in lakes and focusses on physical, chemical, and biological constraints which could potentially result in 'ecological bottlenecks' to macrophyte reestablishment in lake restoration generally.

An assessment of short- (0 – 2 years) and long-term (2 – 10 years) changes in macrophyte composition in lakes following LMB applications revealed that macrophyte communities do not meet European legislative targets, e.g. the Water Framework Directive for *good ecological status*. Low seedbank viability, dominance of pioneering non-native species and scarcity of external propagule sources may be the main restrictions on macrophyte recovery in LMB treated lakes.

A germination trial confirmed that an LMB layer, formed on surface sediments following application, did not impede macrophyte germination success. However, LMB did significantly reduce benthic algal growth which was species-specific.

Bioassay experiments revealed that macrophyte species responded differently to LMB under different light conditions. Desirable and non-native invasive species and nationally rare protected species responded in-line with their strategy traits. All species grew when applied with LMB in light conditions, however, all species grew less when applied with LMB in dark conditions.

The findings presented demonstrate that additional measures may be required alongside sediment capping to force ecologically recovery, especially where restoration planning is designed to meet ecological targets for desirable vegetation composition. Transplantation of macrophytes may be needed to ensure the establishment of desirable species if viable seedbanks no longer occur following improvements in water quality, or for waterbodies that are isolated from propagule distribution sources, or where pioneering macrophyte species dominate communities.

**Contents**

## List of Figures

## List of Tables

# 1. Introduction

## 1.1. Freshwaters

Freshwaters are considered one of the most vital of all natural resources (Wetzel, 2001). They are extremely important ecosystems which support 9.5% of all described animal species world-wide, despite only covering 0.8% of the total surface area of the globe (Balian et al., 2008) and making up 0.01% of the world's water (Dudgeon et al., 2006). However, fresh water is a limited resource that is being exploited and degraded at an accelerating rate by humanity (Wetzel, 2001). Since the latter half of the 20$^{th}$ century freshwater biodiversity across the globe has declined, or is under threat from anthropogenic activities and this loss far exceeds biodiversity loss in terrestrial systems (Dudgeon et al., 2006).

Fresh waters provide cultural, aesthetic and ecosystem service benefits to humans (Costanza et al., 1997; MEA, 2005). Lakes, in particular, are one of the most vulnerable freshwater systems to anthropogenic disturbance but their functioning and water quality is important to sustain a healthy diverse water environment. Multiple stressors such as: nutrient pollution, climate warming, invasive species, and habitat destruction and modification have become some of the greatest threats to lakes

(Dudgeon et al., 2006; Jeppesen et al., 2017). Many of these threats are causing declines in the diversity of freshwater biota (He et al., 2019) including aquatic macrophytes (hereafter, macrophytes) (Chambers et al., 2008). Macrophytes have shown global diversity and abundance declines as a result of eutrophication over recent decades (Lauridsen et al., 2015; Zhang et al., 2017). Declines in macrophyte will ultimately threaten the faunal diversity of aquatic ecosystems (Chambers et al., 2008) and the provision of ecosystem services (Lauridsen et al., 1994; Scheffer and Jeppesen, 1998). It is, therefore, critical that macrophyte communities are preserved or restored where they are threatened, declining or have been lost.

## 1.2. Importance of macrophytes in lakes

The role of macrophytes in sustaining lake ecosystem structure and function should not be underestimated and they are useful indicators of water quality (Lauridsen et al., 1994; Penning et al., 2008; Søndergaard et al., 2005). Their presence contributes to habitat complexity which accommodates functional diversity and heterogeneity making colonised areas the most productive regions of a waterbody (Chambers et al., 2008). Macrophyte richness is also linked to increased biodiversity of other freshwater groups (Law et al., 2019).

Shallow lakes support feedback mechanisms (Figure 1.1) that can buffer the effects of stressors (Carpenter and Cottingham, 1997) but when critical nutrient thresholds are exceeded, macrophytes decline and a waterbody can shift to a phytoplankton dominated state (Scheffer et al., 1993; Scheffer and Jeppesen, 1998) where macrophytes largely disappear (Jeppesen et al., 1997; Scheffer et al., 1993; Søndergaard et al., 2007). However, this shift can take a long time and is considered to be more of a gradual change with macrophyte species compositions shifting from being dominated by charophytes and *Myriophyllum spicatum*, to *Ceratophyllym demersum* and *Rannunculus* species to finally those dominated by *Zannichellia palustris* and fine-leaved *Potamogeton* species at eutrophication end-phase (Sayer, et al. 2010a). Submerged macrophytes have been known to play the most crucial role in promoting clear waters (Scheffer, 1998). Dense macrophyte stands and vegetated littoral areas of a lake are important refuge shelters for large-bodied zooplankton (Blindow et al., 2002; Perrow et al., 1999; Van Donk and Van de Bund, 2002); increasing zooplankton density and promoting greater phytoplankton grazing (Carpenter and Cottingham, 1997). Certain macrophyte species (e.g. *Myriophyllum*

*spicatum*, *Ceratophyllum demersum* and certain Characeae) have been reported to release allelopathic chemicals as a repellent to phytoplankton and epiphyte growth (Hilt and Gross, 2008; Van Donk and Van de Bund, 2002). Macrophytes can significantly alter sediment P - cycling in lakes, for example, dense macrophyte beds (particularly rooted macrophyte species) prevent wind-induced sediment re-suspension (Ibelings et al., 2007), which in turn prevents nutrient release into the water column to be utilised by phytoplankton (Scheffer et al., 2001). Macrophytes can up-take nutrients directly through the sediment and/or from the water column (species dependent) (Van Donk and Van de Bund, 2002) and alter redox conditions and modify pH levels (Wetzel, 2001).

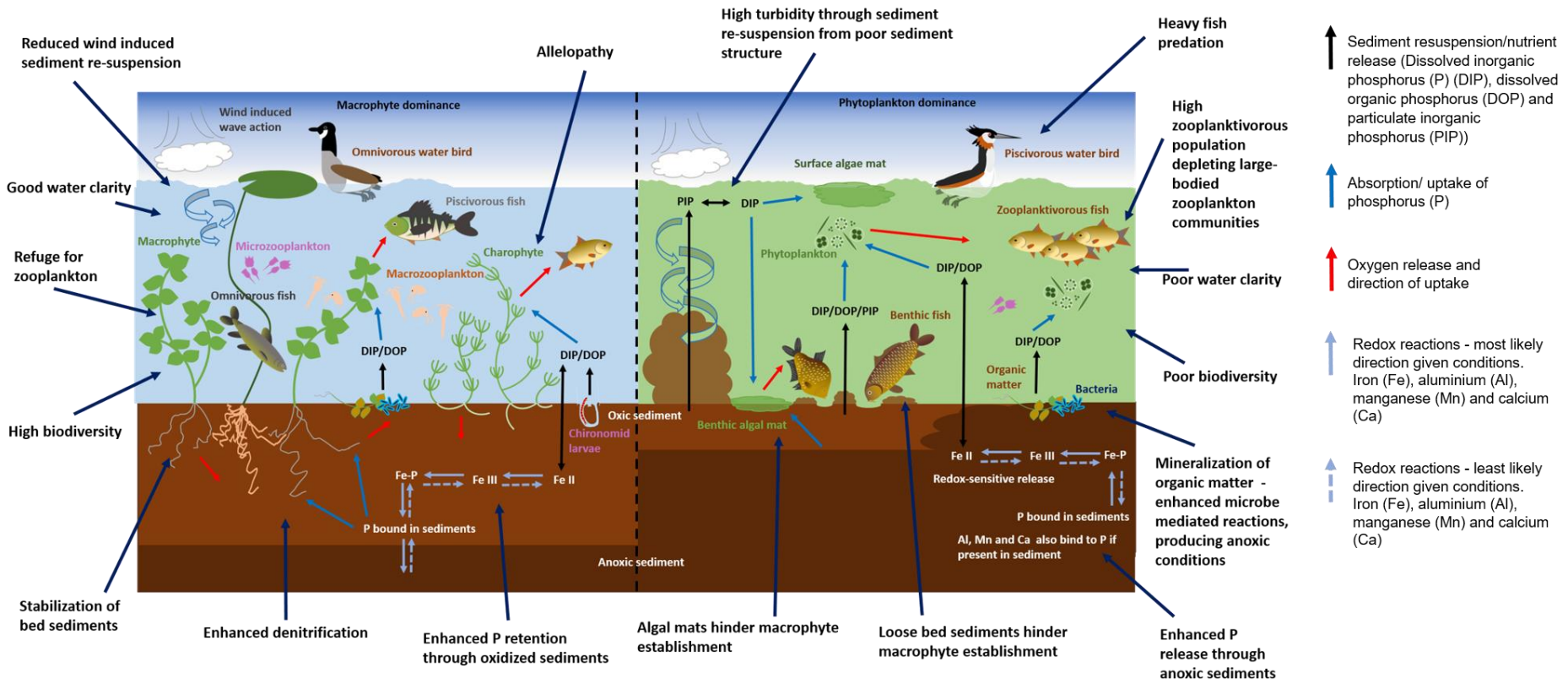



**Figure 1.1** Example of an macrophyte and a phytoplankton dominated system with some of the mechanisms which help to stabilize each system (© Kate Waters-Hart).

## 1.3. Macrophyte ecology in relation to catchment phosphorus load reduction

Macrophytes are a diverse group of aquatic vegetation that can either be emergent, floating or submerged in the water column. The group largely consists of angiosperms (Chambers et al., 2008) but includes bryophytes (mosses and liverworts) and macroalgae (Characeae). Grime (1977) defined two groups of features that influence plant growth forms; the first group includes the stress factors that limit plant growth such as nutrient and light availability, the other group includes disturbance factors. These are identified as biomass loss from disturbances such as wave exposure or grazing. Various macrophyte species have different traits that enable them to live in their respective habitats. Macrophytes that grow together will compete for resources e.g. nutrients, space and light they share within their immediate environment, which can impact the growth outcomes of one individual versus another. Some species are specific in their tolerance to stress and therefore maybe more adaptable than other species (Table 1.1) with more tolerant species dominating communities in more changeable or disturbed systems.

Different macrophyte species have different growth forms which allow them to compete with other species for nutrients, light and space (Murphy et al., 1990). Elodeid species such as *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch.) H. St. John can overwinter and produce vegetatively which allows for quick establishment in spring. They outcompete other species for space and light as they are generally large, taller and faster growing which allows them to colonise before other species (Trémolières, 2004). *Elodea* species generally like nutrient-rich sites and can also uptake nutrients via shoot and roots which also allows for faster growth (Trémolières, 2004) in comparison with other species that only up-take via roots or shoots. They have been known to remain during high nutrient loads and following reduced P loading (Sand-Jensen et al., 2017). Potamogetonaceae species such as *Potamogeton crispus L*. can occur in mesotrophic or eutrophic waters and mainly grow through rhizomes (Preston and Croft., 2001). As a species group, they are mainly dominant in having competitive-based traits (Murphy et al., 1990) such as having an ability to produce high biomass quickly and are canopy forming. Haloragaceae species such as *Myriophyllum spicatum L*. also have competitive characteristics (Murphy et al., 1990) to outcompete other species. In contrast, Isoetid species such as *Littorella uniflora* (L.) Asch. are short, slow-growing and can grow in low nutrient conditions, they therefore do not require competitive traits to survive but

are more stress-tolerant e.g. due to changes in water fluctuation (Robe and Griffiths, 1998). Macrophytes with stress-tolerant traits occur in lakes stressed by low nutrient availability and pH. Competition and disturbance tolerant traits are seen amongst species that are more frequent in productive lakes impacted by water level fluctuations (Murphy et al., 1990; Trémolières, 2004).

In most cases where nutrient concentrations are high in the water column, phytoplankton concentrations are also often high, which reduces water transparency and can restrict macrophyte communities to favour species with greater tolerances in low light which are also often more nutrient tolerant species. Summer mean total phosphorus (TP) concentrations of 130 – 1,000 µg $L^{-1}$ and total nitrogen (TN) concentrations of > 2 mg $L^{-1}$ see turbid conditions with low submerged macrophyte species in shallow Danish lakes (Gonzalez Sagrario et al., 2005). It is accepted that TP < 50 µg $L^{-1}$ and lower TN (< 2 mg $L^{-1}$) see a high species richness of AMs (Gonzalez Sagrario et al., 2005; Jeppesen et al., 2000). However, there are species-specific TP, soluble reactive phosphorus (SRP) and nitrate ($NO_3$-) tolerances for many species (Table 1.1 and 1.2) and so specific species may dominate over the course of different nutrient concentration changes. Furthermore, constant fluctuations in chemical, and physical parameters acts as a stressful environment (Trémolières, 2004) and so species that are more adaptable or tolerant to stress may dominate in highly changeable environments (Grime, 1979, 1977). Water managers and regulators need to consider the different nutrient tolerances and different morphological traits of different macrophyte species before lake remediation measures are applied, as there is likely to be impacts on community compositions following nutrient load reductions.

**Table 1.1.** The different growth form of common UK macrophyte species and their nutrient growth ranges. Where actual concentrations do not exist, nutrient ranges are presented.

| Species | Growth form | Nutrient uptake | Strategy | Species type | $PO_4$-P (µg $L^{-1}$) | Total P (µg $L^{-1}$) | N range ($NO_3^-$ µg $L^{-1}$) | Number of sites | Nutrient reference |
|---|---|---|---|---|---|---|---|---|---|
| *Nitella flexilis* | O | R | CSD | D | 4 – 58 | 11 – 139 | > 500 | 5 | 1, 2 |
| *Tolypella glomerata* | O | R | CSD | D | 4 | 18 | > 500 | 1 | 1, 2 |
| *Nitellopsis obtusa* | O | R | CSD | D | 2 – 43 | 14 – 78 | > 500 | 2 | 1, 2 |
| *Chara rudis* | O | R | CSD | D | 2 | 14 | > 500 | 1 | 1, 2 |
| *Chara hispida* | O | R | CSD | D | 2 – 13 | 14 – 98 | > 500 | 14 | 1, 2 |
| *Chara vulgaris* | O | R | CSD | D | 2 - 186 | 12 – 270 | > 500 | 12 | 1, 2 |
| *Chara contraria* | O | R | CSD | D | 2 - 186 | 12 – 270 | > 500 | 15 | 1, 2 |
| *Chara globularis* | O | R | CSD | D | 2 – 370 | 12 – 510 | > 500 | 15 | 1, 2 |
| *Chara aspera* | O | R | CSD | D | 2 - 186 | 12 – 270 | > 500 | 11 | 1, 2 |
| *Littorella uniflora* | R | R | SD | D | | O – M | | | 3, 4 |
| *Najas flexilis* | S | R | CSD | D | | M | | | 3, 4 |
| *Myriophyllum spicatum* | RVS | R | CD | DP | | M – E | | | 3, 4 |
| *Potamogeton pectinatus* | RS | R | CD | LD | | E | | | 3, 4 |
| *Potamogeton perfoliatus* | RS | R | CD | LD | | O – M – E | | | 3, 4 |
| *Potamogeton pusillus* | RTS | TS | CD | LD | | M | | | 3, 4 |
| *Potamogeton crispus* | RTS | RS | CD | LD | | M – E | | | 3, 4 |
| *Zannichellia palustris* | RS | R | CD | LD | | E | | | 3, 4 |
| *Callitriche hermaphroditica* | S | S | CD | D | | M – E | | | 3, 4 |
| *Utricularia vulgaris* | RST | RS | CS | DP | | E | | | 3, 4 |
| *Ranunculus aquatilis* | RS | RS | CSD | DP | | E – H | | | 3, 4 |
| *Ceratophyllum demersum* | RVR | RS | CD | DP | | M – E | | | 3, 4 |
| *Elodea canadensis* | VR | RS | CD | U | | E | | | 3, 4 |
| *Elodea nuttallii* | VR | RS | - | U | | E | | | 3, 4 |

Growth form: O - oospore, R – rhizomatous, S – seed, T – turions, V – vegetative (clonal)

Nutrient up-take: R – roots/rhizomes, S - shoots

Strategy: C - competitive tolerant, S - stress tolerance, D – disturbance tolerant. Data taken from Murphy et al., (1990)

Species type: D - desirable, U – undesirable, DP – desirable but can be problematic (dominate), LD – less desirable (based on ecological status from Poikane et al., (2018)

Mean TP concentrations – O – oligotrophic (≤ 10 µg $L^{-1}$), M – mesotrophic (10 – 35 µg $L^{-1}$), E – eutrophic (35 – 100 µg $L^{-1}$), H – hyper-eutrophic (≥ 100 µg $L^{-1}$)

Nutrient references: 1 **-** Blindow (1992); 2 – Lambert (2007); 3 – Preston and Croft (2001); 4 – Thomas et al., (1996)

**Table 1.2.** The comparison of environmental variables and the presence and absence of charophytes with average tolerance concentrations. Data taken from Lambert and Davy (2011).

| Variable | Charophytes present (n = 351) | Charophytes absent (n=115) |
|---|---|---|
| $NO_3$-N (mg $L^{-1}$) | 0.46 | 2.4 |
| $PO_4$-P (µg $L^{-1}$) | 18.4 | 29 |
| Cover of filamentous algae (%) | 4.4 | 22 |
| Submerged macrophyte species richness | 0.93 | 1.83 |
| Floating macrophyte species richness | 0.35 | 0.64 |

## 1.4. Directives and conservation drivers

In order to tackle freshwater deterioration, legally binding directives have been initiated such as the EU Water Framework Directive (WFD) (Directive 2000/60/EC) (European Commission, 2000) which requires European member states to improve the status of ecological and chemical conditions of degraded/at risk waterbodies to "good status" by 2015. In addition, the EU Bathing Water Directive (BWD) (Directive/76/160/EEC) and the revised BWD in 2006 (2006/7/EC) were set up to safeguard public health and protect the aquatic environment in both coastal and inland areas from pollution. The updated directive requires member states to monitor and assess bathing waters for different parameters. Similarly, in the US, the Clean Waters Act was introduced to prevent water pollution and protect human health and the environment (Clean Water Act, 1972). For drinking water, the World Health Organisation (WHO) has a guideline of 1 µg $L^{-1}$ total microcystin-LR (WHO, 2011) as the occurrence of toxin-producing cyanobacterial blooms may need extra purification processes to degrade cells in drinking waters. The EU Urban Waste Water Treatment Directive (UWWTD) (91/271/EEC) (Council Directive, 1991) was implemented in 1991 to protect the water environment through the requirement of primary, secondary and tertiary treatment works for waste in urban areas.

Macrophytes are used to assess conservation-based classification schemes such as the WFD, the European Habitats Directive (JNCC, 2015), for Sites of Special Scientific Interest (SSSIs) and for macrophyte species that have a UK Biodiversity Action Plan (BAP) (HMSO, 1994). The EU Biodiversity Strategy follows on from the 2006 BAP with an aim to halt habitat and biodiversity loss by 2020 (European Commission, 2011). Recent reporting suggest that 60% of surface waters across Europe are failing good ecological status and 46% of lakes within the EU are failing good status for a number of chemical parameters for the WFD (European Environment Agency, 2018). An assessment of over 14,000 waterbodies < 1ha in size across Britain found that 75% of lakes were at risk of failing WFD TP targets to meet 'good ecological status' 2015 (Carvalho et al., 2005). However, the 2013 classification results show conditions have not noticeably improved for WFD lakes over the last few decades in the UK. In England, 74% of lakes and 34% of lakes in Wales need TP reduction to meet 'good ecological status' (Spears et al., 2018).

Meeting legislative targets is a complex challenge with many lakes probably unable to reach WFD legislation without remediation measures (Zamparas and Zacharias, 2014). Suitable nutrient management approaches are critical to meet

ecological targets in the EU (Conley et al., 2009; Poikane et al., 2019) but it is likely that further deadline extensions will be needed to meet the WFD 2027 deadline without further widespread nutrient reduction measures (Carvalho et al., 2019). The understanding of the most suitable measures to reduce internal P loads in lakes that also promote macrophyte recovery is, however, less well known. It is important to address this knowledge gap to ensure desired macrophyte recovery from eutrophication to conform with the deadlines enforced by legislation such as the WFD (i.e. by 2021 or 2027).

## 1.5. The development of metrics based on macrophyte ecology

Macrophytes have significance in the functioning of lake ecosystems and have therefore been integrated into holistic assessments for evaluating the ecological condition of standing waters (Willby et al., 2009). Macrophytes have mainly been used in the UK since the 1970s to assess conservation-based targets e.g. SSSI condition through measurements such as species richness, number of rare species present and the number of *Potamogeton* species to prioritise protection (Nature Conservancy Council, 1989; Willby et al., 2009). Macrophytes have also more recently been used in developing further advanced metrics to assess the ecological condition of waterbodies against nutrient enrichment for the WFD, as macrophytes are known to be sensitive to eutrophication (Willby et al., 2009). A series of lake macrophyte metrics have been developed based on a classification analysis using 3,923 macrophyte surveys across the UK (WFD - UKTAG, 2014; Willby et al., 2009) (Table 1.3) to assess lake ecological status using the LEAFPACS lakes macrophyte classification tool (Willby et al., 2012). The classification analysis revealed that TP is the second-best predictor in describing variations in lake macrophyte compositions, following alkalinity. These metrics are the condensed taxonomic and distributional information gained from macrophyte surveys used to reflect water quality in terms of the water's biota (Dudley et al., 2013). Therefore, the development of macrophyte metrics that are sensitive to nutrients have been used to assess changes in macrophyte communities to nutrient status over time. The use of multiple metrics allows for collecting information across a range of pressures that may not be covered through using just one measurement. Additionally, using different metrics allows for the differences in morphological traits across species that may not be picked up through using one metric. Different metrics are used for assessing macrophyte changes across Europe

to evaluate ecological status for the WFD, but no standardized metrics are in use across countries (Poikane et al., 2018). Macrophyte surveys can be very labour intensive particularly for large lakes as several areas need to be surveyed and so this is perhaps where variations in methodologies have been responsible.

**Table 1. 3.** Macrophyte metrics used for water body classification**.** Data taken from Willby et al., (2009).

| Metric | Sensitivity | Use |
|---|---|---|
| Lake Macrophyte Nutrient Index (LMNI) | Nutrients | Assessing the average rank of taxa |
| Number of taxa (NTAXA) | Nutrients | Total number of hydrophyte species |
| | Hydromorphology | |
| | Acidification | |
| Number of functional groups (NFG) | Nutrients | The number of functional macrophyte groups |
| Coverage (COV) | Nutrients | Average percent cover of hydrophyte taxa present |
| | Hydromorphology | |
| Algae (ALG) | Nutrients | Relative cover of filamentous algae |
| Invasive non-native species (INV) | Nutrients | Relative cover of non-native species |
| | Hydromorphology | |

## 1.6. The degradation of macrophytes

### 1.6.1. *Macrophyte declines*

Macrophytes are increasingly threatened through anthropogenic activities (Chambers et al., 2008) with eutrophication being a major contributor to wide scale macrophyte loss (Hilt et al., 2018). Increased industrial pollution, population density, climate change, aquaculture, changes in land-use and the intensification of agriculture and associated fertilizer use are some of the primary causes of macrophyte declines (Phillips et al., 2016; Zhang et al., 2017). Consequently, substantial changes in species composition, species richness and cover has been reported globally (Egertson et al., 2004; Hilt et al., 2013; Kennison et al., 1998; Körner and Nicklisch, 2002; Lauridsen et al., 2015; Perrow et al., 1994; Phillips et al., 2016, 1978; Sand-Jensen et al., 2000; Zhang et al., 2017).

Macrophytes are declining on a global scale and at an accelerated rate, especially within the last 40+ years (Zhang et al., 2017) but declines have been documented for more than 100 years (Sand-Jensen et al., 2000) and there have been substantial changes in composition and abundance of submerged macrophytes in European lakes (Ayres et al., 2008). For example, Egertson et al., (2004) found that

species richness of macrophytes declined from 30 in 1951 to 12 in 2004, shifting from a submerged community (99%) to emergent-dominated community (84%) in Clear Lake, USA. Phosphorus concentrations went from < 20 µg $L^{-1}$ in 1934 up to 190 µg $L^{-1}$ in 2000. *Potamogeton praelongus* was the first species to disappear along with other *Potamogeton* spp. *Potamogeton pectinatus* was one of the few species that remained throughout the century of water quality decline and is attributed to its high nutrient tolerances. Perrow et al., (1994) evidenced that changes in TP concentrations influenced the submerged plant biomass in Alderfen Broad from 1979 – 1991. High biomass (~ 60 g dry weight $m^{-2}$) was recorded following reduced TP concentrations of ~ < 50 µg $L^{-1}$ but as TP concentrations increased in subsequent years macrophyte biomass disappeared after reaching TP concentrations of ~500 µg $L^{-1}$. The community was dominated by *Ceratophyllum demersum* over this period and resulted in fluctuations in its biomass. Palaeolimnology has shown that the rate of UK and EU macrophyte decline has been gradual over the last 100 years with reduced abundance and diversity as opposed to sudden macrophyte losses (Phillips et al., 2016; Sand-Jensen et al., 2000; Sayer et al., 2010a). In eutrophic lakes, declines are often seen first in small, slow-growing, rosette-leaved species and charophytes (Blindow, 1992; Phillips et al., 2016; Sand-Jensen et al., 2000) which are replaced with fast-growing, canopy-forming species such as *Myriophyllum*, *Ceratophyllum* and *Potamogeton* species as a reaction to reduced light availability (Blindow, 1992; Phillips et al., 2016). Largely, communities are changing from systems dominated by submerged macrophyte species towards domination by floating and emergent macrophyte species (Zhang et al., 2017) with very few rare submerged species remaining (Egertson et al., 2004). The rate of extinction or decline of macrophytes is not well monitored or reported. However, since the 1800s 14% of stoneworts have been lost from England and for vascular plants (both aquatic and terrestrial), 20 native species macrophyteare now lost which accounts for a < 2% decline (Natural England, 2010).

### 1.6.2. *EU and UK endangered macrophytes*

There is currently only one submerged macrophyte that is endangered in both the UK and EU (Table 1.4); *Najas flexilis* (Wild.) Rostk. & Schmidt, and as such is protected under the Habitats Directive (Annex II & IV) (92/43/EEC) (Council of the European Union, 1992), under the 1981 Wildlife & Countryside Act (Schedule 8) and has a

Biodiversity Action Plan (BAP) (HMSO, 1994) for its preservation and of its habitat. *Najas flexilis* is also in global decline (Figure 1.2.) and has been declining gradually over the last few decades in the UK becoming extinct from its only remaining English site, Esthwaite Water in 1982, despite extensive subsequent searches for individuals. *Najas flexilis* populations are now confined to Scotland (Figure 1.3.) and Ireland with a western distribution. In Scotland there are only seven remaining mainland sites where it has been recorded in the last ten years and European extent is declining by ≤ 1% every year across its 44 recorded sites (European Community, 2019) (Figure 1.3). There have been a few instances where *Najas flexilis* has been re-recorded at sites from which it has been lost in Scotland, although this has generally been attributed to sampling artefacts relating to monitoring efforts (European Community, 2019). Restrictions in range and local extinctions have been attributed to insufficient habitat requirements which are linked to eutrophication and non-native species. The release of P from lake-bed sediments (internal loading) and the presence of *Elodea* species are more specifically linked to an absence of suitable habitats for this species (European Community, 2019).

**Table 1.4.** European protected aquatic vascular plant species under the Habitats Directive (Annex II & IV) (92/43/EEC) (Council of the European Union, 1992).

| Species | Growing location | EU status | UK status | Occurs in UK (number of sites) | UK trend in status | Rate of decline (per annum) |
|---|---|---|---|---|---|---|
| *Najas flexilis* | Submerged | V | R | Scotland (44) | Deteriorating | ≤1% |
| *Apium repens* | Flooded meadow | NT | CE | England (4) | Deteriorating | >1% |
| *Luronium natans* | Floating | LCD | SE | England (45), Wales (123) | Deteriorating | Stable (England) 0.49% (Wales) |

V – vulnerable, NT – near threatened, LCD – least concern declining, R – rare, CE – critically endangered, S - scarce

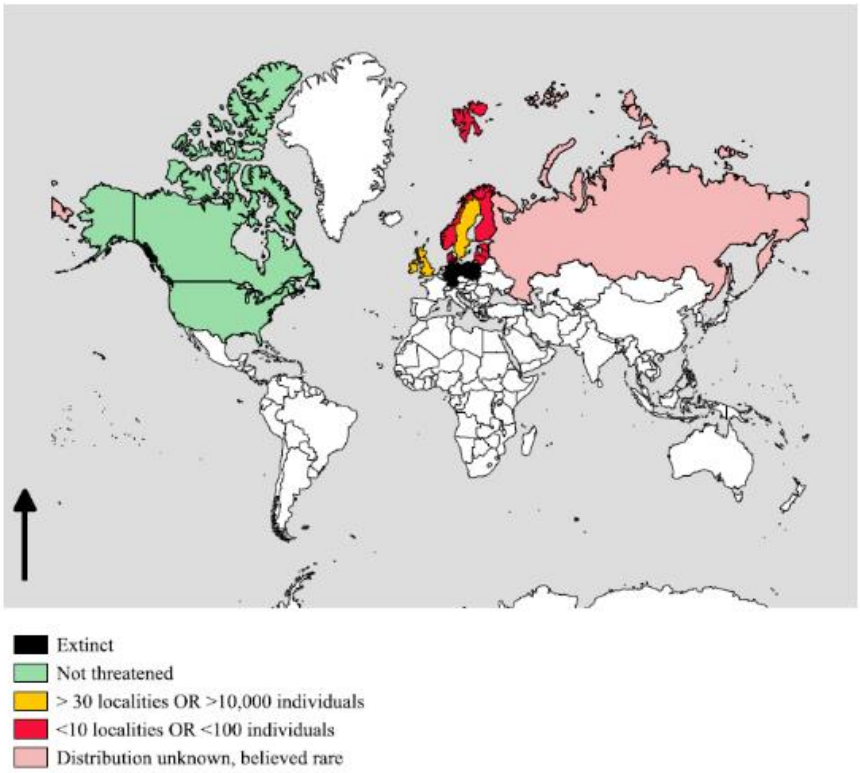



**Figure 1.2.** Global distribution of *Najas flexilis* and status (European Commission, 2009).

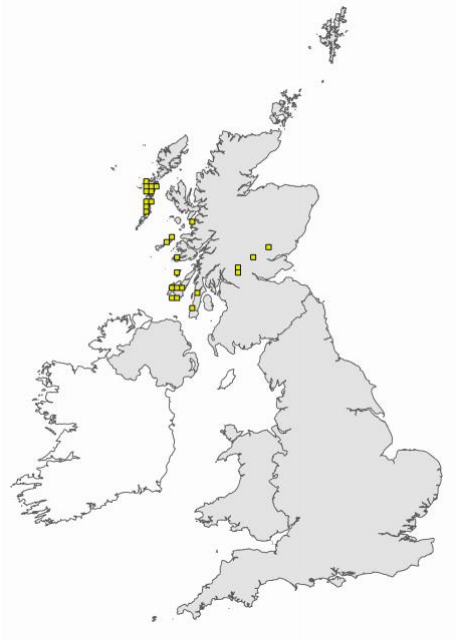


**Figure 1.3.** *Najas flexilis* distribution in the United Kingdom (European Community, 2019).

There are several vascular submerged and charophyte species that are protected under the 1981 Wildlife and Countryside Act (Schedule 8) and have associated BAPs due to their UK conservation status (Table 1.5). Thirteen out of twenty-one of these listed aquatic plants are submerged and have different conservation statuses with some more at risk of UK extinction than others. Despite legislative protection for *Najas flexilis* and other macrophyte species protected under EU and UK legislation, the ability to explain macrophyte recovery is not well understood (Bakker et al., 2013; Lauridsen et al., 2003a; Phillips et al., 2016; Søndergaard et al., 2007). This is mainly due to the fact that there is little knowledge on how to conserve species that are at risk of extinction which is a major issue for the conservation of macrophyte species. The loss of macrophytes can also result in the loss of other endangered species through habitat loss (Bakker et al., 2013; Carpenter and Lodge, 1986; Zhang et al., 2017) and if the current rate of macrophyte decline is sustained or accelerates further, it will impact and threaten the wider diversity and water quality of freshwater systems, which consequently, places a great ecological significance on this species group.

**Table 1.5.** United Kingdom aquatic vascular and non-vascular (stoneworts) plant species that are protected under the 1981 Wildlife & Countryside Act – Schedule 8 and have Biodiversity Action Plans (HMSO, 1994).

| Species | Growing location | UK conservation status |
|---|---|---|
| *Ranunculus ophioglossifolius* | Semi-permanent ponds and marsh | E |
| *Viola persicifolia* | Wet fens and formerly river valleys | E |
| *Lythrum hyssopifolia* | Wetlands | E |
| *Scenecio paludosus* | Fen and ditches | CE |
| *Sium latifolium* | Marginal | S |
| *Pilularia globulifera* | Marginal | V |
| *Damasonium alisma* | Temporary ponds | E |
| *Tolypella intricate* | Submerged | E |
| *Oenanthe fistulosa* | Emergent | V |
| *Hottonia palustris* | Submerged/emergent | V |
| *Alisma gramineum* | Submerged | CE |
| *Chara canescens* | Submerged | E |
| *Chara baltica* | Submerged | V |
| *Chara connivens* | Submerged | E |
| *Chara intermedia* | Submerged | E |
| *Nitella gracilis* | Submerged | V |
| *Nitella tenuissima* | Submerged | E |
| *Nitellopsis obtusa* | Submerged | V |
| *Lamprothamnium papulosum* | Submerged/coastal | NT |
| *Najas marina* | Submerged | V |
| *Crassula aquatica* | Submerged/emergent | V |

E – endangered, CE – critically endangered, S – scarce, V – vulnerable, NT – near threatened

## 1.7. Evidence of macrophyte community responses following nutrient load changes

The community composition, abundance, and extent of macrophytes in lakes varies widely depending on a number of abiotic and biotic conditions. These include altitude, water depth, alkalinity, water clarity, substrate, fish communities, bird grazing, nutrients and bathymetry (Carpenter and Lodge, 1986; Capers et al., 2009; Bornette and Puijalon, 2011). Nutrient status and light availability have been described as arguably the most important factors that govern macrophyte diversity (Jeppesen et al., 2000). Water clarity is often dependant on dissolved organic matter, suspended solids and phytoplankton.

Macrophyte re-establishment following restoration is essential to allow establishment of other associated species (Hilt et al., 2006). However, little is known about macrophyte community responses following lake remediation (Coops and Doef, 1996; Jeppesen et al., 2005). The limited peer-reviewed literature covering this topic largely focuses on multi-lake studies investigating macrophyte responses (Hilt et al., 2018; Jeppesen et al., 2005; Körner and Nicklisch, 2002; Lauridsen et al., 2003a; Søndergaard et al., 2008; Spears et al., 2016) as a result of a paucity of long-term monitoring data spanning both degradation and recovery periods in lakes.

Eutrophication management studies have found macrophyte communities to be more diverse following reduced nutrient inputs (Dudley et al., 2012; Hilt et al., 2013, 2010; Kennison et al., 1998; Murphy et al., 2018). Hilt et al., (2013) examined long-term (100 years) macrophyte data to assess community changes in Lake Müggelsee. Species declined gradually from 24 to 5 over 70 years, which then followed 20 years of turbid conditions where the lake was dominated by phytoplankton. When external loads were reduced by 50%, high P concentrations remained due to internal loading and communities remained dominated by *Potamogeton pectinatus*, *Potamogeton perfoliatus* and *Nurphur lutea*. After three years, although *Najas marina*, *Zannichellia palustris* and *Potamogeton friesii* were reported as new species, the community remained dominated by *Potamogeton pectinatus*. Murphy et al., (2018) found higher macrophyte diversity in Lake Constance following external P and N load reduction. During eutrophication the lake was dominated by filamentous algae and tall Elodied species. Following external load reductions, in-lake TP concentrations reduced from 90 µg $L^{-1}$ in 1975 down to < 7 µg $L^{-1}$ in 2010 – 2015. Charophyte abundance rose from two species in 1978 to eight species in 1993, three years after external load reductions, which the rose to ten in 2016. By 2016 charophytes became the most

dominant group, occupying 62% of the overall relative abundance. This was attributed to the declining TP levels in the lake. Since the 1980s, using long-term data (100 years), community shifts were seen following external nutrient reductions in Loch Leven, UK. The relative number of taxa, taxa richness, evenness and maximum growing depth all showed an improvement since 1972. This indicated the lake was returning to its community that was present in 1907 with relative abundance dominated by charophyte spp. (Dudley et al., 2012). However, paleolimnological work has highlighted changes in macrophyte communities during eutrophication which revealed that Loch Leven remains a long way from its reference state (pre-1900), dominated by oligotrophic soft water macrophytes (Salgado et al., 2010).

Despite evidences of fluctuating community composition following nutrient load reductions, there are many examples in the literature where no macrophyte recovery has been reported (Hilt et al., 2018; Jeppesen et al., 2005). Macrophytes are amongst the most under-represented freshwater groups in broad-scale studies of freshwater biodiversity despite their importance in maintaining structure and function in lakes (Alahuhta et al., 2017; Chambers et al., 2008). The ability to assess long-term ecological change using macrophyte community composition is also hampered through the availability of site-specific long-term monitoring programmes and as such, focus has been on multi-lake macrophyte responses to nutrient enrichment/reduction, utilising spatial data sets (Jeppesen et al., 2005, 2000; Körner and Nicklisch, 2002) or long-term data from the few individual lakes with monitoring programmes (Dudley et al., 2012; Hilt et al., 2010; May and Carvalho, 2010; Phillips et al., 2015). This lack of data constrains the ability to assess long-term declines and community changes as a result of nutrient pollution and other stressors across lakes (Penning et al., 2008; Poikane et al., 2018; Zhang et al., 2017). In addition, macrophyte community responses to nutrient load reduction are commonly not recorded (Coops and Doef, 1996; Jeppesen et al., 2005). Of what studies have monitored macrophyte rehabilitation, recovery time is variable following restoration techniques (Hilt et al., 2018; Jeppesen et al., 2005; Lauridsen et al., 2003a; Søndergaard et al., 2007) (Appendix 1, Table 1). However, full ecological recovery is infrequently reported due to the absence of desirable species (Bakker et al., 2013; Verdonschot et al., 2011).

## 1.8. Nutrient loading and the factors confounding macrophyte recovery

### 1.8.1. *Internal loading and phosphorus release mechanisms*

The accumulation of surplus nutrients from decades of nutrient pollution can be retained within lake sediments, as it is well known that freshwater lakes can act as sediment sinks (Sharpley et al., 2013). Phosphorus can be both deposited and cycled in freshwater lakes and lake sediments play an important role in regulating in-lake chemical and biological processes. The retention and release of P between bed sediments and the overlying water column (Søndergaard et al., 2003) can hamper or delay the recovery of lakes for years to decades following catchment management (Jeppesen et al., 2005; Søndergaard et al., 2013, 2007). The delay in recovery can depend on multiple factors which can make internal loading severity lake specific (Jeppesen et al., 1999). Loads can be increased through high sediment re-suspension rates, high rates of bioturbation, high temperatures, high pH, reducing redox conditions, low iron (Fe):P ratios and through increased microbial activity (Table 1.6.).

**Table 1.6.** Factors influencing phosphorus release from lake-bed sediments.

| **Influencing factors** | **Author(s)** |
|---|---|
| Sediment resuspension | Ekholm et al., (1997); Jones & Welch, (1990); Søndergaard et al., (2001; 2003); Sereda et al., (2008); Tarvainen et al., (2005) |
| Bioturbation | Fukuhara & Sakamoto (1987); Lewandowski and Hupfer, (2005); Lewandowski et al., (2007); McMahon et., (2015); Chaffin and Kane (2010); Tarvainen et al., (2005) |
| Submerged macrophytes | Horpilla & Nurminen, (2003); Ibelings et al., (2007) |
| Temperature | Jensen & Anderson, (1992); Søndergaard et al, (1999b); Spears et al., (2012) |
| Redox | Ekholm et al., (1997); Hupfer & Lewandowski, (2008); Nürnberg (1984) |
| pH | Jensen & Anderson, (1992) |
| Iron:P ratios | Petticrew & Arocena, (2001); Søndergaard et al., (2003) |
| Microbial processes | Hupfer & Lewandowski, (2008); Søndergaard et al., (2003) |

The foraging behaviour of benthivorous fish species can significantly increase sediment re-suspension and the release of P from sediments (Folke et al., 2004; Jeppesen et al., 1997; Scheffer, 2001). Phosphorus release rates appear to be driven by species behaviour and can range from 0.93 for multiple species to 22.5 (µg $g^{-1}$ $h^{-1}$) for individual species such as *Rutilis rutilis* L. (Sereda et al., 2008b; Tarvainen et al., 2002). When *Dorosoma cepedianum* L.(Gizzard shad) was modelled as detritivores, they were accountable for 40% of the total P (SRP g $ha^{-1}$ $day^{-1}$) released by the fish community (of which they made up 23% of the total fish biomass) from sixteen published studies (Sereda et al., 2008a). Additionally, if fish biomass exists between 150 and 250 kg $ha^{-1}$ then macrophyte communities can no longer be sustained as a result of increased turbidity in the water column (Smith et al., 1999).

The pumping activity of tube dwelling macroinvertebrate species (e.g. chironomids) can increase P release from organic-P pools (Lewandowski et al., 2007). However, mesocosm experiments have shown that benthic organisms (*Hexagenia* nymph species) can enhance P flux of total reactive P and SRP across the sediment-water interface by 1.4 mg/$m^2$/day and by 1.02 mg/$m^2$/day, respectively (Chaffin and Kane, 2010). Another mesocosm experiment has shown that chironomids (n = 8 individuals) can reduce SRP concentrations of pore water by up to 1.3 mg P $L^{-1}$ when compared to mesocosms without chironomids. This was due to increased water circulation at the sediment surface which increases the immobilization of P onto Fe-complexes, causing a larger oxidised surface area (Lewandowski et al., 2007).

Higher temperatures explained >70% of the gross TP release rates from three Danish lakes (15 - 100 mg P $m^{-2}$ $d^{-1}$ (average summer values for four lakes) (Jensen and Andersen, 1992).

Higher pH values (pH 9.5 and 9.7) from two core experiments significantly increased SRP release from lake sediments (Jensen and Andersen, 1992). Increased pH also lowers the efficiency of Fe(III) hydroxides in oxic surface sediments to absorb P (Hupfer and Lewandowski, 2008).

The capacity of sediments to bind P depends on their chemical composition. The presence of Fe, aluminium (Al), manganese (Mn) and calcium (Ca) can control the P-binding or P-cycling sediment-water interactions (Søndergaard et al., 1996b). Anoxic conditions can release P into the water column through the reduction of Fe-P and other hydroxides (Hupfer et al., 2008; Nürnberg, 1984).

The mineralisation of organic matter mediated through microbe activity also increases sediment P release through the consumption of oxygen, reducing hydroxides and making surface sediments anoxic (Hupfer and Lewandowski, 2008).

Lakes that suffer from internal P loading often follow seasonal patterns of higher water column P concentrations in the summer months, with P retention often negative during this time (Søndergaard et al., 2013, 2003). In addition, high hydraulic retention time, high nutrient inlet concentrations and lake depth can all have an increase in the residence time of P in lake systems and the sensitivity of a given lake to internal loading (Søndergaard et al., 2003, 2001). The duration of recovery will depend on the magnitude and severity of historic external nutrient load inputs (Jeppesen et al., 1999).

All of these release mechanisms can also interact and can influence the rate of sediment-P release. Sediment TP release rates (RR) for North American and European oligotrophic lakes have an average RR of 0.4 mg/m$^2$/d (n=3), for mesotrophic lakes an average of 4.1 mg/m$^2$/d (n=11), for eutrophic lakes an average of 11.6 mg/m$^2$/d (n=31) and for hypereutrophic lakes an average of 20.4 mg/m$^2$/d (Nürnberg, unpublished studies).

### 1.8.2. *Impact of climate change on internal load*

Climate change is expected to exacerbate internal loading (Bormans et al., 2016) through hydrological changes including, changes in the volume, timing and frequency of precipitation leading to an increase in the frequency and severity of floods (IPCC, 2014, 2007). These changes will modify the quality of standing waters with consequent alterations in biodiversity (Strayer and Dudgeon, 2013). Increased flooding events will change the timing and forms of nutrient delivery to lakes resulting in pulses and potentially, an increase in overall catchment and internal P-loads (Mooij et al., 2005). In addition, predicted global temperatures are set to increase by 1.4 – 5.8°C until the year 2100 (Mooij et al., 2005). Higher temperatures will reduce water transparency through higher summer chlorophyll a (Chl-*a*) concentrations and favour the dominance of cyanobacteria during summer which will lead to reduced zooplankton abundance (Mooij et al 2007). Higher temperatures will also accelerate bacterial mineralization which will enhance sediment P release (Søndergaard et al., 2003). Turbid conditions are likely to be more severe which will impact macrophyte

species-richness and change community compositions to more low-light tolerant species.

### 1.8.3. *Evidence of macrophyte recovery confounded by internal loading following external load reduction*

The most common and logical first step to approach eutrophication management is to reduce external nutrient loading to lakes (Verdonschot et al., 2011). There is ongoing debate on whether reduction of N, P or both should be prioritised as a general approach for eutrophication management (Conley et al., 2009; Paerl et al., 2016; Schindler et al., 2016). In most lakes, P is considered the limiting nutrient and is found at lower concentrations in relation to N requirements for phytoplankton growth (Carpenter, 2008) and so, typically, efforts have focussed on reducing P loads from catchment sources to lakes. As a result, in recent decades external nutrient loads to some freshwater lakes have declined (Jeppesen et al., 2007b; Sas, 1989; Schindler et al., 2016; Spears et al., 2012) due to efforts in catchment management (Schindler, 2006; Smith and Schindler, 2009). The modernization of wastewater treatments driven through policies such as the UWTT has been effective at reducing nutrient loads in some instances but for many, sewage treatment works are still discharging P-rich effluent but diffuse sources have been harder to control (Bennion et al., 2015; Carpenter et al., 1998; Schoumans et al., 2014). Agricultural-based restoration schemes such as the promotion of good soil management practices and field buffer margins have also contributed to declines in diffuse pollution (Sharpley et al., 2000). Nutrient inputs of N and P to Danish lakes has declined by 44% for N and 30% for P from atmospheric, land and urban sources from 1992 – 2011 (Bjerring et al., 2014). However, many watershed reduction programmes in the US are not achieving reduction in P concentrations because of internal loading (Sharpley et al., 2013).

The water quality of some lakes has improved quickly following external nutrient P load reduction (Sas, 1989) but in many cases, water quality improvements have been slow (Carvalho and Kirika, 2003; Jeppesen et al., 1991; Mardsen, 1989; Søndergaard et al., 2007, 2003) ranging from years to decades following reductions but typically being 10 – 15 years in response to reduced TP loading and < 5 years to reduced N loading (Jeppesen et al., 2005; Welch and Cooke, 1995). This delay can be caused by confounding factors (Verdonschot et al., 2013), for example, internal loading (Carvalho and Kirika, 2003; Jeppesen et al., 1991; Mardsen, 1989; Sharpley et al., 2013; Søndergaard et al., 2007).

There is evidence that internal loading confounds macrophyte recovery through continued high P concentrations following external nutrient-P load reduction (Jeppesen et al., 2005; Lauridsen et al., 2003a; Sand-Jensen et al., 2017) (Appendix 1, Table 1). For example, Jeppesen et al., (2005) reported recovery of macrophyte species was lake-specific 16 years after external nutrient load reductions across 35 Danish lakes. Over the recovery period some lakes showed an increase in species abundance, coverage and colonisation depth, whilst others had reduced species richness. Some exhibited no change in species composition. The TP concentrations ranged from 27 – 3500 µg $L^{-1}$ at maximum loading in shallow lakes (n=22) and 8 – 350 µg $L^{-1}$ in deep lakes (n=13) which reduced to 37 – 513 µg $L^{-1}$ in shallow lakes and to 4 – 132 µg $L^{-1}$ in deep lakes at the end of the study. In 77% of shallow lakes and 82% of deep lakes secchi depth (m) increased with nutrient reduction.

Lauridsen et al., (2003a) also attributed internal loading as a reason for a lack of macrophyte recovery eight years following external P reduction in 5 Danish lakes and in 4 lakes in which P load was reduced and biomanipulation of the fish community was conducted. TP concentrations declined from 66 µg $L^{-1}$ to 42 µg $L^{-1}$ (n = 5) following P load reduction, alone, and from 150 µg $L^{-1}$ down to 90 µg $L^{-1}$ (n=4) for P load reduction and biomanipulated lakes. Secchi depth increased from 1.9 to 2.5m and from 0.6 to 1.45 for P-reduced and P-reduction through biomanipulation, respectively.

Hilt et al., (2018) assessed macrophyte recovery in lakes and whether sites had reached full and sustained recovery following external load reduction. Mean summer TP concentrations of 59 µg $L^{-1}$ were reached post- reduction. However, only three from ten sites had achieved clear stable conditions with macrophyte establishment 14 – 26 years following initial interventions. The remaining six waterbodies either had not yet reached a clear water stable period with macrophytes present or the recovery trajectory was unknown. Recovery times since first interventions ranged from 21 – 29 years across these six sites. The most commonly colonising species following external load reduction studies were *Potamogeton spp*., particularly *Potamogeton pectinatus* (16 lakes), *Potamogeton crispus* (9 lakes) and *Potamogeton perfoliatus* (8 lakes) (Hilt et al., 2018).

The continued supply of sediment P from internal loading in lakes is a barrier to achieving chemical and macrophyte recovery following reduction in catchment P load. The TP ranges achieved following catchment load reductions reported in the studies above may not be expected to result in macrophyte responses. Jeppesen et al., (2000) (Figure 1.4), suggest that TP and Chl-*a* concentrations should be < 50 µg

$L^{-1}$ to see higher submerged macrophyte richness. Jeppesen et al., (2000) reported that at TP concentrations of 0 – 50 µg $L^{-1}$ there was a mean of 11.7 species which declined to only 0.5 species at TP concentrations of > 400 µg $L^{-1}$. Lower Chl-*a* concentrations (< 50 µg $L^{-1}$) also increased secchi depths and the maximum colonisation depth of submerged macrophytes with a mean of 3 m across 71 lakes. In light of this, in-lake P reduction measures are assessed in section 1.9 to compare if measures designed to control internal loading provide macrophyte responses more quickly than external P-load reduction studies in line with TP and Chl-*a* concentrations, where reported in the literature.

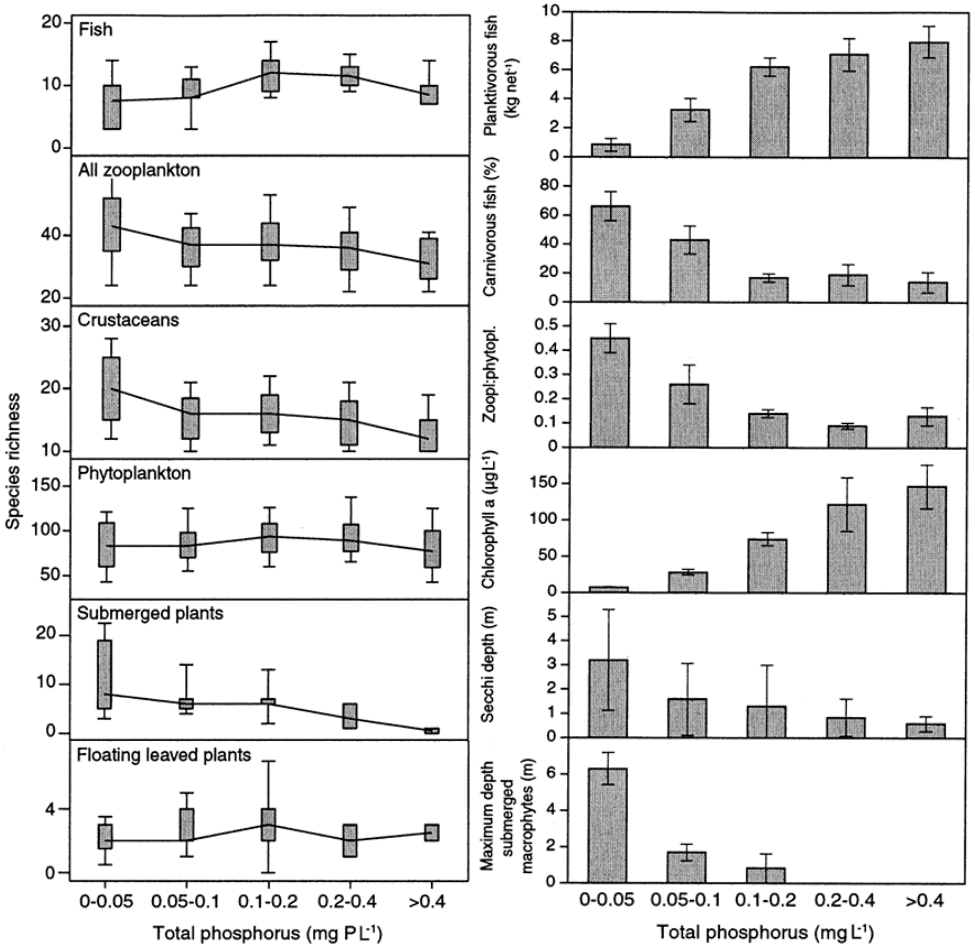



**Figure 1.4.** The expected increase in submerged macrophytes species and maximum macrophyte growing depth in response to secchi depth and total phosphorus concentrations. Data taken from Jeppesen et al., (2000).

## 1.9. Macrophyte responses to the recovery of nutrients from in-lake restoration measures

Additional internal corrective measures, such as the use of physical, chemical and geo-engineering techniques have been developed to help 'speed up' chemical recovery by controlling internal loading and increasing depth (e.g. dredging). The initial choice of internal remediation measures requires a good knowledge of the waterbody (Hickey and Gibbs, 2009; Huser et al., 2016a; Lürling et al., 2016; Spears et al., 2016) and significantly reduced external nutrient loads (Jeppesen et al., 1990; Søndergaard et al., 2003, 2000) so in-lake TP and Chl-*a* concentrations are < 50 µg $L^{-1}$ (Jeppesen et al., 2000) to have best possible success of increased desirable macrophyte species (Jeppesen et al., 1990; Søndergaard et al., 2003, 2000). The most common internal loading control measures are reviewed in-light in the context of macrophyte recovery to see if in-lake measures are evidencing similar macrophyte timescales, responses and species as external P-load reduced lakes.

### 1.9.1. *Biomanipulation*

Biomanipulated lakes in their majority are manipulated using fish populations, to revert a lake back to clearer water conditions. This is often performed using two main methods which can be applied singularly or in unison; removing zooplanktivorous and benthivorous fish (bottom-up control and/or stocking lakes with piscovourous fish (top down control. The removal of zooplanktivores allows zooplankton to thrive, promoting top down control on phytoplankton. The addition of piscivores aims to slow down phytoplankton production. The feedback of eliminating benthivores can be seen relatively quickly as re-suspension of bed sediments is decreased, which also limits the amount of P released into the overlying water column. Despite biomanipulation methods being rewarding in the early 1950s (Scheffer and Jeppesen, 1998) there is some contrast in the recent literature as to whether this technique provides more restoration successes or failures, in practice. Successes and failures have been well documented over the last decade (Gulati et al., 2008; Meijer et al., 1999; Søndergaard et al., 2008, 2007) with Dutch lakes in particular seeing more documented failures than successes (Gulati and Van Donk, 2002). Biomanipulation successes in the short term can be effective by reducing Chl-*a* concentrations and increasing transparency within the first few years (Søndergaard et al., 2007). The long-lasting responses of biomanipulation in many cases are uncertain (Jeppesen et al., 2007a) but generally the benefit is short lived, with many lakes returning to turbid conditions within 10 years

or less (Søndergaard et al., 2007). Insufficient removal of zooplanktivores and/or benthivores is often accountable as the main cause for unsuccessful studies (Søndergaard et al., 2007; van Donk et al., 1994). A fish reduction of >75% is often required to have a successful impact (Meijer et al., 1999). It can be difficult to get the right balance particularly in large lakes where the chance of failure is increased (Jeppesen et al., 2007a; Perrow et al., 1997). Restoration failures could also be attributed to the return of zooplanktivoures and/or P release from sediments and sediment re-suspension (Søndergaard et al., 2007). Jeppesen et al., (2012) showed that biomanipulation is only effective when nutrient concentrations are low. It has been advised that bio-manipulation methods be delayed for some years until sediment P loads have been reduced (Søndergaard et al., 2003).

The biomanipulation of 70 lakes revealed high variability amongst restored lakes as mean TP concentrations ranged from 50 µg $L^{-1}$ – 140 µg $L^{-1}$ and Chl-*a* concentrations ranged from 21 – 300 µg $L^{-1}$ following treatment (Appendix 1, Table 1). Only 14.3% of these 70 lakes saw TP reductions (50% decline in summer concentrations) and 21.4% saw Chl-*a* concentration reductions (50% decline in summer concentrations) post- intervention. We would expect site-specific macrophyte recovery in this case according to Jeppesen et al., (2000) which was observed across studies (Appendix 1, Table 1). Lakes with higher TP and Chl-*a* concentrations had lower macrophyte species richness compared to those with lower concentrations (Appendix 1, Table 1). Macrophyte recovery can be rapid (Meijer et al., 1999), followed by large-annual fluctuations with responses been seen typically between 2 - 4 years post remediation (Lauridsen et al., 2003a; Søndergaard et al., 2007). The need to repeatedly biomanipulate due to insufficient fish removal or from continued sediment P release could confound macrophyte recovery longer-term as macrophyte communities need to establish repeatedly over again due to unfavourable P concentrations and low light availability. Evidence of macrophyte recovery is lake-specific with charophytes, *Potamogeton crispus*, *Elodea canadensis* and *Ceratophyllum demersum* as new species gains < 1 – 12 years reported across 89 lakes (Ibelings et al., 2007; Lauridsen et al., 2003a; Søndergaard et al., 2007) (Appendix 1, Table 1).

### 1.9.2. *Sediment removal*

Sediment dredging reduces the P concentrations in the water column by removing top sediment layers (Søndergaard et al., 2001). Sediment dredging has been used extensively in the Norfolk Broads, UK for both sediment removal and for channel-deepening for navigation purposes. The exposure of a less nutrient-rich sediment layer is thought, over time to reverse the trophic structure of lakes (Pandey and Yaduvanshi, 2005), as it removes the source of internal loading. However, a newly exposed sediment layer with high P concentrations can be exposed after dredging which prevents recovery through the re-initiation of P cycling (Annadotter et al., 1999; Does et al., 1992). Despite this, successes have been documented in reducing P concentrations but positive effects are often short lived, lasting on average < 5 years but it is highly site specific (Phillips et al., 2015). This may be due to the dredging approach e.g. the amount of sediment removed from depth, the lake type or a combination of both. The suitability of appropriate sediment disposal sites can also prevent its use (Born, 1979; Cooke et al., 2005), particularly if sediments are contaminated (Bortone et al., 2004; Cooke et al., 2005).

TP concentrations three years post- dredging ranged from 25 – 75 µg $L^{-1}$ (mean = 55.8 µg $L^{-}1$) and Chl-*a* concentrations ranged from 18 – 40 µg $L^{-1}$ (mean = 24 µg $L^{-1}$) in six and seven lakes, respectively in the Norfolk Broads (Phillips et al., 2015). At these reported concentrations it is not expected that macrophyte species would respond positively (Jeppesen et al., 2000; Phillips et al., 2015). Additionally, in some lakes increases in Chl-*a* were seen following dredging (n = 3) by 10 – 15 µg $L^{-1}$. Dredging is not considered a long-term restoration measure as TP concentrations are rarely reported to remain < 50 µg $L^{-1}$ for consecutive years after dredging, with TP concentrations generally increasing within five years of removal (Phillips et al., 2015). It is likely that macrophyte diversity will remain low or be dominated by more species that can remain *in situ* across a wider TP range.

Charophytes are known to establish rapidly following sediment removal (Wade and Edwards, 1980) as dredging can expose propagule rich layers under buried sediment (Phillips et al., 2015; Sayer et al., 2012). Successful establishment of charophytes and in some cases other species that have a high seed production (*Zannichellia paulustris*, fine-leaved pondweeds and *Najas marina*) have colonised in the Norfolk Broads following dredging (Phillips et al., 2015) (Appendix 1, Table 1). However, the sediment depth removal is important as removing sediment from too deep in the sediment profile can impact colonisation potential with regard to light

compensation points for individual species (Phillips et al., 2015). Dredging can also negatively impact freshwater biota through disturbance and physical damage, e.g. the process can significantly reduce macrophyte propagule banks (Bakker et al., 2013) and impede ecological recovery (Hilt et al., 2006; Zhang et al., 2010). Paleolimnology can be used to assess where propagule-rich layers lie to ensure desirable communities are not removed and to ensure that viable seed banks are exposed (Sayer et al., 2012). The reported timescale of macrophyte responses from lakes in the Norfolk Broads can be quick but filamentous algae is usually first to respond to the effects of dredging (Appendix 1, Table 1), which has occurred in eleven out of fifteen dredged waterbodies (Phillips et al., 2015). Charophytes are largely the first desirable species to respond following dredging but they are particularly sensitive to disturbance and can germinate in response to this. Therefore, the germination of oospores could be due to disturbance , rather than the impact of dredging itself (Phillips et al., 2015).

### 1.9.3. *Chemical and geo-engineered P-sorbing/capping materials*

Chemical P-sorbing/capping methods are the second most widely used group of in-lake restoration methods for the control of internal loading in lakes (Verdonschot et al., 2011). Geo-engineered P capping agents are designed to manipulate the biogeochemical processes in lakes which are known to improve ecological structure and function (Mackay et al., 2014). Lake managers' focus and target has been to rapidly reduce water column P concentrations and control P release from bed sediments; designed to force a phytoplankton dominated waterbody towards a clear-water system. There are numerous products available to control internal loading in lakes which includes engineered materials, industrial-by-products and salts (e.g. Douglas et al., 2016; Gibbs and Özkundakci, 2011; Hickey and Gibbs, 2009; Lürling et al., 2016; Mucci, 2019; Spears et al., 2013b). P capping agents vary in chemical make-up but the most widely used materials are Aluminium (Al), Iron (Fe), Calcium (Ca) and engineered materials such as Phoslock® (a lanthanum (La)-modified bentonite clay (LMB)). Materials can be applied through direct injection into the hypolimnion in deeper lakes but they are most commonly applied to surface waters as a slurry where the materials bind dissolved P as they travel through the water column and 'cap' the bed sediments, where the product continues to bind P released from sediments.

### 1.9.4. *Aluminium*

Aluminium (Al) in the form of aluminium sulfate ($Al_2(SO_4)_3$) and aluminium chloride ($AlCl_3$) is one of the most widely used chemical P inactivation and flocculation products applied to lakes with most applications applied in the US (Huser et al., 2016a; Welch and Cooke, 1999). Al has been a popular choice to control internal loading largely due to its ability to bind P under anoxic conditions (Cooke et al., 1993; Hickey and Gibbs, 2009). Its application has resulted in reduced TP and Chl-*a* concentrations (Cooke et al., 1993, 2005; Huser et al., 2016a; Reitzel et al., 2003) with the longevity of treatments lasting between 0-45 years (Huser et al., 2016a). Effects of applications to deeper stratified lakes can last a mean of 21 years with polymictic lakes lasting an average of 5.7 years (Huser et al., 2016a). Successes outweigh the failures, with 56 out of 75 being successful and 19 unsuccessful (Jensen et al., 2015). Failures using Al is often reported to be caused by poor understanding of P-loading to waterbodies and inaccurate dose calculations (Huser et al., 2016b; Lewandowski et al., 2003) or from benthic fish disturbance (Huser et al., 2016a). The maximum dose is often calculated based on the pH of a lake, but applications have been successful when dose is based on sediment P concentrations (Reitzel et al., 2005). A waterbody's pH should not fall below pH 6.5 to prevent the formation of soluble $Al(OH)^3$ ions (Hickey and Gibbs, 2009) which can be toxic to fish communities (Reitzel et al., 2005).

As Al can decrease the pH of receiving waters, pH buffers such as calcium hydroxide have been used to reduce these effects. The ecological responses to Al is not well understood (Pacioglu et al., 2016) and there have been reports of suppression to zooplankton communities (Reitzel et al., 2003) and bioaccumulation in fish (Wauer and Teien, 2010).

Across 83 lakes, Al reduced mean TP concentrations from 100 µg $L^{-1}$ pre-treatment to 36 µg$L^{-1}$ post-treatment. Secchi depth increased from 1.6 m pre-treatment to 2.4 m post-treatment. Chl-*a* decreased from 42.7 to 16.3 µg $L^{-1}$ (Huser et al., 2016a). With these reported concentrations post-treatment we would expect a higher macrophyte species-richness across Al treated lakes in comparison to biomanipulated and dredged waterbodies, according the results presented by Jeppesen et al., (2000). However, Al has had very few reported macrophyte responses with most reviews completed on terrestrial plants (Gensemer and Playle, 1999) but there are two trials looking at growth effects on *Chara hispida*. In a mesocosm experiment, poly aluminum chloride (PAC) was applied in doses of 50,

100 and 200 ml $m^{-3}$ to 0.8 $m^{-3}$ *insitu* mesocosms. The application inhibited growth and reduced the length of *Chara hispida.* Length was reduced by 10 – 20 cm across the doses. Branchlet length increased and internode cells became elongated. This was attributed to a pH reduction from 9.4 to 6.5 through application and the high Al solubility and toxicity as necrosis was observed on individuals. Elongation of internode cells was attributed to reduced light availability through charophytes' thallus being covered with coagulated suspension precipitated from the water (Rybak and Joniak, 2018). The second trial demonstrates that *Chara hispida* is a poor accumulator of Al after the application of PAC in a laboratory trial where 2 mg $g^{-1}$ dry weight accumulated in cells. The degree of accumulation did not increase with higher Al loads and the minimum applied load of 6.1 g $m^{-3}$ saw degradation after 24 hours (Rybak et al., 2017). However, marginal macrophytes have been found to have concentrations of Al 50-fold higher in tissues following treatment with *Typha domingensis* and *Schoenoplectus califonicus* having concentrations 4- and 2-fold higher, respectively (Malecki-Brown et al., 2010). It is clear there needs to be further trials on the impacts of Al on other submerged vegetation (Malecki-Brown et al., 2010) to assess macrophyte potential toxicity and physiological stress. Charophyte species are known to be quite stress-tolerant (Murphy et al., 1990) so it might be expected that species that are less-tolerant to Al addition would be worse off than these results reported for *Chara hispida*.

### 1.9.5. *Iron*

Fe salts as iron sulfate ($FeSO_4$) or iron chloride ($FeCl_3$) are commonly used P-sorbing products due to Fe's strong affinity to naturally bind to available P in freshwaters (Immers et al., 2015). Despite its high P binding capacity, Fe addition has had limited successes reported in the literature to control sediment P-release in waterbodies (Cooke et al., 1993) and fewer reported field trials compared to Al (Bakker et al., 2016; Lürling et al., 2016). It is only advised to be used under aerobic conditions (Cooke et al., 2005) as the redox sensitivity of Fe is a major flaw in its use to improve water quality in lakes and a lack of desired impacts are normally due to diminished P-binding potential under reduced conditions (Hickey and Gibbs, 2009; Smolders et al., 2006). The breakdown of organic matter through microbe mediated reactions can cause anoxic conditions through increased oxygen consumption which can reduce Fe to form Iron sulphide ($FeS_x$) when sulphur is sufficiently present, which reduces the

binding sites for P (Smolders et al., 2006). If there are high concentrations of $SO_4^{2-}$ in the water column, P can compete for anion absorption sites and therefore Fe should only be applied where $SO_4^{2-}$ concentrations are low (Bakker et al., 2016). Under anoxic conditions Fe (III) is reduced to Fe (II) and Fe relinquishes its binding capacity, liberating P into the overlying water column (Kleeberg et al., 2013) contributing to the cycling of P within the system. Commonly, most restoration interventions fail through increased TP and Chl-*a* concentrations following the cessation of Fe applications (e.g.Boers et al., 1994; Foy and Fitzsimons, 1987; Immers et al., 2015) whilst some have failed through ineffective calculated dose (Kleeberg et al., 2013). The overall success of Fe applications in achieving improved water quality and longevity has been variable and is lake dependent (Bakker et al., 2016).

Immers et al., (2015) reported that TP concentrations fell to 20 µg $L^{-1}$ through the year that Fe was applied to Lake Terra Nova, The Netherlands. However, as soon as Fe addition ceased, summer TP concentrations increased dramatically to pre-application concentrations (maximum of ~ 90 µg $L^{-1}$). Chl-*a* concentrations also reduced by ~20% of the five year average preceding the application but 1 - 2 years post-remediation, Chl-*a* began to increase (maximum of ~ 175 µg $L^{-1}$). Foy and Fitzsimons, (1987) found summer TP concentrations reduced to 35.7 µg $L^{-1}$ in the year after Fe application to White Lough, Northern Ireland U.K, but concentrations increased to 47.4 µg $L^{-1}$ and 48.3 µg $L^{-1}$ one and two years after treatment, respectively. Summer Chl-*a* was 23.1 µg $L^{-1}$ in the year post-treatment which increased to 27.8 µg $L^{-1}$ two years post-treatment. Given the short-lived successes of treatments which is approx. one year for these studies, it is unlikely that macrophyte establishment could occur given the quick increase in TP and Chl-*a* concentrations reported.

There has only been one reported field trial which assessed both the effects of Fe on chemistry but also macrophytes. Immers et al., (2015) found that *Elodea nuttallii* established two years following Fe treatment to Lake Terra Nova, occurring in 63% of sampling points, however declines were then witnessed two years later, only occurring in 51% (Appendix 1, Table 1). Lab trials using tanks (0.3 $m^3$) to assess the macrophyte responses of *Elodea nuttallii*, *Potamogeton pectinatus* (Immers et al., 2014) and *Chara virgata* and *Chara globularis* (Immers et al., 2013) to Fe addition did not directly impact their growth and physical appearance. In laboratory trials using glass beakers (500 ml) which were filled with 6 cm of sediment and 8 cm of water, $FeSO_4$ decreased the number of roots of *Hydrilla verticillate* and decreased the

malondialdehyde content in plant leaves which expressed physiological stress in the plant after 30 days (Lin et al., 2017). There needs to be more lab and field trials assessing the responses to other macrophyte species given these species-specific responses and the declines reported, as Fe toxicity could be a possible reason for declines (Immers, 2014) .

### 1.9.6. *Calcium*

Ca is usually applied in the form of calcium carbonate/lime ($Ca(OH)_2$) or calcite ($CaCO_3$) and has been mainly applied to acidified lakes with the majority of restoration projects having occurred in Sweden and Norway (Gulati and Van Donk, 2002). If a waterbody is very productive and pH values increase above 9, calcite will precipitate with available P and form hydroxyapatite molecules thus P binding is relatively efficient, but it is less effective than Al and Fe additions (Smolders et al., 2006). The longevity of treatments is variable (Prepas et al., 2001a; 2001b; Reedyk et al., 2001) and is not considered to be a long-term method to control internal loading or for use in soft water lakes (Smolders et al., 2006).

In Figure Eight Lake, Canada, TP concentrations reduced by 91% and Chl-*a* reduced by 70% one year after application, but six years after the first initial Ca dose, TP concentrations were ~80 µg $L^{-1}$ and Chl-*a* concentrations were ~10 µg $L^{-1}$ from summer to October (Prepas et al., 2001b). Immediate reductions in TP and Chl-*a* concentrations were seen one week after Ca was applied to Halfmoon Lake, Canada. TP and Chl-*a* concentrations reduced by 77% from pre-treatment values. However, TP concentrations were at ~90 µg $L^{-1}$ and Chl-*a* at ~40 µg $L^{-1}$ six years after the first initial Ca treatment. These reported TP and Chl-*a* concentrations in combination would not be sufficient to see a high species richness of submerged macrophyte species post-treatment according to Jeppesen et al., (2000).

Ca has also had varied outcomes when looking at biological responses to treatment (Angeler and Goedkoop, 2010). The biomass cover (%) of macrophytes initially declined by 95% at 2 m depth and by 88% at 1 m depth one year after treatment in Halfmoon Lake but then gradually increased at 1 m depth three years post- application. Only five years after the first application did macrophyte biomass return at 2m depth. Macrophyte species shifted from mainly floating species (*Lemna* trisulca) to *Potamogeton* spp. The cover of macrophytes declined in Lofty Lake, Canada post- application and growing depth decreased by 0.5 m compared to pre-

treatment values (Reedyk et al., 2001) (Appendix 1, Table 1). However, TP concentrations were 78 µg $L^{-1}$ post-treatment which also were too high to support submerged macrophyte diversity (Jeppesen et al., 2000).

### 1.9.7. *Lanthanum-modified bentonite*

La-modified bentonite (LMB) was developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) (Douglas, 2002). It is marketed for the removal of phosphate and oxyanions, depleting them and making them unusable by algae (US Patent 63508383 B1, (Douglas, 2002). La is known to bind strongly with P (Haghseresht et al., 2009) however, free La ions ($La^{3+}$) in soluble form can be toxic to some aquatic biota (Barry and Meehan, 2000; Douglas et al., 2004). To combat this, the merging of La with bentonite was necessary to prevent toxic effects when LMB was applied to waterbodies (Haghseresht, 2006). The reaction of La with phosphate ions produces rabdophane ($LaPO_4$), a form of La phosphate which is a stable mineral and is not altered under anoxic conditions or by fluctuations in pH at the lake scale (Ross et al., 2008) making it a versatile product to use for different lake types. LMB has been applied to over 200 aquatic systems (Copetti et al., 2016; Grant B Douglas et al., 2016), on a global scale (Mackay et al., 2014) and has been applied to lakes (Crosa et al., 2013; Lürling and van Oosterhout, 2012; Meis et al., 2013), reservoirs (Meis et al., 2012), impounded rivers (Novak and Chambers, 2014; Robb et al., 2003), has been trialed for usability in saline waters (Mucci, 2019; Reitzel et al., 2013) and in drinking waters (N Traill 2019, pers. comm.). It has had many successful water chemistry improvements in mesocosm trials (Crosa et al., 2013; Márquez-Pacheco et al., 2013), and in field trials (Epe et al., 2017; Gunn et al., 2014; Haghseresht et al., 2009; Meis et al., 2013) which has generally resulted in reduced TP, SRP, Chl-*a* and increased transparency across treated lakes (Copetti et al., 2016; Lürling et al., 2016; Spears et al., 2016). Longevity of treatments can be variable but are likely due to continued external P-load inputs (Lürling and van Oosterhout, 2012). Even with the merging of La with bentonite, there have been documented release rates of La following applications (Lürling and van Oosterhout, 2012; Meis et al., 2012) which initially caused concerns. However, La concentrations in the water column of treated lakes only appear to be temporary post- application with La release rates declining 3 -12 months after treatments (Spears et al., 2013a). There have been several reports and peer reviewed literature examining the toxicity of La to freshwater biota and risk

to human health (Afsar and Groves, 2009; Behets et al., 2019; Clearwater, 2004; Copetti et al., 2016; D'Haese et al., 2019; Lürling and Tolman, 2010; NICNAS, 2001; Spears et al., 2013a). In combination the reports and published literature demonstrate that in most cases species expressed a low sensitivity to LMB.

TP concentrations across 15 lakes were reduced from 80 µg $L^{-1}$ to 30 µg $L^{-1}$ two years post-treatment and Chl-*a* concentrations from 119 µg $L^{-1}$ to 74 µg $L^{-1}$ in thirteen lakes and secchi depth increased from 4 m to 5.1 m in fourteen lakes post-applications (Spears et al., 2016). In the same multi-lake study, Spears et al., (2016) reported that two years after LMB treatment, lake specific responses were witnessed across six lakes with only weak improvements (Appendix 1, Table 1). Maximum growing depth increased from 1.8 m to 2.5 m and species richness increased from 5.5 to 7 species but responses were highly lake specific. However, the concentrations that were reported post-treatment are within the ecologically relevant ranges that should see more macrophyte submerged species establish (Jeppesen et al., 2000). In other whole-lake trials, colonisation depths increased, and macrophyte coverage extended after LMB application to Loch Flemington (U.K), in comparison to colonisation depths two years pre- and one year post- application (Gunn et al., 2014) (Appendix 1, Table 1). Despite this, macrophyte communities were dominated by *Elodea canadensis* and community composition remained similar to pre-treatment communities. This was also the case for Lake De Kuil (The Netherlands) where, before the application of $FeCl_3$ and LMB ('Flock & Lock'), there were low numbers of macrophyte species. Two years after the application, however, 12% of the lake's area ($m^2$) was covered in macrophytes dominated by *Elodea nuttallii* and *Chara vulgaris* compared to pre-application (Waajen et al., 2016a). In laboratory core trials *Elodea nuttallii* growth was not inhibited by LMB addition (Chrzanowski, n.d.) but root:shoot ratios were higher in LMB treatments compared to other P removal materials ($FeCl_3$, $AlCl_3$, PAC). Lin et al., (2017) found reduced root length and root number in *Hydrilla verticillata* in comparison to controls when applied with LMB in 500 ml glass beakers after 30 days and hypothesised that this was caused by reduced oxygen supply to roots following the 1g LMB addition. The most common species colonising after LMB treatment are *Elodea* spp. and charophytes (Appendix 1, Table 1) and it is unclear why more species are not occurring despite improved water chemistry.

## 1.10. Possible reasons for a lack of macrophyte recovery

It is possible that desired water quality conditions are still insufficient to encourage macrophyte recovery, despite improvements following different external and in-lake restoration methods (Hilt et al., 2006). Persistent high external nutrient loads, insufficient reductions or continued sediment P release and difficulties in calculating 'effective doses' of P-capping products (Meis et al., 2013) could be accountable. Desirable abiotic conditions may be absent following intervention; light, nutrient availability and sediment composition are all very important for re-colonisation and successful germination from seed propagules (Bornette and Puijalon, 2011; Hilt et al., 2006). Connectivity between waterbodies is particularly important in the re-establishment of macrophytes to a site. Often this relies heavily on the ability of wind (Soomers et al., 2010) and other organisms such as wildfowl to transport macrophytes, particularly for macrophytes that reproduce vegetatively. Biotic constraints exist such as, waterbird grazing on newly establishing macrophyte communities (Lauridsen et al., 2003b, 1993; Søndergaard et al., 2000, 1996a) and buried propagules deep in sediment profiles where they can no longer respond to germination cues (Boedeltje et al., 2003) are potential causes for a lack of recovery of desirable macrophyte communities. The absence of a viable seedbank (Bonis and Grillas, 2002) could also be responsible. The rapid colonisation by undesirable or non-native invasive macrophyte species can also be to blame as they can prevent native species to colonise by competing for space, light and nutrients, leaving low probability for colonisation by more desirable species (Bakker et al., 2013).

There is little information in the literature if additional management-specific barriers in addition to general barriers could prevent desirable species/more species to establish following in-lake P-capping techniques (Figure 1.5). The timescale of full recovery from P-capping agents is uncertain due to insufficient long-term monitoring of macrophytes generally. It is unknown if P-capping products can impact on macrophyte germination rates by causing an extra barrier to germinate through (Hilt et al., 2006) causing a burial effect and if this could impact community structure, as it is possible only certain species would be able to germinate from deeper sediment depths. In addition, the P capping products that are applied could potentially alter macrophyte communities through P limitation in both the water column and sediments. The direct impacts of P-capping products applied to freshwaters where established macrophytes are present is also unknown.

## 1.11. Knowledge gaps

The success of lake remediation is assessed by how quickly macrophytes reappear to treated waterbodies, however we still struggle to explain the ecological mechanisms of their slow response to nutrient reduction (Bakker et al., 2013; Lauridsen et al., 2003a; Phillips et al., 2016; Søndergaard et al., 2007). It is clear from this literature review that macrophyte recovery from external nutrient P-reduction alone maybe confounded by internal loading. However, the majority in-lake measures are unable to reduce in-lake TP and Chl-*a* concentrations to within ecologically relevant ranges to promote macrophyte recovery post-treatment. Much of the research on these materials has been conducted on their efficiency to reduce internal P loads but little evidence has been presented on the ecological recovery following improved chemical conditions. Meeting ecological legislative targets will be difficult using these techniques without further knowledge how macrophyte communities are impacted following treatments. LMB can reduce TP and Chl-*a* concentrations sufficiently to provide more desirable conditions for macrophyte species to establish but the reasons why they are not colonising following treatments are unknown. This is clearly a 'bottleneck' to achieving macrophyte recovery. Some of the possible reasons for a lack of recovery are presented in Figure 1.5. Without the re-establishment of macrophytes following restoration measures, restoration goals are not fully met which prevents reaching ecological set targets. There is no evidence in the literature of the ability of geo-engineered/P-capping products to improve ecological lake quality in the context of legislation (e.g. the WFD) (Spears et al., 2018). The main scope of this study was therefore, to investigate the impact of LMB on submerged macrophyte recovery.

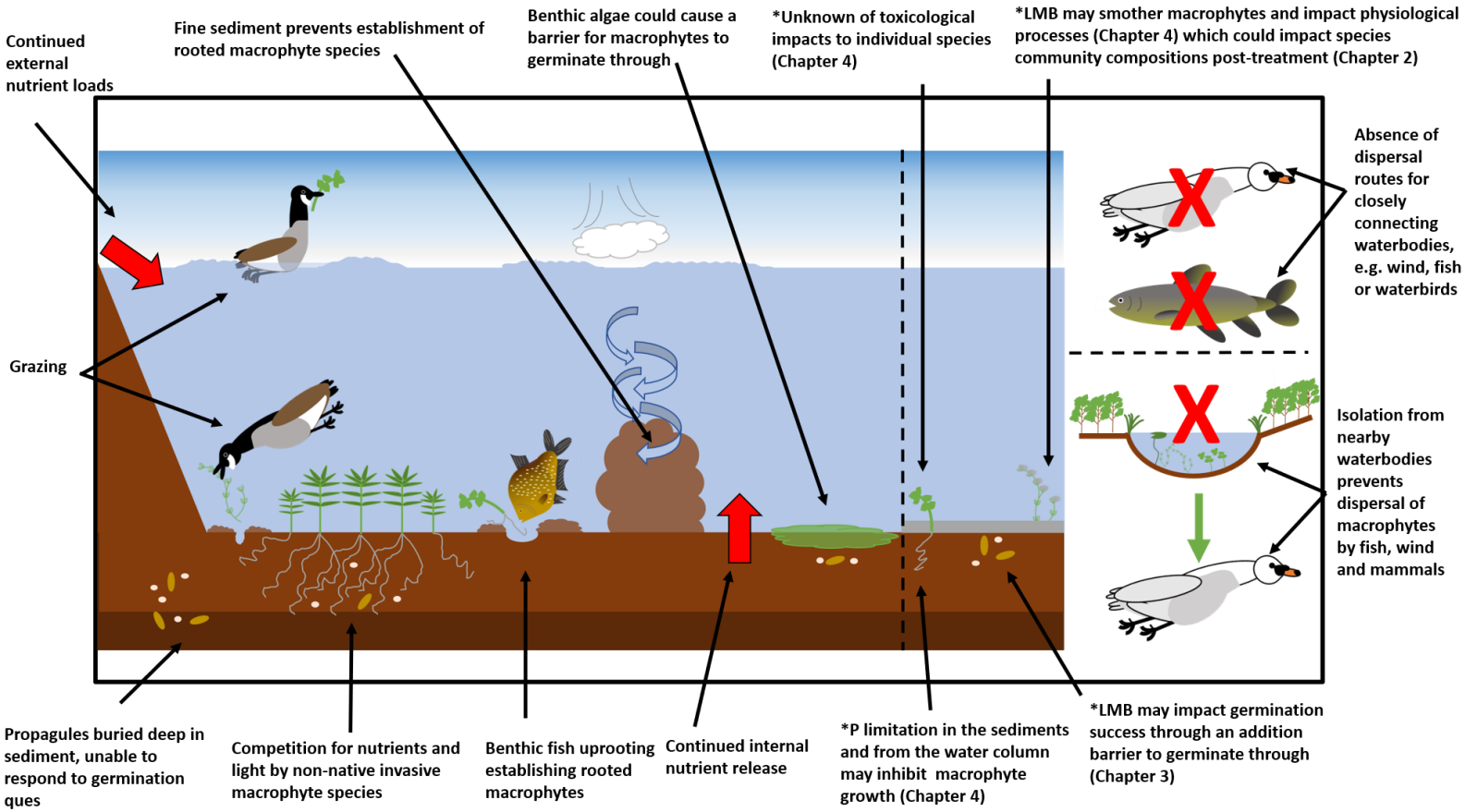



**Figure 1.5.** Diagram of general and lanthanum-modified bentonite (*) reasons why desirable macrophyte species may not be establishing once chemical recovery has been achieved in treated lakes (© Kate Waters-Hart).

## 1.12. Introduction to the key hypothesis to be addressed in each chapter and thesis structure

The aim of this thesis was to use standard and novel techniques to provide an evidence-base for understanding some of the mechanisms behind the delay in macrophyte recovery in LMB treated lakes. These were investigated using multiple existing short and long-term data sets, field surveys conducted across multiple sites and a range of different experimental approaches.

This thesis is structured around three paper-like chapters (chapters 2-4) with the main questions to be addressed which are summarised in Figure 1.6 and are as follows:

- What is the recovery time of macrophyte's to a lake following an LMB treatment?
- Does LMB cause a barrier following application, preventing germination success of macrophytes?
- Are there macrophyte species-specific growth responses following an LMB application?

Chapter 2 assess the impact of LMB on desirable macrophyte recovery potential, macrophyte species and community composition across multiple lakes following LMB treatments. Both short term (1-3 years) and longer term (3+ years) impacts have been investigated. Several WFD macrophyte metrics (species richness, Lake Macrophyte Nutrient Index scores (LMNI's), Number of Functional Groups (NFG's) etc.) and community composition assessments were used to assess against non-treated lakes to gauge if LMB treated lakes are meeting legislation. The chapter's objectives were to use existing data alongside new data to investigate macrophyte recovery timescale trajectories and community compositions across multiple LMB treated lakes with an emphasis on assessing whether treated lakes meet ecological legislative set targets since application and their potential colonisation origin.

**Hypothesis:** Building on results from Spears et al., (2016) it is expected that with more sites and longer runs of data that LMNI scores will decline due to a reduction in nutrients. NTAXA, and NFG should increase . If the same findings as Spears et al., (2016) hold, poor monitoring could be a reason for a lack of macrophyte recovery.

Chapter 3 assesses if LMB inhibits macrophyte propagule recovery from lakebed sediments. A 21-week germination trial was conducted comparing LMB treated sediments to un-treated sediment and extant populations to assess if the additional layer formed on the sediment surface through an LMB application confounded germination of desirable macrophytes. The main objective was to assess macrophyte recovery potential from existing seedbanks and to investigate if LMB has an impact on germinating community compositions.

**Hypothesis:** LMB may alter community composition as an extra layer added to the sediment profile may push some macrophyte species out of their germination cue ranges, e.g. less light hitting the sediment surface maybe detrimental for some species that require higher light levels.

Chapter 4 investigates general and species-specific growth responses of already growing macrophytes to LMB application. A series of bioassay experiments were conducted to aid understanding of the mechanisms that may impact already growing species in LMB treated lakes and how desirable, invasive and rare endangered species might respond. Numerous growth parameters were assessed in a controlled laboratory environment under different light levels to mimic different growing depths. This chapter's main objective was to explore macrophyte species general and species-specific responses to LMB and the mechanisms which might be responsible for a delay in ecological recovery for a number of species with different morphological traits.

**Hypothesis:** There will be species-specific responses to LMB due to differences in species strategy traits in response to an LMB application.

Chapter 5 brings together the findings from the three data chapters and discusses them in the context of wider literature on achieving macrophyte recovery using LMB. Future research within the field is identified, supported by concluding remarks.

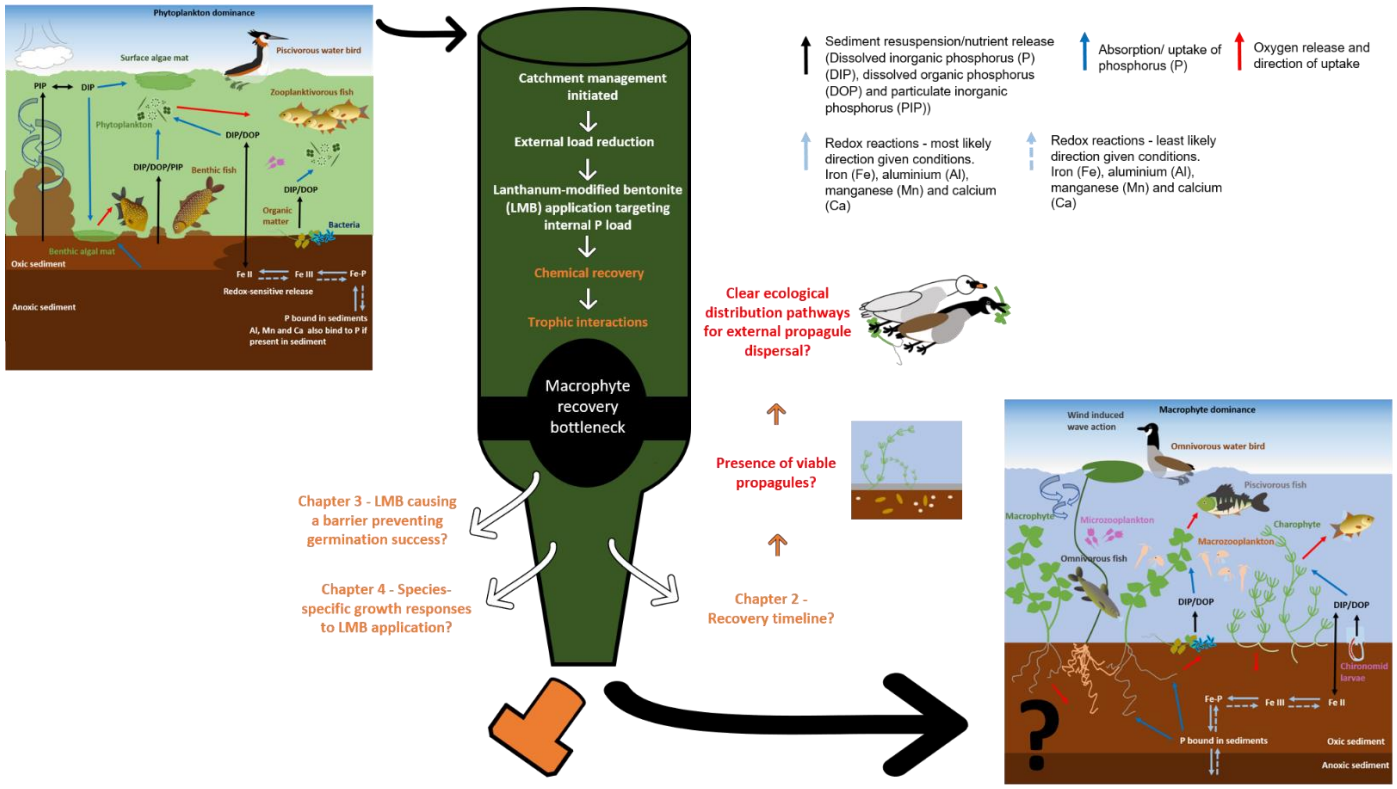



**Figure 1.6.** Diagram illustrating the current macrophyte recovery bottleneck following lanthanum-modified bentonite applications and the thesis questions to be addressed (orange text) to help understand the reasons behind a lack of macrophyte recovery (© Kate Waters-Hart).

# 2. Chapter 2: Aquatic macrophyte responses in lakes treated with lanthanum modified bentonite (Phoslock®)

Kate E. Waters-Hart [a, b, *], Nigel Willby [b], Said Yasseri [c], Iain D.M. Gunn [a], Stephanie Cole [d], Tim Epe [e], Andrea Kelly [f], Miquel Lürling [g, h], Genevieve Madgwick [i], Sebastian Meis [j], Frank van Oosterhout [h], Jo-Anne Pitt [k], Iain Sime [l], and Bryan M. Spears [a]

[a] UK Centre for Ecology & Hydrology, Penicuik, Midlothian, EH26 0QB, UK

[b] University of Stirling, Stirling, FK9 4LA, UK

[c] Limnological Solutions International Pty Ltd, Admiralstr. 150a, 28215, Bremen, Germany

[d] Environment Agency, Red Kite House, Howbery Park, Crowmarsh Gifford, Wallingford, Oxford, OX10 8BD, UK

[e] Institut Dr. Nowak GmbH & Co. KG, Mayenbrook 1, D-28870, Ottersberg, Germany

[f] Broads Authority, Yare House, 62-64 Thorpe Road, Norwich, NR1 1RY, UK

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

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

[i] Natural England, Eastbrook, Shaftesbury Road, Cambridge, CB2 2DF, UK

[j] lanaplan GbR, Lobbericher Str. 5, D-41334 Nettetal, Germany

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

[l] Scottish Natural Heritage, Great Glen House, Leachkin Road, Inverness, IV3 8NW, UK

The candidate, as lead author, performed a number of the macrophyte surveys, the data analysis and writing were carried out by the candidate. Co-authors provided data, support and guidance on the scope and design of the project. Co-authors also contributed to the editing of the manuscript.

Acknowledgements: Thanks to Stephen Ives, Justyna Olszewska and John Pottie for help with macrophyte surveys and to Jan Brückmann for help with macrophyte identification for some of the German surveyed lakes.

## 2.1. Abstract

Lanthanum modified bentonite (LMB) has been used in over 200 waterbodies across the world to control phosphorus (P) release from bed sediments to overlying waters. It has been found to significantly reduce in-lake P and chlorophyll-a concentrations and improve water transparency at the individual lake scale and collectively across lakes with varying conditions. Despite the success of chemical recovery in LMB treated lakes, a comprehensive assessment of aquatic macrophyte (macrophyte) responses following improved chemical conditions is lacking. We examined twelve lakes with pre- and post-LMB application data to assess macrophyte recovery in both the short-term (1-3 years) and long-term (3-10 years), where data allowed. Recovery was quantified using the following measures: common species occurrences pre- and post-LMB application (8 -12 lakes), timescale of newly colonised species (5 lakes), lake macrophyte Water Framework Directive (WFD) metrics (7-8 lakes), macrophyte community composition (5 lakes), lakes meeting UK ecological targets (2 lakes) and lakes meeting European ecological targets (2 lakes). The macrophyte monitoring methods and frequency of monitoring were highly variable between and within lakes. macrophyte recovery following LMB addition was lake-specific with the majority of lakes expressing weak signs of ecological recovery over the short- and long-term. *Elodea canadensis* was the most commonly occurring species following LMB applications, with the majority of lakes remaining dominated by species that represent unacceptable conditions under the WFD (i.e. less than 'good' status). Community composition saw little change three years post-application compared to pre-application conditions. LMB treated lakes in this study are currently not meeting ecological targets over the time scales used for assessment. We call for standardised macrophyte monitoring methodologies and well-designed monitoring of sufficient treated and control lakes over pre- and post-application phases to track macrophyte changes in the long-term, given the apparent time-lags in recovery timescales. The limitations and reasons for macrophyte suppression are discussed.

## 2.2. Introduction

Aquatic macrophytes (macrophyte) perform fundamental functions in lakes (Jeppesen et al., 1997; Scheffer et al., 1993). macrophyte sustain clear waters through nutrient uptake (Van De Haterd and Ter Heerdt, 2007; Van Donk and Van de Bund, 2002) and

they stabilise bed sediments which can prevent sediment re-suspension and release of sediment phosphorus (P) to the water column (Blindow et al., 2002; Ibelings et al., 2007). macrophyte also provide oxygen, food and shelter thus sustaining aquatic food webs (Perrow et al., 1999). Despite their critical role in lake health, the diversity and cover of submerged macrophyte is in global decline, with accelerated deteriorations over the last 40+ years owing to multiple stressors such as climate change and eutrophication (Lauridsen et al., 2015; Zhang et al., 2017). The increase in excess nutrients entering lakes (mainly P and nitrogen) can deteriorate both water quality and lake function; in shallow lakes this can cause a switch from submerged macrophyte dominance to phytoplankton dominance, which leads to a deterioration in water quality (Smith and Schindler, 2009; Søndergaard et al., 2003). To address the major issue of world-wide nutrient pollution, environmental policies have been implemented to prevent further chemical and ecological declines in freshwaters, e.g. the European Water Framework Directive (WFD) (European Commission, 2000).

In order to meet policy targets, management actions are required to reduce nutrient loads to lakes (Zamparas and Zacharias, 2014). Large-scale catchment management programmes have been successful in many cases in reducing nutrient loads to lakes (Jeppesen et al., 2007a; Schindler et al., 2016). However, there is often a time-lag, typically years to decades in lake chemical recovery due to historic P cycling from sediments to the overlying water column (Jeppesen et al., 2005; Meis et al., 2012; Søndergaard et al., 2007). In-lake remediation measures to control internal loading have been used to try to force chemical recovery with the expectation that ecological improvements will also occur, including the recovery of macrophyte.

Lanthanum-modified bentonite (LMB) is a P-sorbing material used to control internal loading and is designed to strip P from the water column and from bed sediments to inhibit P release once the material ‘caps’ the sediment (Mackay et al., 2014). LMB’s use is increasing and is global in extent (Mackay et al., 2014). Applications of LMB have provided successful water quality improvements at the individual lake scale (Bishop et al., 2014; Crosa et al., 2013; Lürling and van Oosterhout, 2013; Meis et al., 2013) and generally across many treated lakes (Spears et al., 2016). Limited studies have examined macrophyte recovery in the short term (1-3 years post LMB addition), at the individual lake scale (Gunn et al., 2014; Waajen et al., 2016a) and across multiple lakes (Spears et al., 2016) with only weak signs of recovery so far reported using crude indicators. Little is known of macrophyte community composition responses following LMB treatments designed to achieve

conservation targets (Spears et al., 2016) but following LMB applications we would expect to see increases in macrophyte species richness and cover according to results from other nutrient reduction studies (Jeppesen et al., 2000; Søndergaard et al., 2010). From the nutrient reduction seen in 18 lakes following LMB applications (Spears et al., 2016) we would expect increases in submerged macrophyte species, as, Jeppesen et al. (2000) found that mean submerged macrophyte species number increased from 0.5 to 11.7 species. This is a relative increase in 22.4 species as TP concentrations declined from > 400 µg $L^{-1}$ to < 50 µg$L^{-1}$ following nutrient reduction in 71 lakes. Instead, only an increase of 1.6 species to a mean of seven were observed across six LMB treated waterbodies from Spears et al., (2016). This is a relative increase in 0.27 macrophyte species post-LMB application from a reduction in TP concentrations from 80 µg $L^{-1}$ to 30 µg $L^{-1}$. We would also expect to see improvements in WFD macrophyte status, as Poikane et al. (2019) found that "good" macrophyte status was reached in EU high alkalinity shallow lakes (3 – 15 m deep) at TP concentrations ≤ 48 – 53 µg $L^{-1}$ and for very shallow lakes (<3 m) at ≤ 58 – 78 µg $L^{-1}$. We would assume that the weak signs of recovery, reported by Spears et al., (2016), could be due to biological constraints or from poor monitoring data. A lack of macrophyte diversity could be due to dispersal barriers. Waterbodies that are more isolated may have difficulty in establishing angiosperm populations from vegetative propagules or this process may take longer due to reliance on organisms (e.g. fish or waterbirds) or wind/water for dispersal. Therefore, in the short-term we might expect macrophyte recovery following LMB to rely on in-situ seedbanks, particularly for isolated waterbodies. We expect that species such as charophytes, which are known to survive longer in the seedbank (De Winton et al., 2000), may return more quickly than vegetative propagule species that, in the majority, rely on external dispersal routes for establishment. Furthermore, as eutrophication is a worldwide phenomenon operating over decades or centuries, some desirable historically abundant species may have become locally rare making their re-establishment difficult. This in-turn will affect macrophyte community composition, e.g., lakes that are well connected to other waterbodies/waterways would potentially have more similar and diverse communities than lakes that are more isolated in terms of their connectivity to water sources. These isolated waterbodies may need to rely more on seedbanks in the short-term following an LMB treatment. This study builds on that of Spears et al., (2016), by using longer data sets and more LMB-treated sites and comparing macrophyte changes to control sites to see if the findings of weak macrophyte

responses post-application from Spears et al., (2016) hold. Additionally, we have developed several analytical approaches to try to determine macrophyte responses using WFD lake macrophyte metrics that are known to be sensitive to eutrophication (Willby et al., 2009). Table 2.1 summarises these macrophyte metrics and our hypothesised response for each metric generally across LMB treated waterbodies.

**Table 2.1.** Macrophyte metrics to assess macrophyte recovery following LMB addition and hypothesised responses.

| Macrophyte metric | Description | Reference | Hypothesis |
|---|---|---|---|
| Lake macrophyte nutrient index (LMNI) | A taxon-specific nutrient response score | Willby et al., (2009); (WFD - UKTAG, 2014) | LMNI scores to decline due to a reduction in nutrients after LMB applications |
| Number of macrophyte taxa (NTAXA) | A diversity metric, the number of scoring taxa recorded in the field survey | Willby et al., (2009); UKTAG (2014) | NTAXA to increase based on evidence from Jeppesen et al. (2000) and the nutrient reductions seen across treated LMB treated lakes (Spears et al., 2016) |
| Number of functional macrophyte groups (NFG) | A diversity metric, individual taxa are allocated to one of 18 "functional groups (group of organisms that share similar morphological traits)" | Willby et al., (2009); UKTAG (2014) | NFG to increase based on evidence from Jeppesen et al. (2000) and the nutrient reductions seen across treated LMB treated lakes (Spears et al., 2016) |
| Macrophyte total occupancy (%) | Relative percent cover of macrophyte | Søndergaard et al., (2010) | We would expect an increase in % cover to a median of approx. 57 - 60% cover for shallow lakes and 8 – 10% for deep lakes given the TP concentrations of <0.05 $mg/L^{-1}$ following LMB applications seen generally across treated lakes according to Spears et al. (2016) |

Comprehensive quantitative assessments of macrophyte community responses to management interventions, including LMB, are rare and limited by variations in methodologies and sampling frequencies (Zhang et al., 2017). As with other approaches, there is currently, therefore, low confidence in the use of LMB to support recovery in line with ecological quality targets. Different macrophyte assessment methods are in use across Europe, few of which are published or can be compared across countries (Penning et al., 2008). Currently, WFD intercalibration exercises are used to overcome the issue of multiple methodologies (Poikane et al., 2018) but the

lack of standardised methods is a widely acknowledged problem. Researchers continue to develop statistical methods with which to explore macrophyte responses to management interventions using disparate data, although issues of pseudo-replication (Davies and Gray, 2015), particularly with lake restoration studies, are common. Finally, it is difficult to get replication at the lake scale or to find appropriate un-impacted reference sites (Poikane et al., 2018), or simply comparable untreated sites, to act as control lakes.

Data from twelve LMB-treated lakes were used for a comprehensive analysis of macrophyte community responses over both short- (1-3 years) and long-time scales (3 -10 years). Data from four control lakes were used to compare macrophyte communities against LMB lakes to account for changes in inter-annual variation. We utilised multiple assessment approaches to address weaknesses in methodologies and monitoring frequencies to address the following questions: (1) what are the most common species returning to LMB-treated lakes that might also give us an indication of their origin; seedbank establishment or from external sources?; (2) do macrophytes take longer to colonise more isolated treated lakes post-application compared to lakes in closer proximity to one another?; (3) does the macrophyte composition of LMB-treated lakes differ from control sites?; (4) does macrophyte species richness and other nutrient sensitive metrics respond, as expected, following LMB treatment, consistent with nutrient reduction?; and (5) are lakes treated with LMB meeting 'good' ecological status as set by the WFD and 'good' condition as set for SSSI's?

## 2.3. Methods

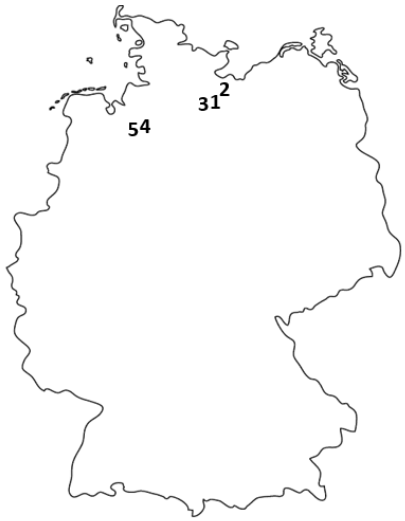
### 2.3.1. *Data availability and study sites*

The following analyses are based on collated data from twelve lakes (Figure 2.1) where LMB has been applied (Table 2.2). macrophyte community data existed for eight of the twelve lakes that had both pre- and post-application data available. Crome's Broad (UK) was included in the study as two separate lakes, as annual macrophyte surveys recorded communities from two separate basins, north (N) and south (S). The remaining four lakes had exclusively post-LMB application macrophyte data available. Some of the lakes received more than one application of LMB (Table 2.2). The LMB application for Lake Rauwbraken (NL) and Lake Eichbaumsee (DE) differed from the other lake application approaches in that LMB was applied with a flocculent; polyaluminium chloride (PAC) and a pH buffer (Spears et al., 2016; Van

Oosterhout and Lürling, 2011).

In some lakes, multiple LMB applications were conducted over several years. All pre-application macrophyte data reported here were collected before the first application of LMB and the post-macrophyte data were collected after the last application of LMB (Table 2.3).


(1) Hatchmere
(2) Mere Mere
(3) Loch Flemington
(4) Crome's Broad N and S basin
(5) Clatto Reservoir




(1) Blankensee
(2) Behlendorfer See
(3) Lake Eichbaumsee
(4) Lake Ottersteder See
(5) Lake Silbersee


(1) Lake Rauwbraken

**Figure 2.1.** Location of LMB-treated lakes in each country.

**Table 2.2** Summary of lakes with macrophyte data available and their LMB application histories and their distances (km) to the nearest waterbody of a similar size as a measure of connectivity potential.

| Lake | Country | Size (ha) | Date and mass applied (tonnes) | LMB load (tonnes $ha^{-1}$) | Distance to nearest waterbody (km) |
|---|---|---|---|---|---|
| Hatchmere | UK | 4.7 | 13/03/2013 (25.2) | 5.3 | 0.8 |
| Mere Mere | UK | 15.8 | 09/03/2013 (79.8) | 5.1 | 1.4 |
| Loch Flemington | UK | 15.7 | 15/03/2010 (1.6) | 1.6 | 7.4 |
| Crome's Broad N and S basins | UK | 3.7 | 19/03/2013 (9.75) | 5.1 | 0.7* |
| Clatto Reservoir | UK | 9.0 | 04/03/2009 (24.0) | 2.7 | |
| Lake Rauwbraken | NL | 4.0 | 22/04/2009 (2.0) | 0.5 | 3.3 |
| | | | 23/04/2009 (16.0) | 4.0 | |
| Lake Blankensee | DE | 22.5 | 16/11/2009 (66.0) | 2.9 | 1.8 |
| Lake Behlendorfer See | DE | 64.0 | 02/12/2009 (214.0) | 3.6 | 2.0 |
| Lake Eichbaumsee | DE | 23.2 | 17/11/2010 (148.0) | 6.8 | 0.1* |
| | | | 18/10/2011 (12.0) | 0.04 | |
| | | | 20/03/2012 (16.0) | 0.7 | |
| | | | 26/06/2012 (12.0) | 0.04 | |
| | | | 15/12/2012 (50.0) | 2.6 | |
| | | | 25/11/2013 (50.0) | 2.6 | |
| Lake Ottersteder See | DE | 4.5 | 30/10/2006 (11.0) | 2.4 | 13 |
| Lake Silbersee | DE | 7.0 | 08/11/2006 (21.5) | 3.1 | 2.1 |
| | | | 06/10/2009 (4.0) | 0.6 | |
| | | | 09/10/2012 (3.0) | 0.4 | |

UK – United Kingdom, NL – The Netherlands, DE – Germany.

*Waterbodies with connectivity, e.g. through dykes/next to rivers

**Table 2.3**. Summary of data for the twelve LMB study lakes and control lakes with accompanying macrophyte data.

| Lake | Total number of macrophyte surveys | Number of pre-LMB application macrophyte surveys | Number of post-LMB application macrophyte surveys |
|---|---|---|---|
| Hatchmere | 5 | 2 | 3 |
| Mere Mere | 5 | 2 | 3 |
| Loch Flemington | 6 | 3 | 3 |
| Crome's Broad N basin | 32 | 27 | 5 |
| Crome's Broad S basin | 34 | 29 | 5 |
| Clatto Reservoir | 1 | 0 | 1 |
| Lake Rauwbraken | 19 | 9 | 10 |
| Lake Blankensee | 3 | 1 | 2 |
| Lake Behlendorfer See | 6 | 1 | 5 |
| Lake Eichbaumsee | 1 | 0 | 1 |
| Lake Ottersteder See | 1 | 0 | 1 |
| Lake Silbersee | 1 | 0 | 1 |
| Alderfen Broad* | 33 | 28 | 5 |
| Upton Great Broad* | 35 | 30 | 5 |
| Whitlingham Little Broad* | 14 | 9 | 5 |
| Whitlingham Great Broad* | 13 | 8 | 5 |

*Control lakes with pre-and post-hypothetical LMB application annual surveys with an application in 2012 based on Crome's Broad LMB application

Macrophyte surveys, both pre- and post-application were conducted with different macrophyte survey methods, both within and between lakes. Spears et al. (2016) outline macrophyte survey methods for the waterbodies in the Netherlands and in the United Kingdom (U.K.), excluding Crome's Broad N and S basins (UK), for the years 2014 – 2017 and surveys undertaken in Lake Rauwbraken post- 2007. Methods used to assess macrophyte communities in Crome's Broad N and S basins in 2014 - 2017 were different to previous years due to a change from transect-based to point-based surveys (at each point, two throws of a double-headed rake thrown 5m north and south of the boat edge) and followed the method outlined by The Broads Authority (Tomlinson et al. 2019) which assigned a level of abundance for each recorded species. Transitional years occurred (2011, 2013 and 2015) where both transect- and point-based surveys were performed to inter-calibrate the methods. From 2016 onwards, only point-based surveys were in use. Monitoring at Lake Rauwbraken post-2007 used the transect method outlined by Coops et al. (2007) which followed a five-point scale percentage category (Table 2.4). The survey methods for Lake Blankensee prior to 2015, followed the method of Kohler (1978) that assessed macrophyte community composition through diving-based transects on a five-point abundance scale (Table 2.4). Lake Behlendorfer See followed the WFD method outlined by Schaumburg et al (2015) but data was reported using the method of Kohler (1978) (Table 2.4). The survey methods for all other lakes (i.e. excluding Crome's Broad N and S basins, Lake Rauwbraken and Lake Blankensee for the survey year 2015) followed Common Standards Monitoring (CSM) methods (JNCC, 2015). This method encompasses performing boat and wader transects as well as perimeter shoreland searches for macrophytes. Boat transects use a double-headed rake, thrown to produce 20 regularly spaced sample points along a 100 m transect perpendicular to the shore. Wader surveys also use a double-headed rake thrown from the mid-pint of the 100 m sector which is divided into five sub-sector depths (0.25, 0.5, 0.75 and >0.75 m) at 20 m intervals. A bathyscope and doubled-headed rake are used to record macrophyte species present within each sub-sector. No formal survey method was used to assess the macrophyte community at Lake Blankensee in 2015 due to safety issues, it was, therefore, not appropriate to use CSM for this lake and instead the lake was intensively search, via boat, to assess the macrophyte community.

An additional four lakes were included in this study to act as controls (Table 2.3). The control lakes were most appropriate for Crome's Broad N and sS basins

due to their proximity, stable species richness and abundances. Additional control lakes were searched for inclusion in this study but little to no macrophyte data were available from suitable candidates. Survey methods for all four control lakes followed (Kennison et al., 1998) up until 2014 when the point-based survey method replaced the transect-based method for all Broads Authority monitored sites (Harris, 2014). Only submerged macrophyte were included in all analyses, as some surveys did not incorporate emergent taxa. Similarly, algal species were not included in any analysis due to some methods only recording coarse groups that did not have associated WFD lake metric scores. Maximum growing depth differences were assessed previously (Spears et al., 2016) and are not examined here. Species diversity has also been assessed previously by Spears et al., (2016), but only for six lakes, here we add to these original findings.

Species gains were determined for all LMB-treated lakes with pre-and post-application macrophyte surveys (eight lakes).Changes in metric scores were only made over the period where sites were monitored annually. The potential influence of connectivity on colonisation was based on distance to the next nearest waterbody of similar size. Distances were approximate overland distance measured with the use of online mapping tools.

### 2.3.2. *Lake macrophyte indices and data standardisation*

WFD metrics were calculated for each survey year for each lake where data allowed. These metrics are used in the WFD classification tool LEAFPACS2 for lake macrophyte that compares observed macrophyte communities against those expected in the presence of little or no disturbance (WFD - UKTAG, 2014; Willby et al., 2009). They were used here to assess changes to macrophyte communities following LMB application relative to pre-application values. The number of taxa (NTAXA), lake macrophyte nutrient index (LMNI) and number of macrophyte functional groups (NFG) (Table 2.1) are three out of five WFD metrics used here to quantify responses in the macrophyte community. Where a species was not fully resolved in surveys (e.g. macrophyte was either *Chara globularis* Thuill. sensu stricto or *Chara connivens* Salzm. ex A.Braun), the average LMNI score of the two species was used. Not all five WFD metrics could be used to calculate Ecological Quality Ratios (EQR's) in accordance with UK WFD assessment methods (UKTAG, 2014; Willby et al., 2009), due to data restraints. Therefore, a measure of macrophyte total

occupancy (%) or total percentage cover (%) was also calculated (depending on which survey methods were used for each lake), as percentage vegetation cover has also been used as an indicator to assess lake ecological condition elsewhere (Søndergaard et al., 2010). Due to the variation in methodologies across the survey lakes and within lakes between years, it was necessary to convert abundance scores into percentage cover (%) to accommodate diverse comparisons, as some abundance measures were calculated via integer scales and an exact number was needed to calculate total occupancy/total percentage scores. Often, a mid-point of the range was used for this purpose, if no single value was provided (Table 2.4). The Kohler scale, used for pre-2015 macrophyte surveys in Germany had to be converted via the Londo scale (Londo, 1975), as the Kohler scale is purely qualitative (Table 2.4). Percent occupancy scores were calculated for lakes that were surveyed through CSM methods, or surveyed lakes that had the number of transects or point counts recorded. Other lakes that did not follow CSM methods or had no information on the number of transects had an overall total site percent score calculated. CSM methods used survey data from boat, perimeter and wader transects to calculate LMNI, NTAXA, NFG and total percent/occupancy scores. Sites that had both transects and point count data had their data combined to produce these scores.

Averages and standard deviations for all metrics for seven treated lakes and all control lakes were calculated to assess general changes following LMB applications. Only seven lakes were chosen as this covered the most lakes that could be assessed over the longest period that had both pre- and post-application data. Relative percent (%) changes were calculated for all metrics for all years relative to the pre-application values for eight lakes to assess changes in metrics over time during the post-application survey years.

All control sites had relative percent changes calculated; 2012 was considered pre-application and 2013 onwards was considered post-application to provide control comparisons against data from treated lakes; these periods being chosen to reflect the timing of treatment in Crome's Broad N and S basins.

**Table 2.4.** Comparison of macrophyte methods to assess abundance at the survey sites, with grey highlighted values used where conversions were necessary where methodologies used ranges instead of specific values.

| Score | DAFOR scale | Kennison et al. (1998) (%) | Kennison et al. (1988) % used | Broads Authority point method 2014 onwards | Broads Authority point method 2014 onwards, % used | Londo scale (1976) | Londo % (1976) | Kohler scale (1978) | Kohler scale % (1978) | Kohler scale (1978) % used | Coops et al. 2007 | Coops et al.2007 % | Coops et al. 2007 % used | CSM (JNCC, 2015) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | - | | | | | | | | | | | | Absent/bare substrate |
| 0.1 | | | | <1% | 0.5 | | | | | | | | | |
| 1 | Rare | <5 | 3 | 1-10% | 5.5 | r-m | 0 – 0.2 | Very rare | 0 - 0.2 | 0.1 | Bad | 0 -1 | 0.5 | <25 |
| 2 | Occasional | >5-25 | 15 | 11-20% | 15.5 | 0.1 | 0.2 – 1 | Rare | 0.2 - 1 | 0.6 | Poor | 1 -5 | 3 | 25 – 75 |
| 3 | Frequent | >25-50 | 37.5 | 21-30% | 25.5 | 0.2 - 1 | 1 – 10 | Common/frequent | 1 - 10 | 5.5 | Moderate | 5 -25 | 15 | >75 |
| 4 | Abundant | >50-75 | 60 | 31-40% | 35.5 | 1+ - 5 | 10 – 50 | Abundant | 10 - 50 | 30 | Good | 25 – 50 | 37.5 | |
| 5 | Dominant | >75-100 | 88 | 41-50% | 45.5 | 5+ - 10 | 50 - 100 | Very abundant | 50 - 100 | 75 | Very good | 50 – 65+ | 57.5 | |
| 6 | | | | 51-60% | 55.5 | | | | | | | | | |
| 7 | | | | 61-70% | 65.5 | | | | | | | | | |
| 8 | | | | 71-80% | 75.5 | | | | | | | | | |
| 9 | | | | 81-90% | 85.5 | | | | | | | | | |
| 10 | | | | 91-100% | 95.5 | | | | | | | | | |

### 2.3.3. *Assessing macrophyte community composition responses*

In order to assess similarities in macrophyte community composition across different lakes before and after LMB applications, we performed a Non-metric Multidimensional Scaling Ordination (N-MDS) analysis using the Bray-Curtis similarity matrix (Bray and Curtis, 1957) calculated on species proportion data (recorded macrophytes percent cover/occupancy divided by the total macrophyte species percent cover/total occupancy). Proportional data was used to allow the most robust possible comparisons between data collected by different approaches. The dataset was standardized to one year pre- and three consecutive years post-application. This data range was selected due to the limiting long-term data available for all sites and the desire to maximise the number of lakes in the analysis; five lakes were included in the analysis that met these criteria. All proportional data were used from CSM boat, wader and perimeter transects and boat and diver transects from other methods. Point counts, from Crome's Broad N and S 2015 surveys were also included in the analysis. All analyses were performed in R version 3.5.3 (R Development Core Team, 2019), with the additional R package vegan (v. 2.3-0) (Oksanen et al., 2019).

### 2.3.4. *Overall macrophyte recovery compared against UK and EU WFD targets*

Assessments of overall waterbody status post-application were evaluated based on the net directional relative change across all metrics for each site, including all post-application data. The most current UK and EU ecological condition and status for designated lakes were used to assess whether treated lakes met targets post-application. For Sites of Special Scientific Interest (SSSI) in the UK that had 'Standing Open Water Habitat' as a reportable feature condition data were taken from Natural England's 'Designated Sites View' webpage (Natural England, n.d.). The survey frequency of SSSI condition monitoring programmes are, on average, every seven years, depending on habitat type. Post-application SSSI condition data was not available for some of the qualifying lakes. WFD status data for UK WFD monitored lakes were gathered from the Environment Agency's 'Catchment Data Explorer' webpage (Environment Agency, n.d.). Data were collated on macrophyte classification status which is one of four biological quality elements that contribute to define ecological status for 2016, reporting cycle 2, for relevant UK WFD lakes. Data

were collated in the same way for control lakes whose status was reported under the WFD.

## 2.4. Results

### 2.4.1. *Common species occurrences pre- and post-application of LMB*

Overall, a greater number of taxa emerged across the sites following LMB application. Data from eight lakes were used to characterise pre-application and post-application species. The most commonly occurring macrophyte taxa before LMB application was *Lemna minor* L., (occurring in 6 out of 8 lakes), followed by *Elodea canadensis* Michx. (5 lakes), *Ceratophyllum demersum* L. and *Nuphar lutea* (L.) Sm. (4 lakes) (Figure 2.2a). The most commonly occurring post-application taxa across the eight surveyed lakes was *Elodea canadensis* (occurring in 6 out of 8 lakes). *Lemna minor*, *Potamogeton pectinatus* L. *and Ceratophyllum demersum*, all occurred in five of the eight surveyed lakes.

All pre-application taxa were also found across waterbodies, post-application. All post-application lakes (12) (Figure 2.2b), showed that *Elodea canadensis* was still the most common species occurring (7 lakes) followed by *Potamogeton pectinatus*, *Ceratophyllum demersum* and *Potamogeton pusillus* L. (5 lakes). Species gained were collectively dominated by charophytes, which were recorded fourteen times across six lakes post-treatment, with six occurrences in four lakes where they were not recorded prior to LMB treatment (grouped species data not shown). In addition to charophytes, *Nitella* spp., established in four lakes and *Potamogeton pectinatus* arose in three lakes post- LMB application which were not recorded pre-application (Figure 2.1a and b).

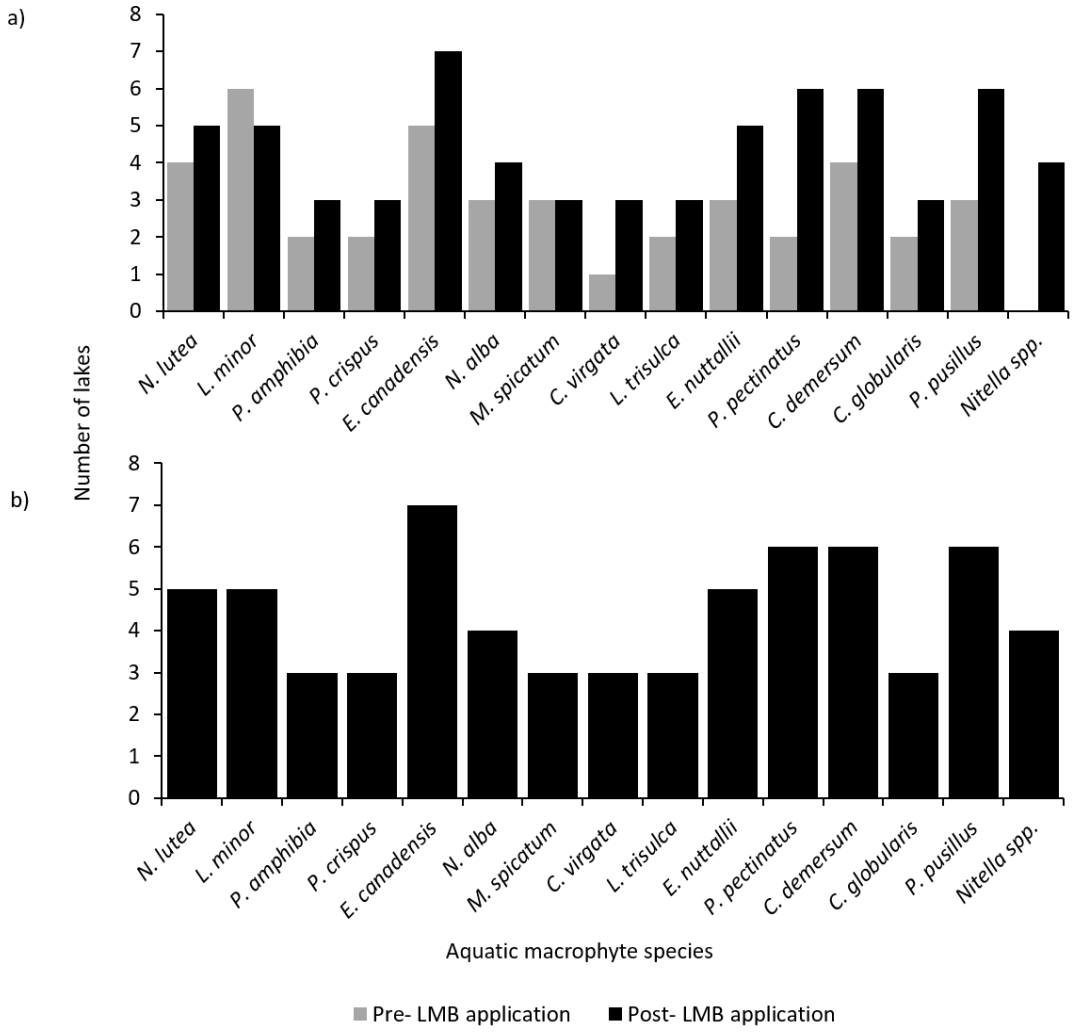



**Figure 2.2.** The fifteen most common macrophyte taxa across eight lakes (pre- and post-) treatment of LMB (a) and across twelve lakes post-application only (b).

### 2.4.2. *Timescale and potential origin of macrophytes gained in LMB treated lakes*

Typically, it took <1-year post-application for some lakes to gain macrophyte species, but waterbodies were specific in timescales with some lakes not gaining any species post-treatment . Loch Flemington was the most isolated waterbody out of the included lakes, with only *Apium inundatum* (L.) Rchb. f. colonising the lake in the first-year post-application. This species can grow via seed or tubers, so it is difficult to say if the propagules were in-situ before application or arrived from external sources. Lake Rauwbraken was the second most isolated. Its community pre-application was a monoculture of *Elodea nuttallii* (Planch.) H. St. John, while *Nitella* spp. established <1-year after LMB treatment. The site became more diverse with time post-treatment,

gaining species such as *Ceratophyllum demersum*, *Potamogeton* and *Utricularia* spp. (data not shown). *Ceratophyllum demersum* does not re-produce via seeds so must have arrived from an external source. Hatchmere and Mere Mere did not gain any macrophytes one-three years post-treatment. Crome's Broad N and S is in close proximity to other similar sized waterbodies and post-treatment both basins had more diverse macrophyte communities compared to the other treated lakes. The aquatic moss *Fontinalis* antipyretica Hedw. established <1-year post-treatment in Crome's Broad N basin. This species can reproduce through spores (Ares et al., 2014) but the establishment of new colonies can originate from detached shoots, leaves and stem fragments (Ares et al., 2014) and is therefore difficult to suggest if it remained *in situ* or was transported to the site. *Potamogeton obtusifolius* Mert. & W. D. J. Koch, *Chara hispida* L., *Chara globularis/connivens* and *Stratiotes aloides* L. all established in the first-year post-LMB application across the N and S basins. These species establish from oospores (Bonis and Grillas., 2002) and vegetatively (asexual reproduction) (Preston and Croft., 2001), respectively, so it is possible they have originated from the seedbank but as Crome's Broad is also in very close proximity to other waterbodies it is difficult to ascertain the origin of these species.

### 2.4.3. *Assessing responses in macrophyte community composition*

The N-MDS exposed few changes in macrophyte community composition following LMB applications (Figure 2.3). macrophyte communities in most lakes were dominated by *Elodea* spp. and *Ceratophyllum demersum*, both before and after LMB applications. Crome's Broad S basin was the only waterbody to express a shift in community composition, with a shift in dominance from *Utricularia vulgaris* L. sensu lato to *Chara virgata* Kütz. three years following LMB application. All other lakes expressed minor, if any, community changes. Some lakes exhibited cyclical shifts in dominant taxa, regardless of application date as indicated by the direction of change in Figure 2.3. Most lakes (including control lakes) exhibited cyclical shifts in dominant taxa.

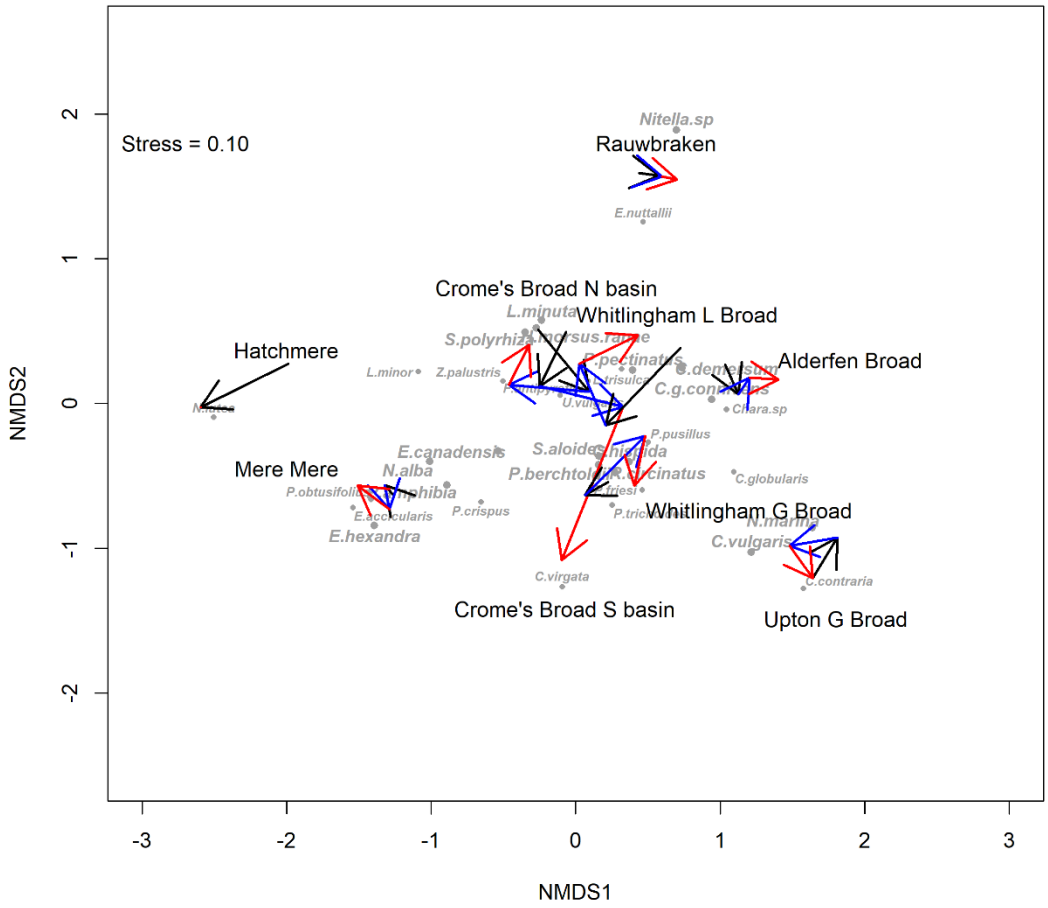



**Figure 2.3.** N-MDS of one year pre- and three years post-LMB application in macrophyte composition (proportion data). The base of the black arrow indicates pre-application conditions and the tip of the arrow indicates the first-year post-application. Blue arrows indicate changes from year one post-application to year two post-application. Red arrows indicate post-application community shifts in years two to three.

### 2.4.4. *Lake macrophyte indices relative to ecological targets*

Slight improvements were observed for seven lakes over the first two years post-application across all lake metrics (Table 2.5). By the third post-application year, all metrics had largely returned to pre-application values. Total percent/total occupancy was the only metric to be consistently higher following the LMB application (Table 2.5 and 2.6). The control lakes exhibited similar minor fluctuations in metrics over the

survey periods with metric values improving slightly in 2013 relative to the pre hypothetical LMB treatment year 2012. Similarly, the total percent/total occupancy values in the control lakes increased post-2012. In general, the improvements noted would be too small to amount to a clear shift in macrophyte-based ecological status in all treated lakes.

The response recovery matrix (Table 2.6) displays each lake's ecological variability across the different macrophyte lake metrics used in this study. Each lake expressed a different ecological response with only Mere Mere demonstrating overall poor macrophyte condition following LMB application. The conditions of Hatchmere and Lake Blankensee did not change over their survey periods. The condition of the remaining treated lakes (62.5% of treated lakes) improved following LMB application and 50% of the control lakes also improved in the same way in their macrophyte responses since 2012. This means only an additional 12.5% improvement in macrophyte responses in lakes treated with LMB in comparison to control lakes that expressed improvements since 2012.

None of the LMB treated lakes monitored as part of the SSSI and WFD programmes reached their set targets following LMB applications (Table 2.6). Crome's Broad declined in its SSSI condition following LMB application and failed to achieve favourable condition status. Alderfen Broad did not change in its SSSI condition over the monitored period. Hatchmere macrophyte classification remained at poor status post-LMB addition and Mere Mere declined from moderate status pre-application to poor status post-application. Upton Great Broad improved from moderate to good macrophyte status post-2012 but was the only control site to be monitored as part of the WFD.

**Table 2.5.** Average metric scores and standard deviations for seven lakes (Mere Mere, Hatchmere, Loch Flemington, Crome's Broad N and S, Lake Rauwbraken and Lake Belhendorfer See) pre- and post- application and hypothetical pre- and post- application values for all control lakes

| Survey number (lake number) | LMNI | NFG | NTAXA | Total % cover/% occupancy | Range of year of survey |
|---|---|---|---|---|---|
| Pre- application survey (7) | 6.3 ± 2.1 | 5 ± 3 | 7 ± 4 | 84.2 ± 54.4 | 2004 – 2012 |
| 1st year post- application (7) | 6.1 ± 1.8 | 5 ± 3 | 7 ± 4 | 136.5 ± 101.6 | 2008 – 2013 |
| 2nd year post- application (7) | 6.2 ± 1.8 | 5 ± 3 | 8 ± 4 | 164.4 ± 168.6 | 2009 – 2014 |
| 3rd year post- application (7) | 6.3 ± 1.8 | 5 ± 2 | 7 ± 3 | 98.4 ± 149.1 | 2010 – 2015 |
| All years post- application (12) | 6.7 ± 0.8 | 5 ± 2 | 7 ± 4 | 131.8 ± 122.9 | 2008 – 2017 |
| Hypothetical pre- application survey for control lakes (4) | 6.5 ± 0.8 | 4 ± 1 | 6 ± 3 | 61.4 ± 42.1 | 2012 |
| 1st hypothetical year post- application for control lakes (4) | 6.7 ± 0.5 | 5 ± 2 | 7 ± 4 | 114.4 ± 6.2 | 2013 |
| 2nd hypothetical year post- application for control lakes (4) | 6.6 ± 1.0 | 3 ± 1 | 7 ± 3 | 173.1 ± 62.3 | 2014 |
| 3rd hypothetical year post- application for control lakes (4) | 6.6 ± 0.9 | 4 ± 2 | 7 ± 6 | 158.7 ± 85.7 | 2015 |
| 4th hypothetical year post- application for control lakes (4) | 6.2 ± 0.8 | 5 ± 1 | 9 ± 3 | 164.3 ± 34.7 | 2016 |
| 5th hypothetical year post- application for control lakes (4) | 6.5 ± 0.7 | 4 ± 1 | 8 ± 2 | 148.1 ± 31.3 | 2017 |
| All hypothetical years post- application for control lakes (4) | 6.5 ± 0.7 | 4 ± 1 | 8 ± 3 | 151.7 ± 50.6 | 2012 – 2017 |

**Table 2.6.** Responses relative to pre-application conditions in macrophyte WFD metrics and UK and EU ecological targets.

| Lake (number of surveys, number of years) | Lake metrics | | | | Overall lake response summary | Meeting SSSI objectives post- app. | Meeting WFD macrophyte status objective post-app. |
|---|---|---|---|---|---|---|---|
| | LMNI | NTAXA | NFG | Total % cover/ % occupied | | | |
| | Overall post- app. | Overall post-app. | Overall post-app. | Overall post- app. | | | |
| Hatchmere (3, 3) | → | → | → | ↑ | → | - | → Poor |
| Mere Mere (3, 3) | ↓ | ↓ | ↓ | ↑ | ↓ | - | ↓ Poor |
| Loch Flemington (3, 6) | ↑ | → | ↑ | ↓ | ↑ | - | - |
| Crome's Broad N basin (5, 5) | ↑ | ↑ | ↑ | ↑ | ↑ | ↓ UD | - |
| Crome's Broad S basin (5,5) | ↑ | ↑ | ↑ | ↑ | ↑ | ↓ UD | - |
| Lake Rauwbraken (10, 10) | ↑ | ↑ | ↑ | ↑ | ↑ | - | - |
| Blankensee (2, 5) | → | → | → | ↑ | → | - | - |
| Behlendorfer See (5, 6) | ↑ | ↑ | ↓ | ↑ | ↑ | - | - |
| Alderfen Broad (5, 5) | ↓ | ↓ | → | ↑ | ↓ | → UR | - |
| Upton G Broad (5, 5) | ↓ | ↑ | → | ↑ | → | - | ↑ Good |
| Whitlingham G Broad (5, 5) | → | ↑ | ↑ | ↑ | ↑ | - | - |
| Whitlingham L Broad (5, 5) | ↑ | ↑ | ↑ | ↑ | ↑ | - | - |

For all metrics ↑ /green = improved score, → /amber = no change, ↓ /red = decrease in score.
For SSSI and WFD designations, arrow indicates a change in status ↑ = increase in condition/status objective, → = no change in condition/status, ↓ = not meeting condition/status objective. UD = Unfavourable declining condition, UR = Unfavourable recovering, Poor = Poor WFD status, Good = Good WFD status.
Colour indicates if lake is meeting targets, red = no, amber = not yet, green = meeting target.

## 2.5. Discussion

### 2.5.1. *Species changes*

Macrophytes typically associated with ‘moderate’, ‘poor’ and ‘bad’ WFD statuses (Poikane et al., 2018) were present at seven out of the twelve treated lakes up to ten years post-treatment. Predominantly eutrophic/mesotrophic species dominated LMB treated lakes after application, as they had done pre-treatment. LMB lakes did, however, have more eutrophic/mesotrophic species appearing at sites where they were not present before application. *Elodea canadensis* remained the most dominant submerged macrophyte species following LMB treatments. Species gains, typically, took greater than one-year post-treatment to appear across the six lakes. Charophyte species appeared in some lakes. Charophytes have been recorded as first colonisers in other restored lakes (Hilt et al., 2018; Waajen et al., 2016a) as have *Elodea* spp. (Immers et al., 2015; Perkins and Underwood, 2002; Strand and Weisner, 2001; Waajen et al., 2016a). New colonisations by *Nitella* spp. and other charophytes is most likely due to dormant oospores in the sediment that have germinated due to more favourable conditions. Charophyte oospores and *Potamogeton* spp. can be viable at high densities for many years in lake sediments (Bakker et al., 2013; Alderton et al., 2017 and references therein) compared with the seeds of other aquatic plants (Bonis and Grillas, 2002; De Winton et al., 2000). Species gains, which took on average greater than one year to appear, are more likely due to dispersal from external sources and their survival may reflect improved lake conditions. However, the origin of re-colonising macrophytes following restoration is difficult to discern and is often unknown (Bakker et al., 2013).

Despite some species gains, few changes towards more desirable species were observed across all lakes following the LMB applications. Recovery timescales for macrophyte recorded before major nutrient enrichment began, are estimated to be so long it is possible that lakes, which historically supported species that are now rare, or locally extinct, will never fully recover by natural colonisation (Sand-Jensen et al., 2017). Furthermore, because eutrophication is such a worldwide phenomenon it is likely that certain species are rare nationally and colonisation through dispersal is, therefore, limited. These assumptions are also true for mesotrophic and oligotrophic macrophyte species, as more lakes succumb to eutrophication through human population increase and land-use change, the abundance of these species will become sparser and the ability to re-colonise through normal pathways will become more restricted. Isolation may be inhibiting more advanced signs of macrophyte

recovery by a lack of dispersal vectors in treated lakes, but data insufficiencies make it hard to verify colonisation sources and this theory needs more rigorous testing. LMB offers the opportunity to assess macrophyte recovery (Figure 2.4) from external vs seedbank sources and to compare the speed of colonisation in isolated and more connected waterbodies. Initially, in-lake mesocosm studies maybe be the best way to approach this.

We would have expected to see more diverse communities appearing following improved lake conditions according to the chemical improvements reported for these treated lakes (Spears et al., 2016) which were within ecologically relevant P ranges to witness macrophyte species richness improvements (Jeppesen et al., 2000; Spears et al., 2016). Crome's Broad S shifted in its community from *Utricularia vulgaris* to *Chara virgata* post-treatment (Figure 2.3). Although, *Utricularia vulgaris* reportedly increased again in 2017 and 2018 (data not shown) (Broads Authority., 2018). However, most of the lakes had high community proportions of *Elodea* spp. and *Ceratophyllum demersum* pre- and post-application. Crome's Broad N did, however, exhibit shifts in dominance, switching from *Ceratophyllum demersum* to *Elodea* spp. and back to *Ceratophyllum demersum* in the short-term but this response was also seen in two (Alderfen Broad and Whitlingham Little Broad) out of four of the control lakes (Figure 2.3) and it is, therefore, impossible to attribute this behaviour to the LMB treatment. For the treated lakes that did not have recurring change in macrophyte taxa, it is also difficult to say if there were cyclical shifts pre- LMB treatment and if so, whether LMB has interfered with these changes in macrophyte species dominance post-treatment, due to the lack of long-term pre-application monitoring data or repeated applications for these treated lakes. High community dominance of *Ceratophyllum* and *Elodea* spp. have been found to be unstable, and large populations frequently collapse from one year to the next, as seen in other lake restoration studies (Lauridsen et al., 1994, 2003a; Ozimek et al., 1990; Sand-Jensen et al., 2017) and in one of our control sites, Alderfen Broad (Hilt et al., 2018), which was monitored over a longer period than in our study. Lakes with species-poor macrophyte communities can adhere to long-term cycles influenced by climate with increased abundance in warmer, sunnier years (Phillips et al., 2016; Rooney and Kalff, 2000). The random fluctuations in community composition are likely due to annual changes in precipitation or sunshine hours that favour the growth strategies of different macrophyte species. Species with particularly high production and an ability to overwinter could dominate the next year's community composition, e.g. *Elodea*

*canadensis,* is a better coloniser in summer and autumn (Trémolières, 2004). Changes in water transparency or herbivory also drive inter-annual variability and could, therefore, be to blame (Bakker et al., 2013; Hilt et al., 2018; Søndergaard et al., 2008). All lakes (both treated and control) displayed fluctuations in macrophyte composition between years, and the changes seen across the average metric scores make this noticeable (e.g. Table 2.3). Large inter-annual fluctuations, some of which can last decades have been witnessed elsewhere (Blindow et al., 2002; Hansel-Welch et al., 2003; Lauridsen et al., 2003a; Mäemets et al., 2006; Rip et al., 2007; Sayer et al., 2010b; Strand and Weisner, 2001; Titus et al., 2004). Despite these possibilities for 'boom-and-bust' cycles, repeated collapses often occur unexpectedly (Simberloff and Gibbons, 2004). Changes in abiotic and/or biotic factors are most likely responsible but data are commonly insufficient to confirm cause-effect (Titus et al., 2004).

It is difficult to assign with confidence macrophyte community responses to management interventions from single surveys against pre-application data due to inter-annual variation. This is especially true where stochastic fluctuations have not been characterized (Capers, 2003; Mäemets et al., 2006). The timelines used in our NMDS analysis were short and only show a recovery snapshot. Our results are comparable with the few other single site assessments of macrophyte responses following LMB application (Gunn et al., 2014; Waajen et al., 2016a). They confirm generally that species compositions are unlikely to improve in line with conservation targets up to three years following an LMB application.

Weak responses were observed for all WFD metrics following application. The percent cover/percent occupancy metric, which improved the most in the short and long-term, had high variability, probably due in part to the two different methods used to derive cover values from different survey methodologies. Regardless, the minor improvements reported were insignificant with respect to WFD targets. SSSI targets were also not met for Crome's Broad. This decline was a result of a raised awareness and evidence of high nutrient inputs and risk of this factor, not as a result of the LMB treatment or condition of the lake (Kelly, pers.com 30/04/2020). Given that each lake responds differently to nutrient reduction (Lauridsen et al., 2003a; Spears et al., 2016), there is no surprise that macrophyte recovery also appears lake-specific, regardless of the timescale considered. However, it should be noted that only one site for SSSI condition and two sites for WFD status could be assessed due to designations and data availability.

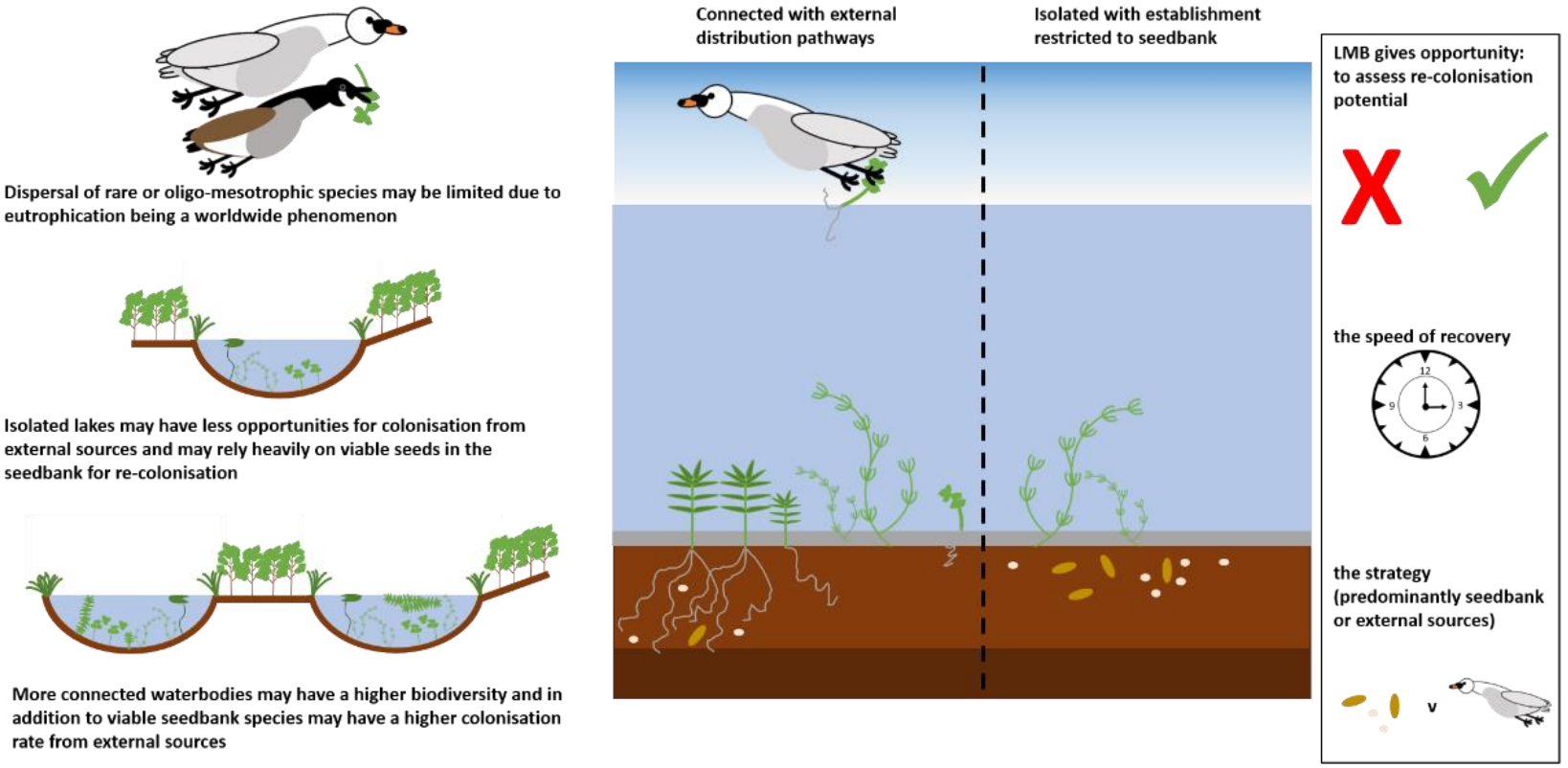



**Figure 2.4.** Diagram of how LMB may give opportunities to study macrophyte recovery potential, the speed of recovery and the strategy (e.g. seedbank versus external sources) through improved water quality conditions following application (© Kate Waters-Hart).

### 2.5.2. *The reasons for recovery delays and limitations*

macrophyte recovery does not always happen quickly, as found in other nutrient reduction studies, which indicate average recovery times of >10 years (Eigemann et al., 2016; Hilt et al., 2018; Jeppesen et al., 2005; Murphy et al., 2018; Sand-Jensen et al., 2017). Some lakes have shown no signs of recovery following water quality improvements (Jeppesen et al., 2005; Lauridsen et al., 2003a) and often lake-specific responses are reported (Eigemann et al., 2016; Jeppesen et al., 2005; Spears et al., 2016). It is extremely difficult, therefore, to predict the effectiveness of lake remediation efforts to restore macrophyte communities to predefined targets, especially where the processes underpinning recovery are often unclear (Søndergaard et al., 2007). Interventions must create improved water quality to encourage re-colonisation and community development. Reduced operational performance of Lanthanum (La) as a result of interactions with humic substances has been reported in other studies (Copetti et al., 2016; Lürling et al., 2014; Lürling and Faassen, 2012a; Spears et al., 2016). Persistent elevated nutrient loading from the catchment may also confound local water quality responses (Lürling and van Oosterhout, 2013), and these factors are discussed in detail for many of the lakes considered in this study by Spears et al. (2016). An absence of viable propagules (Bonis and Grillas, 2002), a lack of external distribution pathways (Sand-Jensen et al., 2017; Soomers et al., 2010), herbivore grazing (Lauridsen et al., 2003b, 1993; Søndergaard et al., 2000, 1996a) from birds (Green et al., 2002) and fish (Pollux, 2011), the presence of invasive non-native species (Bakker et al., 2013) and benthic or epiphytic filamentous algae (Irfanullah and Moss, 2004) may also confound macrophyte recovery, following water quality improvements. It is also important to be patient when tracking recovery; it is suggested that transient recovery periods for macrophyte communities in lakes could last for 2-40+ years (Verdonschot et al., 2013). Monitoring programmes, therefore, need to be designed to last (i.e. use future-proof methods) and not abandoned prematurely.

Most studies choose reference lakes that are ‘minimally impacted’ which is a subjective definition of a reference lake (Growns et al., 2013), but truly un-impacted reference lakes are becoming increasingly rare to find that have both long-term data and that are accessible. Indeed, the lakes presented here as control lakes have their own history of interventions (Kelly, 2008). The responses reported here represent the most comprehensive assessment of macrophyte community compositional changes following LMB addition in the short- and long-term and our results raise issues that

should be considered when using LMB to try to achieve rapid ecological recovery. We acknowledge the limitations of the data available. Specifically, the lack of long-term consecutive annual macrophyte surveys that severely limits detection of recovery in macrophyte communities. The monitoring frequency used for these LMB study lakes and others (Spears et al., 2016, 2013a) has been highly variable, with most restoration programmes recording more post- than pre-application data (e.g. Figure 2.1). Similarly, the variation in methodologies across the LMB study sites was significant, which presents a problem when attempting to draw general responses across lakes (Penning et al., 2008; Poikane et al., 2018; Zhang et al., 2017). Other studies have called for standard survey methodologies in this respect (Spears et al., 2016) and without these we either lose power in our quantitative analyses or the reponse variables that can be compared are constrained.

### 2.5.3. *Implications for the use of LMB to force ecological recovery in other lakes*

It was expected that LMB treated lakes would follow broadly similar recovery trajectories, in line with P reduction, as reported by Jeppesen et al. (2000). Despite the limitations of the data, our results indicate that responses were not consistent across lakes, following LMB applications. As such, it is unlikely that LMB can be used to force ecological recovery in macrophyte communities in the short-term in line with specific ecological quality targets, even though water quality itself may be improved. It is possible that recolonisation is the time-limiting factor here and that most lakes exhibit different responses as they are starting their recovery journey with different pioneering communities and underlying seedbank compositions and different levels of connectivity to external propagule sources. It is possible that these constraints can be addressed, for example, through macrophyte transplantation. There are also uncertainties on how LMB directly impacts macrophyte communities during the recovery period, with particular interest in, for example, the effects of the active layers laid down by repeated applications on the seedbank (Hilt et al., 2006). It is also plausible that LMB may smother growing macrophytes, which may interfere with physiological processes. An application could therefore, potentially negatively impact species that require high light levels as the product may reduce light reaching leaf surfaces. Despite the extensive literature on the toxicity of La to certain freshwater biota (summarised in Copetti et al., 2015; NICNAS, 2001; Spears et al., 2013), there is very little evidence on the toxicity to macrophyte (Copetti et al., 2015) for both La

and the LMB product. There is also potential for LMB to limit recovery of macrophyte through P limitation from applications. All these potential negative impacts need further investigation to either eliminate or contribute to explaining the lack or limited recovery of macrophyte to LMB treated waterbodies. Given the task of reaching ecological quality targets, it is important that both macrophyte recovery successes and failures are reported following lake remediation measures to allow lake managers to assess cost effective measures. Given the long recovery times associated with macrophytes, recovery timescales of relevance should be defined during restoration planning and this should be used to inform appropriate monitoring programmes.

### 2.5.4. *Implications for monitoring*

It was clear that insufficient data hindered our ability to robustly assess macrophyte responses to LMB applications. We call for standard methodologies to be put in place pre- and post-applications to be able to measure responses at the individual lake scale, but also across multiple treated and control lakes across Europe. Methodologies should allow for comparability across countries to maximize the ability to assess responses. WFD member states have had problems with comparing methodologies across countries due to the lack of specified and general methods used to assess ecological status. We advocate simple methods that generate high quality data with minimal effort and low cost. We recommend following CSM methods (JNCC, 2015) for standing open water to assess macrophyte communities. From conducting CSM macrophyte surveys, water quality/lake managers can input annual macrophyte data from surveys into macrophyte WFD metrics to create an annual assessment of whether their lakes are meeting WFD ‘good ecological status’. Annual macrophyte assessments are particularly valuable after implementation of restoration measures to be able to assess macrophyte responses. Segregated and poorly harmonised data inhibits the ability to effectively link ecological change to restoration methods used. Long-term monitoring is necessary given the lack of responses seen in the macrophyte communities of LMB treated lakes and the monitored timescales in this study (0 – 10 years post-application).

## 2.6. Conclusions

Lakes exhibited specific macrophyte recovery pathways following LMB application. Little change in community composition was evident with only a few additional desirable species across twelve treated lakes up to ten years following application. Community composition did not change up to three years following LMB applications (n = 5 lakes) and most lakes showed signs of cycles in dominant taxa, unrelated to LMB. Average macrophyte lake metrics (n = 7 lakes) did not show signs of general improvement 1 – 4 years post LMB application. Individual lake macrophyte lake metrics (n = 8) expressed specific lake recovery trajectories (1 – 10 years post application) with individual lakes failing to meet UK (n = 1) and EU (n = 2) set targets. A lack of external dispersal vectors could impact macrophyte re-establishment, particularly in isolated waterbodies. All lakes (n = 12) varied considerably in monitoring length and methodologies used. It is recommended that standard monitoring protocols and methodologies be adopted to encourage future multi-lake assessments for macrophytes and other biological indicators.

# 3. Chapter 3: To grow or not to grow: assessing the ability of aquatic macrophytes to germinate after lanthanum-modified bentonite (Phoslock®) treatment

Kate E. Waters-Hart [a & b], Nigel Willby [b], Lorna Eades [c], Iain D. M. Gunn [a], Genevieve Madgwick [d], Justyna Olszewska [a], James Watt [a & c], Said Yasseri [e], Iain Sime [f] and Bryan M. Spears [a]

[a] UK Centre for Ecology & Hydrology, Penicuik, Midlothian, EH26 0QB, UK

[b] University of Stirling, Stirling, FK9 4LA, UK

[c] University of Edinburgh, School of Chemistry, Kings Buildings, West Mains Road, Edinburgh, EH9 3JJ

[d] Natural England, Eastbrook, Shaftesbury Road, Cambridge, CB2 2DF, UK

[e] Limnological Solutions International Pty Ltd, Admiralstr. 150a, 28215 Bremen, Germany

[f] Scottish Natural Heritage, Great Glen House, Leachkin Road, Inverness, IV3 8NW, UK

The candidate, as lead author, undertook the experimental design, sample collection, preparation and led the laboratory determination of nutrients and metals. The data analysis and writing were carried out by the candidate. Co-authors provided support and guidance on the scope and design of the project, sediment collection, laboratory analysis of nutrients and provided expertise in inductively coupled ion chromatography (ICP-MS) and ICP-MS methods. The co-authors contributed to the editing of the manuscript.

Acknowledgements: Thanks to Stephen Ives for help with the macrophyte survey of Airthrey Loch.

## 3.1. Abstract

Many Lanthanum-modified bentonite (LMB) applications in lakes have led to reduced phosphorus concentrations resulting in higher water transparency and reduced chlorophyll-*a* concentrations, conditions that should support recovery of aquatic macrophyte (macrophyte) communities. However, a delay or non-recovery of macrophyte is common and the reasons for this are unclear. We assessed the possibility that LMB may cause a barrier for germinating macrophyte propagules in lake bed sediments. A 21-week germination trial using lake bed sediments from a eutrophic lake, Airthrey Loch, United Kingdom (U.K.) confirmed that responses in macrophyte species richness and biomass did not vary following the application of LMB in comparison to untreated controls. However, LMB significantly reduced the biomass of algae ($p < 0.01$; n=24). *Spirogyra* was significantly reduced in both the water column ($p < 0.001$; n=24) and on bed sediments ($p < 0.01$; n=24) in the LMB treatment with higher algal biomass measured where sediment bioturbation was greatest ($p < 0.05$; n=3). Charophytes were the most common species to germinate in both control and LMB treated containers. This laboratory experiment indicated that LMB is unlikely to inhibit macrophyte recovery through limiting germination from a viable seedbank, and, may, ultimately, prove favourable if smothering by algae is reduced. However, we recommend that seedbanks are first investigated and their germination potential confirmed in the laboratory to support the use of LMB in achieving ecological recovery, especially when being considered for use in supporting macrophyte species conservation.

## 3.2. Introduction

One of the commonest objective of any lake restoration project is to establish desirable communities of aquatic macrophytes (macrophytes) due to their essential role in promoting clear water conditions and supporting lake structure and function (Blindow et al., 2002; Carpenter and Lodge, 1986; Coops and Doef, 1996; Jeppesen et al., 1997). However, there have been few reports in the literature of full macrophyte community recovery following lake remediation efforts (Gunn et al., 2014; Immers, 2014; Jeppesen et al., 2005; Lauridsen et al., 2003a; Spears et al., 2016), despite in the majority of cases, nutrient load being reduced sufficiently to promote an increase of macrophytes (Jeppesen et al., 2000). In many cases macrophytes are not factored into monitoring programmes (Hilt et al., 2006) and, therefore, the community

responses to remediation efforts are poorly understood (Bakker et al., 2013). Of the restoration projects that have monitored macrophytes, many only reported short-lived success (Hilt et al., 2018) or failure (Jeppesen et al., 2005; Lauridsen et al., 2003a), often attributed to phosphorus (P) retention and release from lake bed sediments (Søndergaard et al., 2007). Phosphorus can be retained in lake sediments during periods of elevated P loads from catchments and can be subsequently released to the overlying water column (i. e. internal loading) perpetuating poor water quality for decades following catchment load reductions (Jeppesen et al., 2005; Meis et al., 2012; Søndergaard et al., 2007).

Phosphorus-sorbing products such as Lanthanum (La)-modified bentonite (LMB) have been used to limit the effects of internal loading. Reduced total phosphorus (TP), soluble reactive phosphorus (SRP) and chlorophyll-*a* (Chl-*a*) concentrations have been reported across many case studies (Copetti et al., 2016; Spears et al., 2016). Despite chemical improvements, ecological recovery following LMB application is rarely reported. The few studies that have produced data on ecological recovery report only short-lived and weak responses in macrophyte communities (Gunn et al., 2014; Spears et al., 2018, 2016). This apparent lack of recovery following LMB application could be due to several potentially confounding factors related to product efficiency, for example, continued high P loads from the catchment (Lürling and van Oosterhout, 2013; Spears et al., 2018), an ineffective calculated dose of LMB (Meis et al., 2013), and the presence of humic substances that limits the absorption kinetics and capacity of La for SRP (Copetti et al., 2016; Lürling et al., 2014; Spears et al., 2018, 2016). However, where LMB applications have been demonstrated to be effective, ecological factors may constrain macrophyte recovery including limited dispersal pathways (Sand-Jensen et al., 2017; Soomers et al., 2010), sediment re-suspension preventing macrophyte rooting (Bornette and Puijalon, 2011), herbivory reducing biomass (Lauridsen et al., 2003b, 1993; Søndergaard et al., 2000, 1996a), phytotoxic effects of free La ions ($La^{3+}$) (Copetti et al., 2016), a lack of viable seeds (Bonis and Grillas, 2002) and the burial of seeds to depths below which germination can occur (Bonis and Lepart, 1994). A further complicating factor for seed germination is the presence of benthic algae. If benthic algae establishes before seeds can germinate, it gains an advantage in terms of nutrient uptake and subsequent anoxia may cause low germination rates (Asaeda et al. 2007). However, the specific effects of LMB on benthic algae are unknown.

Germination rates following LMB application to lakes have not previously been

reported. macrophytes have returned to LMB treated lakes following applications (Spears et al., 2016: Waajen et al., 2016a) but the origin of these colonisations is unclear; did plants establish from the seedbank, from external sources or from existing populations that were undetected prior to the applications? The addition of a few millimetres of LMB to the sediment surface could bury some propagules enough to impede germination (Hilt et al., 2006). This may also alter community composition in lakes following applications, as species-specific responses should occur. Species, which require lower light levels to initiate germination, may dominate over those that need to be closer to the sediment surface. We would, therefore, expect species such as certain charophytes, which have been known to germinate in low light levels and across different sediment depths (Bonis and Grillas, 2002), to gain an advantage over species such as *Nymphaea alba* L., *Nuphar lutea* (L.) Sm. and *Nymphoides peltata* Kuntze that require higher light levels to germinate (Smits et al., 1990). However, if certain canopy-forming species, such as *Potamogeton pectinatus* L. emerge first they could also suppress other macrophytes and charophyte growth (Van Den Berg et al., 1998a). Additionally, species that have a long-lived 'persistent' seedbank, as opposed to a 'transient' one, may dominate (Grime, 1979). Charophytes, in particular, have persistent oospores in lake bed sediments and produce a high number of oospores, and, therefore, might be expected to dominate (Bonis and Lepart, 1994). Charophytes can tolerate fluctuations in their environment and have been reported to germinate in response to disturbance (Phillips et al., 2015). Since LMB addition could be described as a disturbance, in accordance with the definition of Grime (1979) due to P limitation, charophytes might be expected to dominate emergence. The limitation of P through an LMB application could also alter community composition of germinating macrophytes. Species that require lower P concentrations might germinate more easily than P demanding species; again, charophytes may, therefore, dominate in response to lower P concentrations (Van Den Berg et al., 1998b).

LMB may also cause higher La concentrations in the water column as La concentrations have reported to be elevated in some cases from 8 - 12 months after an application in surface and bottom waters, respectively (Spears et al., 2013a). Higher La concentrations pose the potential risk of $La^{3+}$ in the water column that may cause a toxicity risk to germinating macrophytes but there are very few toxicological experiments on submerged macrophytes in the literature using LMB (Copetti et al., 2016) or any physical signs of toxicity symptoms described for macrophytes. We, therefore, assume that more stress-tolerant species may dominate amongst the

germinated community if higher La concentrations prevail post-treatment.

An assessment of seedbank propagules can inform the prediction of the composition of future macrophyte communities (Leck and Graveline, 1979). The presence of a viable seed bank may be critical to support ecological recovery where contemporary communities have been lost, for example, as a result of eutrophication. These propagule reserves help to preserve lake biodiversity, as some seeds can persist and lay dormant in lake bed sediments for decades (Bonis and Grillas, 2002). Successful germination from the seed bank can be species-specific and regulated by seed burial depth, light and nutrient availability (Sederias and Colman, 2009, 2007). Seedbank germination trials on submerged macrophytes from lakes in the literature are scarce (Bakker et al., 2013) with the majority of studies assessing terrestrial, wetland or riparian seed banks (Bakker et al., 2013; Leck and Graveline, 1979). Despite this, there are a few studies on lakes that have assessed macrophyte germination following nutrient reduction (Ozimek, 2006), changes in water levels (Harwell and Havens, 2003), biomanipulation (Strand and Weisner, 2001), aquaculture management regimes (Xiao et al., 2010) and in response to invasions (De Winton and Clayton, 1996). Other studies have assessed seed dormancy or species-specific germination requirements (De Winton et al., 2000; Smits et al., 1990). Few studies use submerged lake bed sediments, possibly, because common germination emergence methods have been developed for terrestrial vegetation and no standardized method is available for assessing submerged sediment propagule banks (Bakker et al., 2013). In addition, low numbers of emerging seedlings, long seed dormancy, species-specific germination cues and the fact that many submerged species also reproduce vegetatively (De Winton et al., 2000), make working with submerged macrophytes particularly challenging, perhaps, contributing to the lack of evidence in the literature.

We addressed these knowledge gaps by designing a laboratory seed bank germination experiment to assess the effects of LMB on macrophyte community emergence. The experimental system was used to determine whether LMB application altered the composition of macrophyte communities emerging from seed banks and to investigate the effects of benthic algae, nutrient limitation and La toxicity on macrophyte emergence, as outlined above. We discuss the implications of our results in the context of macrophyte species conservation in lakes, a major driver in contemporary lake restoration efforts globally.

## 3.3. Methodology

### 3.3.1. *Sediment propagule bank collection*

Lake bed sediment containing macrophyte propagules was collected in March 2016 before spring germination from three areas of Airthrey Loch, a small (7 ha), shallow (mean depth 1.7 m), eutrophic lake in Stirling, Scotland. The locations of sampling points and the experimental design is detailed in Appendix 2., Figure 1 (Site 1 (S1), Site 2 (S2), Site 3 (S3)). Mean depth varied by sediment collection site (S1 = 1.0 m, S2 = 1.0 m, 0.9 m). A 2 m long 7 cm diameter plastic corer was used to collect the top 4 – 6 cm of sediment from approximately 40 cores in each of the three sampling locations on 21/03/2016 before spring emergence. Different sediment collection sites were used as opposed to whole-lake random sediment sampling as we wanted to compare extant vegetation with germinated populations from roughly the same area of the lake as propagules have a patchy distribution in sediments and can, therefore, incur high variance amongst small sample sizes (Hammerstrom and Kenworthy, 2003). We, therefore, chose specific areas to sample so we would have more confidence that the same species would be in all treatments and present during established vegetation surveying (method described in section 3.3.4.) and not to bias one treatment having a higher macrophyte diversity over another. The sediment from each of the three areas was placed into three containers for homogenisation. Approximately 300 L of lake water was collected prior to sediment collection. Both sediment and water were kept in the dark at 4˚C until processing. The sediment was sieved using a 4 mm sieve to remove large stones, vegetative fragments and organic debris but leaving turions and seeds.

### 3.3.2. *Germination trial*

The homogenised sediments from S1, S2 and S3 were separated into 12 subsamples per site and spread to a thickness of 1.0 -1.5 cm (~289 $cm^3$) over 17.0 cm x 17.0 cm x 27.5 cm clear containers which were previously filled with 4 cm of sterilized fine aquarium sand (~1,156 $cm^3$). Aquarium sand was used to add adequate depth for macrophytes to root. All sand was soaked in boiling water for 10 minutes to remove residue and to ensure sterilisation; this process was repeated three times. Containers were placed on a bench in a greenhouse in a randomised layout. Airthrey Loch water was sieved through a 2 mm sieve before being added to containers to ensure removal of any debris and vegetative parts. Each container had 8 L of sieved water gently

added by pouring over bubble wrap to minimise sediment disturbance. Cling film was placed over each container with five air holes in each to allow gas exchange and to prevent evaporation and the entry of debris into the containers. The containers were then left for 24 hours to settle. The 12 containers from each propagule collection site were randomly assigned a treatment, control, LMB addition or algal removal. An application of 14.7 g of LMB (equivalent to 5.1 tonnes per hectare) was then applied to the LMB treatment containers. The calculated dose was based on surface area loads of LMB to 18 lakes listed in Spears et al. (2016). The 75$^{th}$ percentile of these 18 treated lakes was used here to account for potentially higher P concentrations in the sediment due to homogenisation across sediment depths of up to 6 cm of collected sediment. There is still currently no formal way of calculating LMB dose (Spears et al., 2014); the primary focus here was to assess how LMB impacts germination rates rather than P up-take that is dealt with extensively elsewhere (Copetti et al., 2016; Spears et al., 2016). LMB was applied by taking a small amount of lake water from each container and mixing it with LMB granules to form a slurry. The slurry was then applied to the container.

Control and LMB addition treatments were initiated in April 2016. However, the algal removal treatment was initiated in June 2016. The algal removal treatment was set-up additionally alongside the control treatment as it was noticed soon into the experiment that benthic algal growth had established in control containers. To prevent any treatment bias effects on gemination success due to benthic algae potentially inhibiting germination in the control, the algal removal treatment was set up. This involved preparing another 12 containers filled with sand and the same sediment collected, and water collected in March 2016, as above, both of which had been treated, as above, and stored at 4˚C in the dark until initiation. The algal removal treatment involved removing floating algae or algal growth from the water column with a small aquarium net weekly. Benthic algae growing directly on the sediments were not removed as we did not want to disrupt the sediment layer. All treatments were run for 21 weeks, with the control and LMB treatments finishing in September 2016 and the algal treatments finishing in October 2016, the latter running later to account for the phased treatment initiation. The experiment followed the general method of Thompson and Grime (1979) that allows for an assessment of the ‘active seed bank’ or the ‘ecologically active component of the seed bank’ (Haag, 1983) in lake sediments. At the end of the experiment, the percent volume of the water column inhabited by emergent macrophytes (PVI) was estimated. Algal growth in the water

column and benthic algae growing on the sediment also had PVI scores measured. macrophytes were identified to species level, where possible, and dried at 75˚C for 48 hours, weighed and then re-died for a further 48 hours and then weighed to give dry weight (g), which included leaves, stems and roots.

### 3.3.3. *Chemical parameters and additional measurements*

At the end of the experiment, unfiltered water was removed 1 – 2 cm above the sediment surface for TP (µg $L^{-1}$) and total La (TLa) (µg $L^{-1}$), with subsamples being filtered using a Whatman GF/F filter, pore size 0.7 µm for SRP (µg $L^{-1}$). All filtered and unfiltered samples were immediately frozen at -18˚C until processing. All water sample analysis methods for P (TP and SRP) (Appendix 2.1.1) and TLa (Appendix 2.1.2) are detailed in Appendix 2.1.

Relative depth change of the LMB layer was measured as an indicator of bioturbation at the end of the experiment for the LMB treated containers, only.

### 3.3.4. *Airthrey Loch survey to assess extant macrophyte populations*

Boat survey transects were carried out in September 2016 to assess extant macrophyte populations in Airthrey Loch in accordance with the Common Standing Monitoring (CSM) guidance for freshwater lakes (JNCC, 2015). One boat transect was conducted in each of the three sediment collection areas in order to be able to compare the extant macrophyte populations with the macrophyte populations from the germination experiment. Each boat transect was split into approximately 20 sections. Within each of the 20 sections a double-headed-grapnel was thrown 1 m from the boat and the macrophyte retrieved were recorded as macrophyte abundance (score of 0 – 3) along with a record of all species present and the percent of algal cover (score of 0 - 3). Scores were converted into percent volume inhabited (PVI) along each transect for each recorded species.

### 3.3.5. *Statistical analysis*

Variation in water chemistry determinants with treatment type and sample site was evaluated using two-way analysis of variance (ANOVA). Prior to analysis, data were checked for normality and homogeneity of variance. If models did not meet these

assumptions, data was transformed (log (n+1) or sqrt (n+1)). Where transformations still did not meet model assumptions then the non-parametric Kruskal-Wallis test was performed. If the Kruskal-Wallis test was used, *p* value correction was performed using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) to avoid the event of Type 1 errors occurring, as the control treatment was used twice in statistical testing separately for LMB and algal removal treatments. Tukey's Post Hoc analyses were performed to identify where significant differences were reported between treatments and where interactions were significant ($p = \leq 0.05$). If treatments were assessed using the Kruskal-Wallis test, interactions were assessed using the aligned rank transformation method (Leys and Schumann, 2010) with *p* adjustment. If interactions were significant ($p = \leq 0.05$) the Dunn test was performed to assess treatment effects.

ANOVAs were used to assess if LMB inhibited germination or enhanced germination by comparing control and LMB containers for macrophyte species richness and total macrophyte species biomass (PVI and total dry weight (g)). ANOVAs were also used to assess if LMB reduced algal biomass in the water column and on the sediment surface across sediment collection sites, comparing total, water column, benthic and species-specific algal biomass (PVI). ANOVAs were similarly used to assess if algae impacted germination success in the controls by comparing the parameters above in the controls against the algal removal treatment.

To assess community compositions across treatments a non-metric multidimensional scaling ordination (NMDS) using the Bray-Curtis similarity matrix (Bray and Curtis, 1957) was employed, using macrophyte species percent volume inhabited (PVI) data. Sorensen's similarity index (SSI) was used to assess similarity between the extant macrophyte community in Airthrey Loch using macrophyte percentage cover and the emerging community in the treatments, using the formula $S1 = 2c / a + b$, where *c* was the number of species common to both the seed bank experiment and the extant vegetation and *a* and *b* were the total number of species in the seed bank and extant vegetation, respectively (Sorenson, 1948). SSI enables comparison between percentage cover and PVI data and SSI scores range from 0 – 1, with 0 indicating no shared species. The SSI method has been used extensively to compare propagule communities with established vegetation (Casanova, 2015; Gurnell et al., 2008).

All statistical analyses were carried out using *R* (R Development Core Team,

2019) version (3.6.1) with the packages; vegan (Oksanen et al., 2019) and ARTool ((Kay and Wobbrock, 2019; Wobbrock et al., 2011).

## 3.4. Results

### 3.4.1. *Phosphorus and lanthanum concentrations*

TP concentrations ranged from a mean of 54.4 – 95.5 µg $L^{-1}$ in the control treatment and 50.6 – 68.4 µg $L^{-1}$ in the LMB treated containers across the sediment collection sites (Appendix 2.2., Table 1). There was no significant difference in TP concentrations between control and LMB treated containers (Appendix 2.2., Table 2, Figure 1a). SRP concentrations were all below the level of detection (LOD) (<20 µg $L^{-1}$) in the control treatment and ranged from 21.5 – 26.0 µg $L^{-1}$ in the LMB treated containers across sediment collection sites (Appendix 2.2., Table 2). Data below the LOD were treated as half of the LOD for calculating means and standard deviations and for statistical testing. SRP concentrations were significantly higher in the LMB treatment compared to controls (F = 8.766, df = 1, $p$ = < 0.01) with no significant differences in concentrations across sediment collection sites (Appendix, 2.2., Table 2, Figure 1b).

TP concentrations ranged from a mean of 68.4 – 88.1 µg $L^{-1}$ in the algal removal treatment across the sediment collection sites (Appendix 2.2., Table 1). There was no significant difference in TP concentrations between control and algal removal treatments (Appendix 2.2., Table 3, Figure 1a). SRP concentrations ranged from a mean from below the LOD – 24.3 µg $L^{-1}$ in the algal removal treatment (Appendix 2.2., Table 3). The SRP concentrations were significantly different in concentration between sediment collection sites (F = 8.131, df = 2, $p$ = 0.05), (site 1 << site 3 $p$ = < 0.05) (Appendix 2.2., Table 3, Figure 2b).

Total La was significantly higher in the LMB treatment compared to the control ($F$ = 19.734, df = 1, $p$ = <0.0001) (Appendix 2.2., Table 1 and 2, Figure 1c) and total La concentrations were all below LOD (0.12 µg $L^{-1}$) for all control containers (Appendix 2.2., Table 2). LMB treated sediment from collection sites 1 and 2 had a mean of 36.6 µg $L^{-1}$, whilst site 3 had a mean concentration of 73.3 µg $L^{-1}$ (Appendix 2.2., Table 2), which was higher but not significantly higher than sites 1 and 2 (Appendix 2.2., Table 2).

### 3.4.2. *Macrophyte responses*

Macrophytes germinated across all three treatments and across all three sample sites of Airthrey Loch, with a total of 11 species recorded across all treatments (Table 3.1). These species included floating, submerged and emergent/marginal groups. Several species emerged over the duration of the experiment but died before the end of the experiment (Table 3.1). These individuals were identified at the end of the experiment but were not included in any analysis. By day 12 of the experiment, macrophytes had germinated in all three treatments. The most common species occurring across all treatments and sites was *Chara virgata* Kütz. (Table 3.1, Figure 3.1). *Sparganium* spp. (plants were too small to distinguish between *S. erectum* L. and *S. emersum*) Rehmann was the most commonly occurring taxa in the control treatment, whilst for LMB and algae removal treatments the most common species occurring was *Chara virgata*.

ANOVA indicated no significant differences between control and LMB treatments for species richness, dry weight (g) and total macrophyte PVI scores regardless of site (Table 3.2 , Figure 3.2, 3.3 and 3.4 and Appendix 2.2 Table 4). Total combined algae growth (PVI) (the combination of all algae species recorded in the experiment) was, however, significantly lower in the LMB treatment when compared to the control (Figure 3.5, Table 3.2), both in the water column and on the surface sediment (Figure 3.6, Table 3.2). Algal PVI in the water column of the containers varied with collection site (Figure 3.6; site1 << site 3 $p$ = 0.001; site 2 << site 3 $p$ = 0.001). Algal growth on the sediment surface also varied significantly with collection site (site 1 << site 3 $p$ = < 0.01; site 2 << site 3 $p$ = < 0.05).

**Table 3.1.** Macrophyte and algal species emergence from bed sediments subjected to germination treatments (control (C), Lanthanum-modified bentonite (L) addition and algal removal (A)) across each sediment seedbank collection site (S1, S2 and S3) in Airthrey Loch. The presence and absence of extant species growing in Airthrey Loch at the time of sediment collection are shown as well as a visual assessment of community composition from 2017 - 2019. In the germination treatments, the number of ticks indicate an individual replicate box with emergence success (✓) and emergence, but with individuals dying before the end of the experiment (✓). Ticks for Airthrey Loch macrophyte survey represent the presence of the species growing and recorded within the surveyed boat transect. Ticks for Airthrey Loch visual assessment indicate their presence.

| **Macrophyte species** | **Control** | | | **LMB** | | | **Algae removal** | | | **Airthrey Loch transects 26/09/2016** | | | **Airthrey Loch visual assessment 2017 - 2019** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **S1** | **S2** | **S3** | **S1** | **S2** | **S3** | **S1** | **S2** | **S3** | | | | |
| *Sparganium* spp. | | ✓✓✓ | | | ✓ | | | ✓ | | | | | ✓ |
| *Potamogeton pectinatus* | | ✓ | ✓ | ✓ | | ✓ | | | | | | | ✓ |
| *Chara virgata* | | ✓ | | ✓✓ | ✓✓✓ | ✓✓ | ✓✓ | | | | | | ✓ |
| *Nymphoides peltata* | | | | | ✓ | | | | | | | | ✓ |
| *Chara globularis* | | | | | ✓ | ✓ | | | | | | | ✓ |
| *Eleocharis acicularis* | | ✓ | | | | | | | | | | | |
| *Potamogeton obtusifolius* | | | | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ |
| *Azolla filiculoides* | ✓ | ✓ | | | | | ✓ | | | ✓ | ✓ | ✓ | ✓ |
| *Lemna trisulca* | | | ✓ | | ✓ | | | | | ✓ | ✓ | ✓ | ✓ |
| *Lemna minor* | | | | | | | ✓✓ | | | ✓ | ✓ | | ✓ |
| *Ceratophyllum demersum* | | | | | | | | | | ✓ | ✓ | ✓ | ✓ |
| *Elodea canadensis* | | | | | | | | | | ✓ | ✓ | ✓ | ✓ |
| *Potamogeton natans* | | | | | | | | | | ✓ | ✓ | ✓ | ✓ |
| Moss spp. | | | | | | | | | | | | ✓ | ✓ |
| *Spirogyra* water column | ✓✓ | ✓✓✓ | ✓✓✓✓ | | ✓ | ✓✓✓ | | | | | | | ✓ |
| *Spirogyra* bottom | ✓✓ | ✓✓✓ | ✓✓✓✓ | | ✓ | ✓✓✓ | ✓✓ | ✓✓✓ | ✓✓✓✓ | | | | ✓ |
| Filamentous water column | | | | | | ✓ | ✓ | | ✓ | | | | ✓ |
| Filamentous bottom | ✓✓ | ✓ | | ✓ | | ✓ | ✓✓✓ | ✓✓ | ✓✓✓ | | | | ✓ |
| *Sparganium* spp. | | ✓✓✓ | | | ✓ | | | ✓ | | | | | ✓ |
| *Potamogeton pectinatus* | | ✓ | ✓ | ✓ | | ✓ | | | | | | | ✓ |
| *Chara virgata* | | ✓ | | ✓✓ | ✓✓✓ | ✓✓ | ✓✓ | | | | | | ✓ |

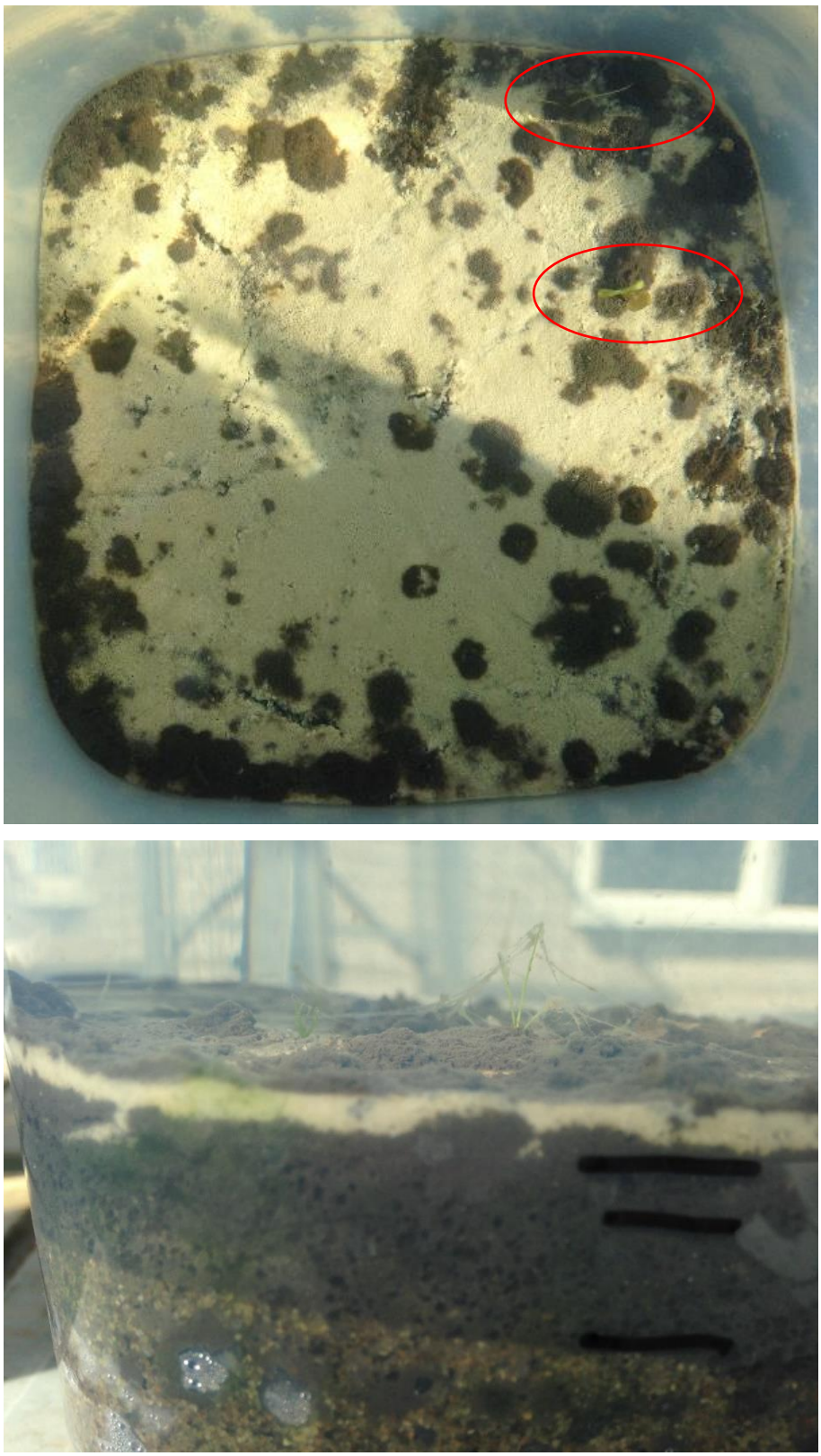


**Figure 3.1.** Emergence of macrophyte species in lanthanum-modified bentonite (LMB) treatment several weeks into experimentation, red circles in top image highlights individuals (© Kate Waters-Hart).

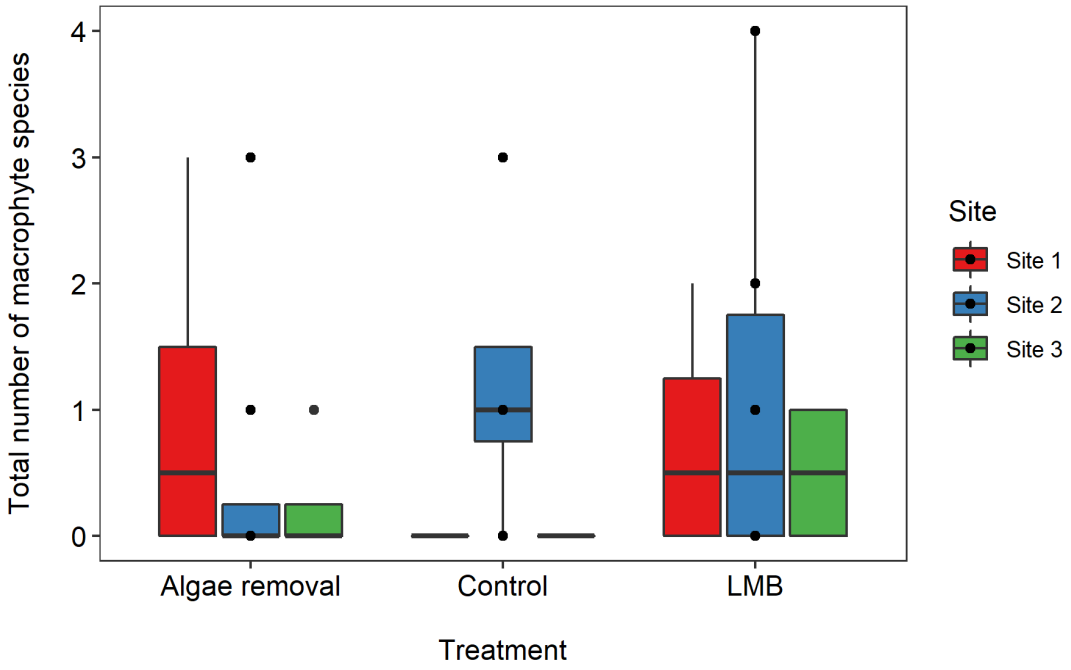



**Figure 3.2.** Total macrophyte species number across each sediment collection site and each treatment.

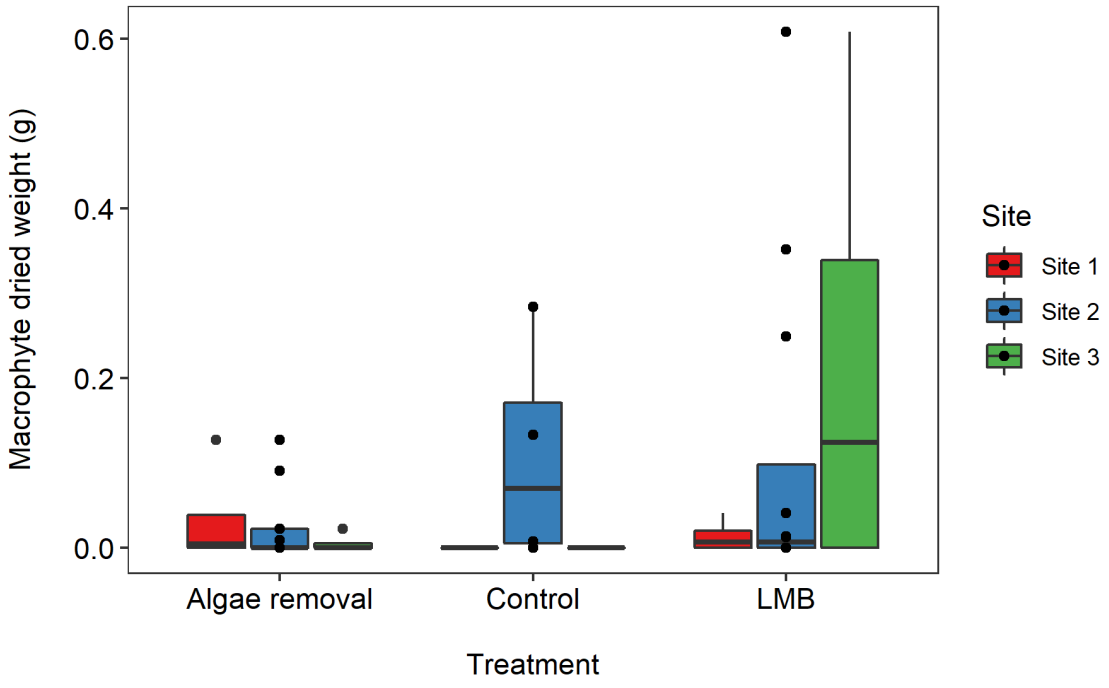



**Figure 3.3.** Macrophyte dry weight (g) at the end of the germination experiment for sediment collection site and treatment.

**Table 3.2.** Results of two-way ANOVA to assess the differences between treatment effects (control (C) and LMB addition) on germination success across the different sediment collection sites (1, 2 and 3). Germination success measured as macrophyte species richness, dry weight (g) and the percent volume inhabited (PVI) by algae communities that grew in the water column or on the surface of the sediment surface.

| **Variable** | **C and LMB** | | | **Sediment collection site** | | | **Treatment: C and LMB * Sediment collection site** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **F** | ***P*** | **Df** | **F** | ***P*** | **Df** | **F** | ***P*** | **Df** |
| Number of macrophyte species ■ | 0.852 | 0.823 | 1 | 0.051 | 0.823 | 1 | 0.051 | 0.823 | 1 |
| Macrophyte dry weight (g) ■ | 1.384 | 0.399 | 1 | 1.135 | 0.412 | 2 | 1.428 | 0.399 | 2 |
| Total combined macrophyte (PVI) (K) | 3.757 | 0.150 | 2 | 1.049 | 0.150 | 2 | 2.572 | 0.150 | 2 |
| Spirogyra water column (PVI) ■ | 11.765 | **<0.01** | 1 | 12.345 | **<0.001** | 2 | 0.432 | 0.656 | 2 |
| Spirogyra bottom sediments (PVI) ■ | 13.453 | **<0.01** | 1 | 11.794 | **<0.001** | 2 | 0.915 | 0.418 | 2 |
| Filamentous algae water column (PVI) ■ | 1.000 | 0.581 | 1 | 1.000 | 0.581 | 2 | 1.000 | 0.581 | 2 |
| Filamentous algae bottom sediments (PVI) ■ | 0.979 | 0.406 | 1 | 1.280 | 0.406 | 2 | 1.152 | 0.406 | 2 |
| Total combined algae (PVI) (K) | 10.580 | **<0.01** | 1 | 4.571 | 0.153 | 2 | 1.221 | 0.318 | 2 |

■: log transformed

(K): Non-parametric Kruskal Wallis test

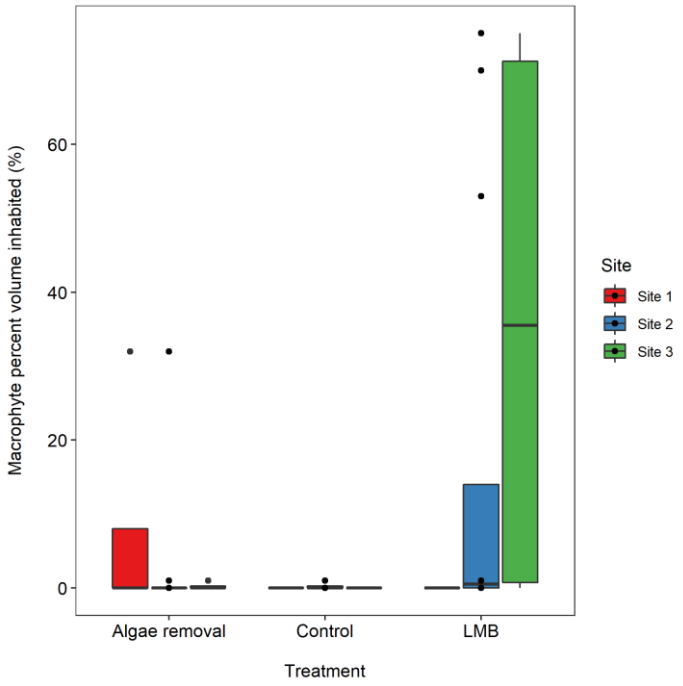



**Figure 3.4.** Total macrophyte percent volume inhabited (PVI) at the end of the germination experiment for sediment collection site and treatment.

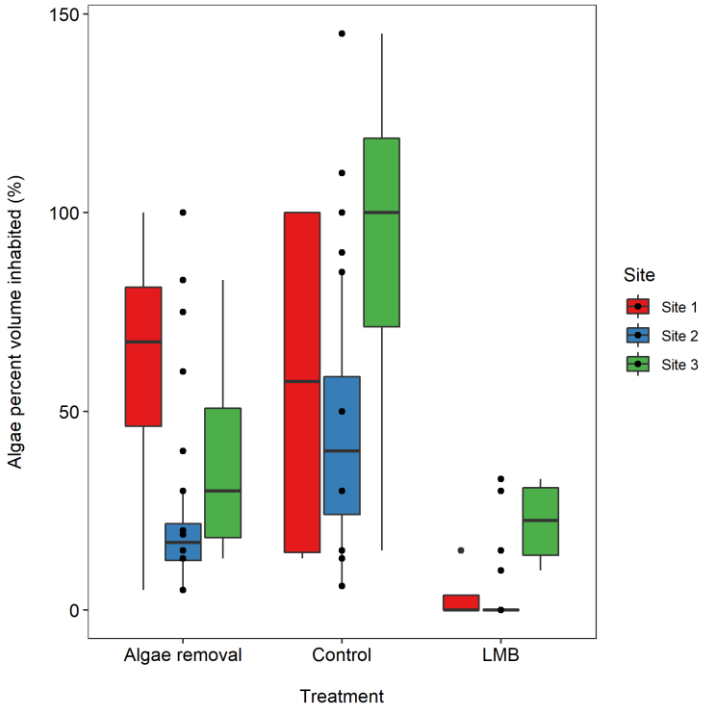



**Figure 3.5.** Total combined algae percent volume inhabited (PVI) at the end of the germination experiment for sediment collection site and treatment.

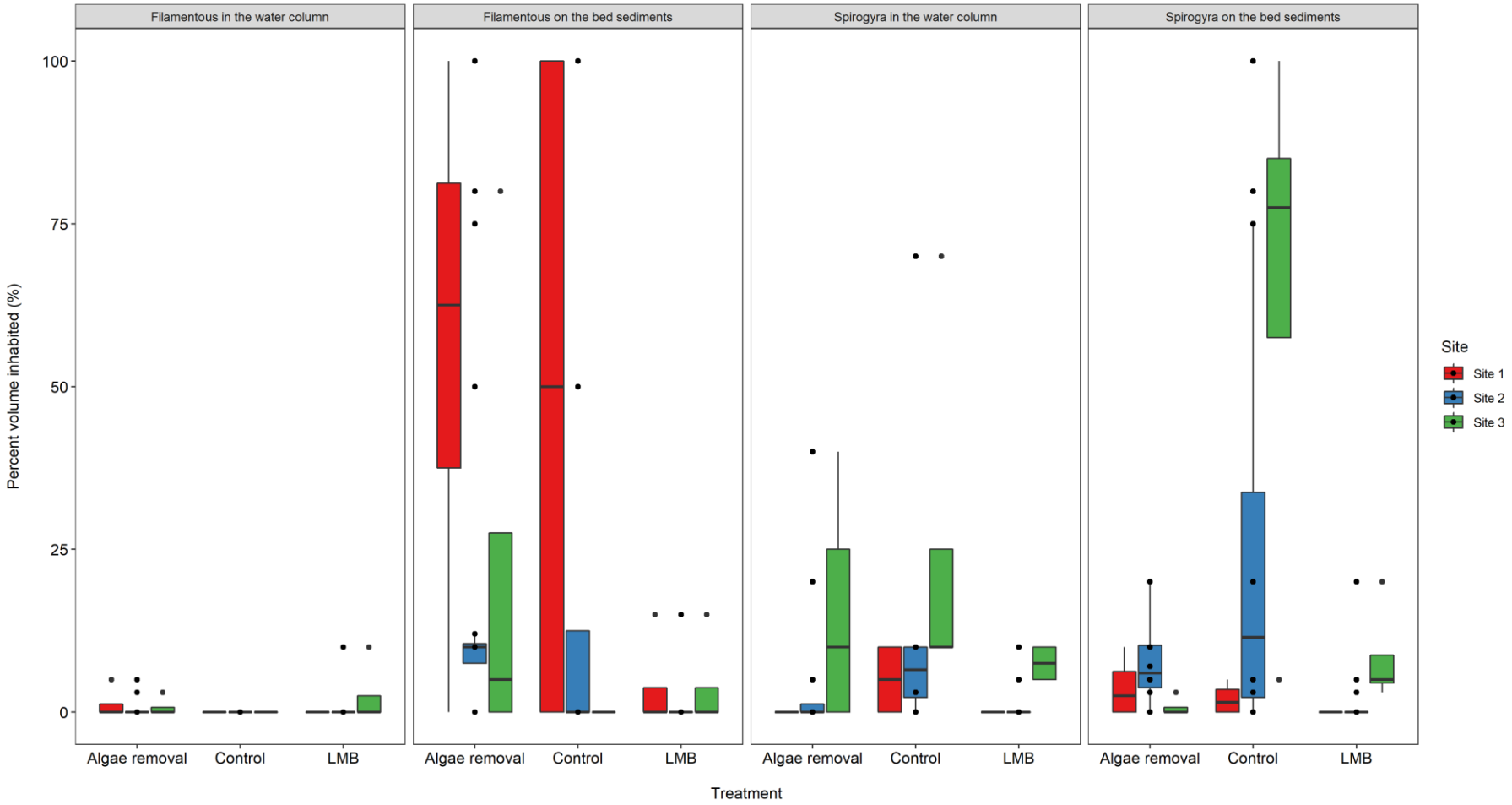



**Figure 3.6.** The percent volume inhibited (PVI, %) for two algal species (filamentous algae and *Spirogyra*) growing in the water column and on bed sediments at the end of the germination experiment for sediment collection site and treatment.

### 3.4.3. *Comparison of the germinating community between control and LMB containers and established vegetation*

The N-MDS showed an overlap in species composition between treatments but did indicate that the control treatment was more dominated by algae species than macrophytes whereas the LMB treatment was more dominated by macrophytes that excluded the growth of *Spirogyra* spp. (Figure 3.7). The species belonging to the same treatment (control and LMB) are grouped by convex hulls and the median species composition is represented by the centre of each orispider. Three containers were not run in the N-MDS analysis, as they did not contain any macrophyte species or algae (containers S1L1, S2L2 and S2L4). The N-MDS showed that some of the LMB treated containers contained other filamentous algae as part of their community composition. Three quarters of LMB containers were more heavily dominated by other filamentous algae than macrophytes. These LMB treated boxes from sediment collection site 3 were affected by a significantly higher bioturbation rate (Figure 3.8, $F = 4.664$, df = 2, $p = < 0.05$), calculated as relative depth change of the LMB layer (mm).

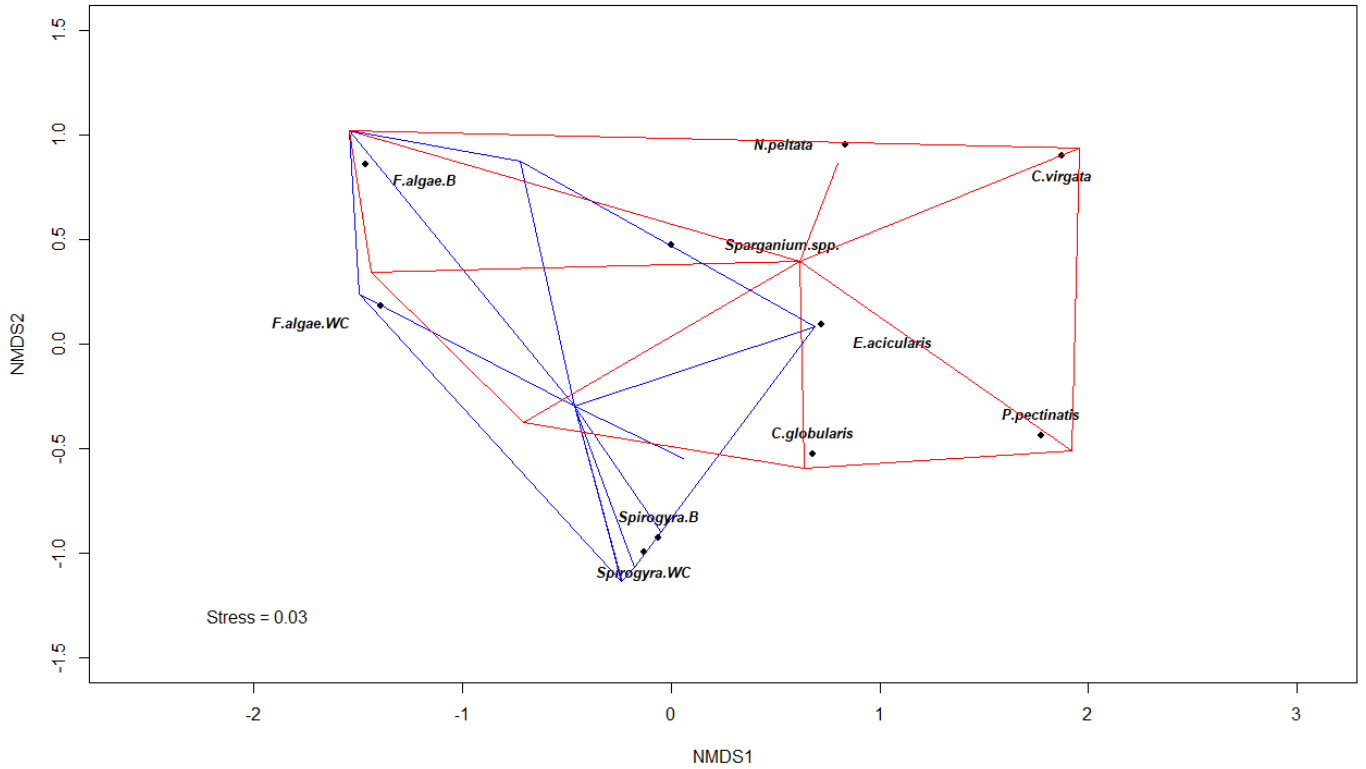



**Figure 3.7.** Non-metric multidimensional scaling ordination of percent volume inhabited (PVI) of macrophyte and algal species and growth location (WC = in the water column and B = growing on the bed sediments). Convex hulls enclose treatments (control (blue) and Lanthanum-modified bentonite (red)) with 'spider' plots showing spread of samples from treatment centroid combined across sediment collection sites. Macrophyte and algal species are labelled in black and dots.

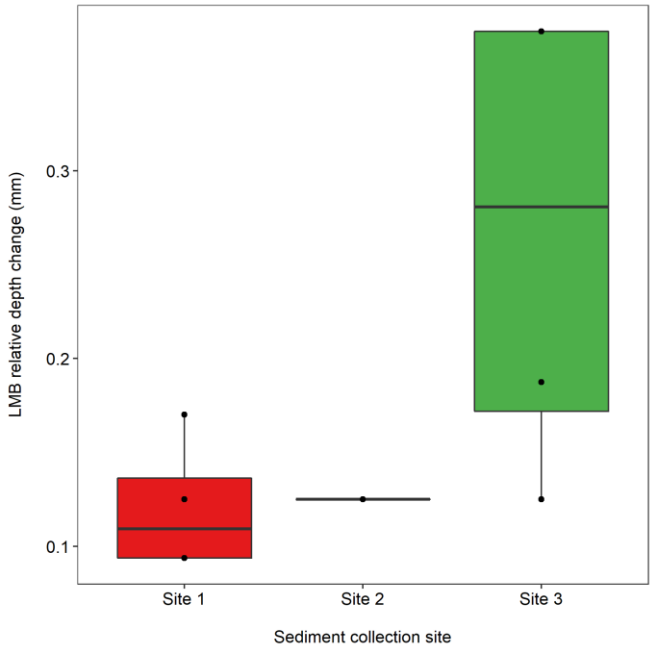



**Figure 3.8.** The relative depth change (mm) of the lanthanum-modified bentonite (LMB) capping layer each day for the duration of the experiment for each sediment collection site.

The SSI revealed that there were no community similarities between the control and LMB treated containers from the germination experiment in comparison to the community composition of the established vegetation in Airthrey Loch (Table 3.3). The algal removal treatment containers had the most similar community composition to *in-situ* communities in Airthrey Loch. The communities that emerged in the algal removal containers from sediment collection site 1 and boat transect 1 had a more similar community than the control or LMB containers with an SSI score of 0.13. Algae removal from site 1 was more similar than the other treatments with an SSI of 0.25 when compared to observations from the established vegetation from the 2016 survey combined with observations of species from 2017 – 2019. The control treatment and the LMB treatment were more similar in composition to the whole basin for sediment collection sites 2 and 3, respectively.

**Table 3.3.** Sorensen's similarity index between established macrophyte populations using Common Standard Monitoring boat survey transects (1, 2 and 3) which corresponded to the three different sediment collection sites in Airthrey Loch, Scotland, U.K., and the communities germinated in the different treatments (control, Lanthanum-modified bentonite and algae removal). Comparison from communities germinated across treatments were also compared to whole basin community composition (grouped species across boat transects and from the visual assessments made from 2017 – 2019).

| | **Established vegetation Site1** | **Established vegetation Site 2** | **Established vegetation Site 3** | **Whole basin** |
|---|---|---|---|---|
| **Site 1** | | | | |
| Control | 0 | | | 0.14 |
| LMB | 0 | | | 0.14 |
| Algae removal | 0.13 | | | 0.25 |
| **Site 2** | | | | |
| Control | | 0 | | 0.28 |
| LMB | | 0 | | 0.25 |
| Algae removal | | 0 | | 0.14 |
| **Site 3** | | | | |
| Control | | | 0 | 0.1 |
| LMB | | | 0 | 0.25 |
| Algae removal | | | 0.08 | 0.18 |

### 3.4.4. *Comparisons between control and algal removal*

ANOVA results indicated no significant differences in germination between the control and algal removal treatments regardless of site for species richness, dry weight and total macrophyte PVI scores (Figure 3.2, 3.3 and 3.4, Table 3.4, Appendix 2.2. Table 4). *Spirogyra* growth in the water column was, however, significantly higher in the control treatment compared to the algal removal treatment (Figure 3.6, Table 3.4). There were no significant differences in S*pirogyra* spp. growing on the bed sediments, other filamentous algae growing in the water column and on bed sediments or between total combined algae PVI values between control and algal removal treatments (Figure 3.5, 3.6 and Table 3.4). Higher PVI scores of S*pirogyra* spp. growing on the bottom sediments were, however, recorded for site 3 with a *P* value of 0.054.

The N-MDS showed an overlap in species composition between the treatments (control and algae removal) (Figure 3.9). The algal removal treatment was less dominated by S*pirogyra* spp. growing in the water column than the control. Treatments are grouped by convex hulls and the median species composition is represented by the centroid of each ordispider.

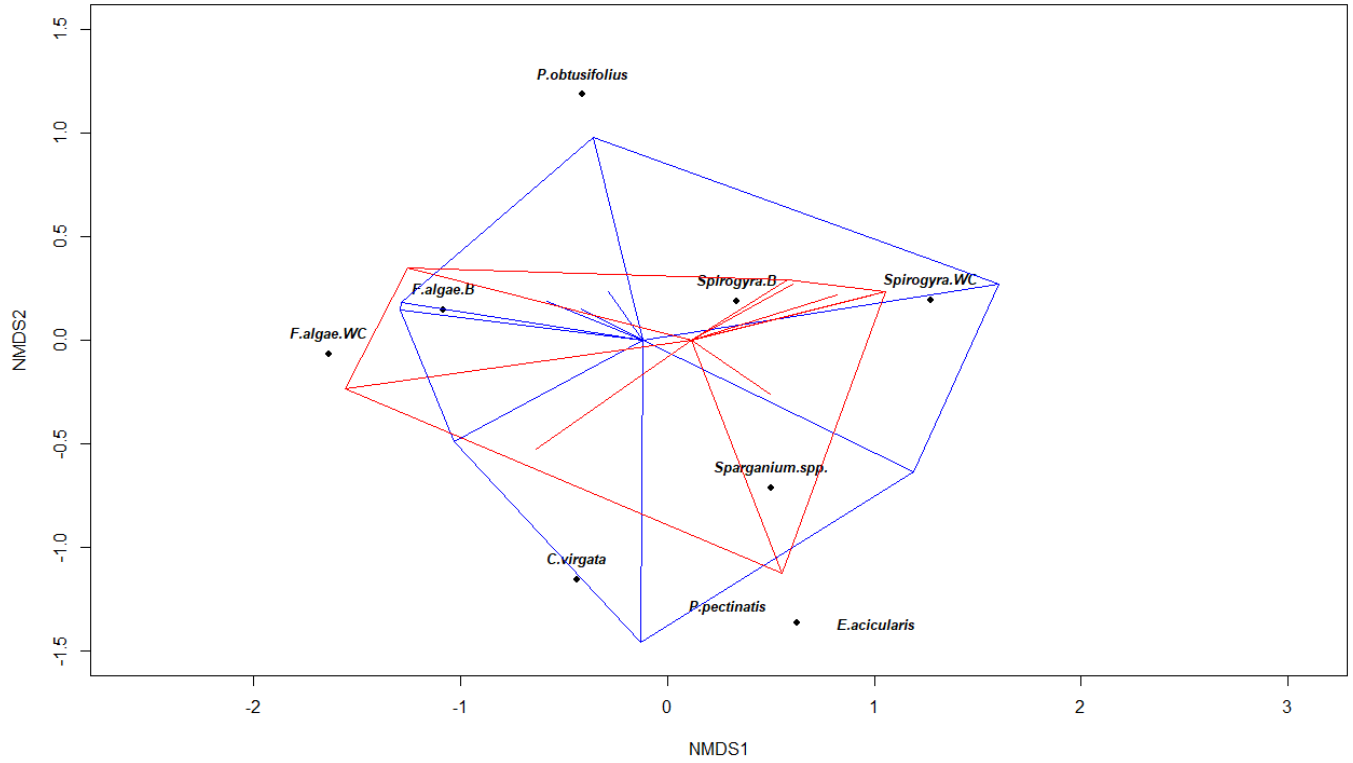



**Figure 3.9.** Non-metric multidimensional scaling ordination of percent volume inhabited (PVI) of macrophyte and algal species and growth location (WC = in the water column and B = growing on the bed sediments). Convex hulls enclose treatments (control (blue) and algae removal (red)) with 'spider' plots showing spread of samples from treatment centroid combined across sediment collection sites. Macrophyte and algal species are labelled in black and dots.

**Table 3.4.** Results of two-way ANOVA to assess the difference between treatments (control (C) and algae removal (A)) across the three sediment collection sites measured as macrophyte species richness, macrophyte dry weight (g) and the percent volume inhibited (PVI) by algae in the water column or on the surface of the sediment.

| **Variable** | **C and A** | | | **Sediment collection site** | | | **Treatment: C and A * Sediment collection site** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **F** | ***P*** | **Df** | **F** | ***P*** | **Df** | **F** | ***P*** | **Df** |
| Number of macrophyte species ■ | 0.912 | 0.823 | 1 | 0.550 | 0.823 | 1 | 0.550 | 0.823 | 1 |
| Macrophyte dried weight (g) ■ | 0.272 | 0.608 | 1 | 2.120 | 0.399 | 2 | 1.893 | 0.399 | 2 |
| Total combined macrophyte (PVI) ■ | 1.115 | 0.304 | 1 | 1.476 | 0.304 | 1 | 1.476 | 0.304 | 1 |
| Spirogyra water column (PVI) ■ | 30.877 | **<0.001** | 1 | 2.273 | 0.158 | 2 | 2.273 | 0.158 | 2 |
| Spirogyra bottom sediments (PVI) ■ | 0.833 | 0.418 | 1 | 4.456 | 0.054 | 2 | 1.297 | 0.418 | 2 |
| Filamentous algae water column (PVI) | 1.882 | 0.581 | 1 | 0.559 | 0.582 | 2 | 0.559 | 0.582 | 2 |
| Filamentous algae bottom sediments (PVI) ■ | 2.219 | 0.406 | 1 | 2.145 | 0.406 | 2 | 0.123 | 0.885 | 2 |
| Total combined algae (PVI) ■ | 1.360 | 0.635 | 1 | 0.102 | 0.752 | 1 | 0.668 | 0.635 | 1 |

■: log transformed

## 3.5. Discussion

### 3.5.1. *Chemical responses following LMB addition*

Although mean TP concentrations were lower collectively across sediment collection sites in the LMB treatment (60.9 µg $L^{-1}$) compared to the control (74.8 µg $L^{-1}$), they were not significantly lower. Mean TP concentrations from the LMB treatment were 27.3% lower than the mean for TP in the control implying LMB did have an impact in reducing TP concentrations. Our post-application TP concentrations in this study were lower than the average pre-applications reported across 18 treated lakes from Spears et al. (2016), making them quite high following an LMB treatment. SRP concentrations were low across all treatments and sites. However, SRP concentrations were, unexpectedly, significantly higher in the LMB treatment compared to all others. This may potentially be explained by the significant algal growth in the control treatment in comparison to the LMB treatment that probably acted to sequester dissolved P directly from the water column. Soluble reactive P concentrations were also significantly higher in the algal removal treatment than the control but this difference was again, not significant for TP concentrations. The up-take rates of TP and SRP by algae in the literature are largely reported for higher P concentrations than observed in our study, however, it can be expected that with a starting mean SRP concentration of between 11 – 18 µg $L^{-1}$ algae such as S*pirogyra* can remove 4 – 5 µg $L^{-1}$ and with TP concentrations between 38 – 53 µg $L^{-1}$ can remove 9 – 10 µg $L^{-1}$ in flowing waters after 4.6 – 12.9 days of growth (Adey et al., 1993). From our study, this same rate of removal of 4 µg $L^{-1}$ every 4.5 – 12.9 days could potentially remove between 130.8 – 163.5 µg $L^{-1}$ of SRP over the course of our experiment. If TP concentrations are reduced by 11 µg $L^{-1}$ every 4.5 – 12.9 days this equates to a potential reduction of 125.4 – 359 µg $L^{-1}$ over the course of experimentation. With the greatly reduced abundance of algae in the LMB treatment, this could be the reason why P concentrations were higher, implying that alage may be more efficient at P-uptake than LMB in this experiment. The post-application SRP concentrations in the LMB treatment ranged from 21.5 – 37.5 µg $L^{-1}$ which is equivalent to, and higher, to the pre-application rates that Spears et al., (2106) reported for 18 treated lakes. It is unclear whether applying LMB to waterbodies that are already quite low in P will actually increase TP and SRP concentrations based on our results. It is also unknown if macrophytes or algae can impact P-uptake by LMB in applications that occur over the growing season.

Total La concentrations were significantly higher in the LMB treatment in

comparison to the control, which confirms our original hypothesis. Concentrations were still high even 21 weeks post-application, with average total concentrations of 36.6, 36.6 and 73.3 µg $L^{-1}$ for sediment sites 1 – 3, respectively. Spears et al. (2013) indicate that the concentrations reported here represent a low probability of elevated $La^{3+}$ concentrations. However, the concentrations we report are higher than the concentrations in the literature when taking the timeframe post-treatment into consideration. Site 1 and 2 were slightly higher than the range of reported TLa concentrations six months post-treatment but site 3 was 42.3 µg $L^{-1}$ higher than the highest recorded value for TLa, some six months after treatment, as reported in the literature in field trials (Spears et al., 2013a). However, our experiment ran just short of six months and so it is possible that these concentrations would have reduced further and to within the reported concentration ranges of treated lakes. Despite this, sediment collection site 3 had the highest TLa concentrations. This may have been due to the significantly higher bioturbation rate associated with chironomids and oligochaetes recorded for this site resulting in translocation of settled LMB back to the water column.

### 3.5.2. *Macrophyte germination responses following LMB addition*

*Chara virgata* was the most common species to germinate across all sediment collection sites and treatments. Comparable lake sediment germination studies are scarce in the literature but the most common species returning in other 'flooded' lake sediment propagule bank studies are charophytes, with 75 – 92% (n = 3) of the community dominated with charophyte species across multiple studies (Bakker et al., 2013; Harwell and Havens, 2003; Strand and Weisner, 2001). Lake sediments are known to have a lower propagule density compared to sediments in riparian systems (Bakker et al., 2013) but it is clear that charophyte propagules are abundant within lake sediments (Bakker et al., 2013) and our study confirms these findings, for Airthrey Loch. Charophytes are generally desirable in ecological restoration projects (Bakker et al., 2013; Blindow et al., 2014) due to their influence on water clarity (Lambert and Davy, 2011) through several positive feedback mechanisms that help to sustain a clear-water state (Bakker et al., 2013). Due to the fact that charophytes germinated in both the control and because LMB treatment and SRP concentrations were generally low in both control and LMB treatments it is likely that charophyte species germinated through disturbance from the experimental set-up and sediment mixing,

rather than through improved water quality.

There were no significant differences in macrophyte species number, dry weight and macrophyte PVI scores between control and algae removal treatments indicating that water column algae did not impact macrophyte germination success (Figure 3.2, 3.3 and 3.4). The effects of benthic algae, however, cannot be tested in our experimental design, as it was not removed due to concerns over bed disturbance. Benthic algal cover may well have restricted seed germination physically or through shading, as indicated, potentially, by the higher *Chara virgata* abundance in the LMB treatment compared to the control. Further examination of the competition between benthic forming algae and macrophyte germination is needed to assess if this might have inhibited germination amongst the controls.

There were no significant differences in macrophyte species richness, macrophyte dry weight or macrophyte PVI scores between control and LMB treated containers. Community composition was, however, different with algal taxa such as *Spirogyra* spp. and other filamentous algae taxa dominating the controls compared with macrophyte dominance in the LMB treated containers, with the exception of LMB treated site 3 where algal species were still present. Site 3 was the sediment collection site that had a significantly higher bioturbation rate recorded to the LMB treatment. A higher bioturbation rate may be attributed to higher SRP concentrations that may have been utilised by the algae explaining why SRP concentrations were lower than expected at this site, given the significant bioturbation rate. Evidence of SRP uptake by *Spirogyra* spp. and other filamentous algae have been reported elsewhere (Adey et al., 1993), although mainly for the uptake of nutrients in wastewaters (Boelee et al., 2011) where concentrations are high. Filamentous algae nutrient uptake potential is considered so good they have been used to harvest nutrients with *Spirogyra* spp. being considered a particularly good candidate due to its easy removal from waters (Mulbry et al., 2010). The higher SRP concentrations within site 3 may have favoured these algae over macrophyte species. Filamentous algae have also been reported as the first colonisers following dredging techniques (Phillips et al., 2015), possibly due to the higher sediment-P concentrations exposed through a removal (Annadotter et al., 1999; Does et al., 1992). This may have happened here through bioturbation where higher sediment-P concentrations are brought to the surface, potentially allowing benthic algae to proliferate. This conflicts with the evidence found by Reitzel et al. (2012) where P concentrations were reduced by a higher bioturbation rate. However, our study is longer-term and ran for a total of 147 days, 4.2 times longer

than that of Reitzel et al. (2012). It is possible that benthic communities are responding to the LMB layer that is encouraging bioturbation in our experiment. Sediment collection site 3 was shallower than the other two collection sites with site 2 being the deepest on average which may explain the higher turnover rate due to possibly more benthic or different communities at this depth. We cannot conclude that the presence of benthic algae suppresses bioturbation, as this could not be assessed in controls. It could be that LMB acts as a benthic algal layer through the equivalent process of P up-take by algae and the rate of bioturbation was obvious due to the visibility of the product. A benthic algal removal versus an LMB treatment designed experiment may help to understand this result further. Based on our results here, we might hypothesise in an experiment like this that SRP concentrations might not differ but differences in the benthic macro-invertebrate community might drive a change in SRP concentrations.

### 3.5.3. *Germinating communities compared to established vegetation*

It was clear that the communities that emerged in the experiment had low similarity with the extant plant community in Airthrey Loch. The algae removal treatment resulted in the highest similarity with the extant community. The extant community was dominated by floating macrophytes and species that are not seed producing such as, *Elodea canadensis* Michx. and low seed producers, *Ceratophyllum demersum* L.. *Potamogeton natans* L. and *P. obtusifolius* Mert. & W. D. J. Koch also occurred in the extant community and are seed producing (Alderton et al., 2017) but mainly re-produce through rhizomes and turions, respectively, (Preston and Croft, 2001) which may be why these species did not emerge in the experiment. Due to the low SSI values in this experiment is most likely that the seedbank contributes little to the established vegetation composition (Abernethy and Willby, 1999). This is most likely because clonal reproduction by large competitive species dominates in Airthrey Loch, e.g. *Elodea canadensis*.

It has been reported in other germination trials that extant populations can be quite different to seed bank communities (Casanova, 2015) and our findings confirm this for Airthrey Loch. Charophytes were the dominant group in the emergent communities of the experiment but were not recorded in the 2016 surveys of the extant vegetation but were recorded in 2017 – 2019 visual assessment. It is possible that the survey methods utilised were insufficient to sample for charophyte species

(Spears et al., 2009). However, it is also possible that the necessary germination cues were not met for oospores in Airthrey Loch, e.g. they could lie too deep in the sediment profile and out of the optimum sediment germinating depth range. There could also be a number of other unfavourable conditions preventing *in situ* germination that were not assessed in this study. For example, the lack of similarity in experimental versus extant communities could be due to the fact that macrophyte species tend to germinate poorly in the field (Bakker et al., 2013), with vegetation propagation normally dominating. It is possible that some recruitment from the seedbank may have been restricted by the presence of species with low propagule longevity, e.g. ≤ 1 year (Bakker et al., 2013). For example, we might never expect certain species, such as *Zannichellia palustris* L. or *Potamogeton perfoliatus* L., to be found germinating due to their transient longevity if they were not present within a year of our survey (Bakker et al., 2013). This has important implications when relying on desirable species with transient propagules to re-colonise lakes where they were previously recorded as any residual seeds may simply no longer be viable. The transplanting of desirable species, following more desirable conditions, maybe the key in bringing back characteristic species to a site in this instance.

*Spirogyra* and other filamentous algal species were not formally recorded in the CSM survey in Airthrey Loch, although anecdotally both can be abundant. Increased light availability to the surface sediments under experimentation may have increased benthic algae growth in comparison with *in situ* conditions and artificially promoted germination amongst macrophyte species. This maybe another reason why certain species were not recorded in the survey sections during the site survey due to lower light levels in the waterbody compared to in the experiment. Germinating light requirements of macrophytes are not widely researched amongst the literature, particularly so for *Potamogeton* species (Hay et al., 2008). Of the light/dark requirements that do exist for macrophytes they are only largely described for marginal/littoral species (Baskin and Baskin, 1998). However, there is evidence that some macrophyte species require well-lit conditions (Forsberg, 1966; Hay et al., 2008; Smits et al., 1990), whilst some require dark conditions (Van Vierssen, 1982), others have variable requirements (Bonis and Grillas, 2002; Hay et al., 2008) or are insensitive to light levels (Kimber et al., 1995). Temperature requirements are more widely evidenced but this is also variable, even amongst the same species (Bonis and Grillas, 2002).

### 3.5.4. *Algae responses following LMB addition*

*Spirogyra* growth was lower in the water column and growing on bed sediments in the LMB treated containers compared with the controls (Table 3.2 and figure 3.6). This confirms our original hypothesis that LMB will reduce benthic algal cover. However, this appeared to be a species-specific response with other filamentous algae not expressing a significant decline in PVI in either water column or surface sediments. *Spirogyra* spp. was significantly lower on surface sediment only from sites 1 and 2 in control and LMB treatments. This may have been due to the higher bioturbation rate reported in the LMB treatment, especially in sediment from site 3. Reports on the effects of LMB on benthic algae or algae in general are extremely sparse (Álvarez-Manzaneda et al., 2019; van Oosterhout and Lürling, 2012), with contrasting results. Van Oosterhout and Lürling (2013) found declines in growth rates of *Scenedesmus obliquus* (Turpin) Kützing and *Microcystis aeruginosa* (Kützing) Kützing at > 0.5 g $L^{-1}$ of LMB and after prolonged exposure and *Anabaena* spp. were controlled, whilst Álvarez-Manzaneda et al. (2019) found no decline in the growth rate of *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J. Kristansen & O. M. Skulberg at < 2 g $L^{-1}$ of LMB. Our results add to this evidence base where LMB reduced the biomass of *Spirogyra* at a dose of 1.84 g $L^{-1}$. It is stated by Álvarez-Manzaneda et al. (2019) that the results could be due to different species sensitivity to LMB and this could be true given it had no impact on reducing general filamentous algae biomass in our study. The algae removal treatment did significantly reduce *Spirogyra* growing in the water column compared to controls but did not affect *Spirogyra* growing on the bed sediments. Higher PVI scores of *Spirogyra* growing on the bottom sediments were, however, recorded for site 3, indicating that a higher bioturbation rate was most likely driving biomass accrual through nutrient liberation from sediments.

### 3.5.5. *Implications for macrophyte conservation measures*

It is advised that lake seedbank communities are assessed prior to the implementation of costly internal nutrient management measures, especially where macrophyte conservation is the primary aim of restoration. For example, paleoecology represents a powerful tool with which to assess the potential for re-emergence from the historic seedbank (Bishop et al., 2019; Sayer et al., 2012) but it does not assess propagule viability. Consideration of propagule longevity should also be taken into account when designing restoration plans as many macrophyte species have transient propagule

viability periods that may last ≤1 year (Bakker et al., 2013). If 'ecologically active' seedbanks are no longer present at a site, the site may need to rely more on dispersal from external sources for re-colonisation, but this may add to the recovery time. For example, if donor sites are isolated it may take some time for macrophyte recruitment following chemical recovery of the waterbody. However, seedbank viability may not always be the limiting factor as some macrophyte propagules and oospores can remain viable for several decades (Bonis and Grillas, 2002). Some macrophyte species have also been restored to agricultural land within < 6months of excavation of 'ghost ponds' (Alderton et al., 2017) and after sediment removal (Sayer et al., 2012) which gives hope to rely on seedbanks in some instances, but it is important to understand that every individual lake system undergoing restoration will have different constraints for macrophyte establishment. Reports of successful macrophyte transplantations are becoming more common in the literature (Knopik and Newman, 2018; Lauridsen et al., 2003b, 1994). However, there has currently only been one macrophyte transplantation study using LMB and that resulted in the loss of all transplanted macrophytes after four months in two river trials (Novak and Chambers, 2014). Given the threats to fresh waters in the future (Brownlie et al., 2017; Dudgeon et al., 2006; Jeppesen et al., 2017) and the pressures to meet legislative water quality targets, macrophyte transplantation may become a more common part of restoration programmes in the future, particularly due to the long ecological recovery times that are commonly encountered even when chemical recovery is achieved (Jeppesen et al., 2005; Phillips et al., 2005).

### 3.5.6. *Forcing macrophyte recovery from the seedbank with LMB*

We have shown in this study that LMB does not restrict macrophyte germination (Table 3.2 and Figure 3.2). So why do macrophytes not rapidly recolonise LMB treated lakes? It is possible that seedbanks in individual lakes have low viability, perhaps as a result of burial following many years of eutrophication. It is, therefore, important to consider seed bank viability and longevity timelines. LMB reduced the coverage of *Spirogyra* algae growing in the water column and on the sediment surface in experimental conditions but this was impacted by bioturbation rates, with increased rates limiting LMB's potential to control coverage. Particularly in shallow lakes or lakes where light reaches the sediment surface, LMB may potentially facilitate plant

establishment by reducing smothering of emerging seedlings by benthic algae, a hypothesis that requires further experimentation.

## 3.6. Conclusion

Using a 21- week germination experiment, we demonstrate that LMB did not cause a barrier or hamper the germination success of macrophyte propagules in the seedbank. LMB addition had no statistically significant effect on macrophyte diversity and biomass in treated containers when compared with untreated controls. However, algal growth was significantly lower following LMB treatment, which may have longer term implications for macrophyte re-establishment, although variation in the severity of this effect was apparent between algal species. A higher bioturbation rate appeared to alter algal responses to LMB. La concentrations remained elevated in the LMB treatment but concentrations did not present an ecotoxicological risk to macrophytes, based on published ecotoxicology reports.

# 4. Chapter 4: Assessing aquatic macrophyte general and species-specific stress responses to lanthanum-modified bentonite (Phoslock®)

Kate E. Waters-Hart [a & b], Nigel Willby [b], Rupert Perkins [c], Nick Cowan [a], Lorna Eades[d], Iain D. M. Gunn [a], Genevieve Madgwick [e], Roseanne McDonald [a & b], Justyna Olszewska [a], Amy Pickard [a], James Watt [a & d], Said Yasseri [f], Iain Sime [g] and Bryan. M. Spears [a]

[a] UK Centre for Ecology & Hydrology, Penicuik, Midlothian, EH26 0QB, UK

[b] University of Stirling, Stirling, FK9 4LA, UK

[c] Cardiff University, Cardiff, Wales, CF10 3AT

[d] University of Edinburgh, School of Chemistry, Kings Buildings, West Mains Road, Edinburgh, EH9 3JJ

[e] Natural England, Eastbrook, Shaftesbury Road, Cambridge, CB2 2DF, UK

[f] Limnological Solutions International Pty Ltd, Admiralstr. 150a, 28215, Bremen, Germany

[g] Scottish Natural Heritage, Great Glen House, Leachkin Road, Inverness, IV3 8NW, UK

The candidate, as lead author, performed the experimental design, sample collection, preparation and was involved in the laboratory determination of nutrients, dissolved organic carbon and metals. The data analysis and writing was carried out by the candidate. Co-authors provided support and guidance on the scope and design of the project, expertise in phyto-physiology, laboratory analysis of nutrients, dissolved organic carbon content and expertise in inductively coupled ion chromatography mass spectrometry (ICP-MS) methods. The co-authors also contributed to the editing of the manuscript.

Acknowledgements: Thanks to Stephen Ives and Alanna Moore who helped with sediment and water collection and to Zarah Pattison for initial discussions on statistics.

## 4.1. Abstract

Nutrient pollution is a global phenomenon, causing the loss and declines of aquatic macrophyte (macrophyte) communities worldwide. Even following catchment management, phosphorus (P) can be retained within waterbodies and cycled from the bed sediments to overlying waters (internal loading) to be used by phytoplankton, causing excessive and often toxin-producing cyanobacterial blooms and the loss of macrophytes. In-lake remediation measures such as Lanthanum (La)-modified bentonite (LMB) have been used to control internal loading in an attempt to promote macrophyte recovery. However, macrophyte recovery in response to LMB application in lakes has often been lacking and the mechanisms behind this lack of recovery have not been assessed. We studied the effects of LMB addition on five macrophyte species (*Elodea canadensis*, *Littorella uniflora, Myriophyllum spicatum, Najas flexilis* and *Potamogeton perfoliatus*) using sediment core incubations (30 days) under light and dark conditions (n=5 replicates per treatment). Responses in water chemistry and macrophyte growth (or stress) indicators (*Fv/Fm*, shoot length (cm), root length (cm), wet weight (g), dry weight (g) and macrophyte wash weight (g)) to LMB and light treatments were assessed. Generally, across all five species there was no significant impact of LMB under light conditions. However, stress responses were highly species-specific. *Elodea canadensis* exhibited a positive growth response to LMB/light conditions but this was considered a negative ecological response given the fact it is an invasive species in the U.K. All species responded negatively to LMB treatment under dark conditions. Total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations, generally, decreased significantly ($p$ = <0.001, $p$ = <0.01, respectively) compared with controls following LMB addition, although responses were species-specific. This may be due to apparent macrophyte senescence that may have inhibited the effectiveness of LMB in controlling available P in the water column. La concentrations remained high following LMB application for the duration of the incubation but varied across species and with light treatment. The implications are that LMB addition to macrophytes may alter community composition by favouring more adaptable and change-tolerant species, in this case, *Elodea canadensis*. It may be difficult, therefore, to achieve recovery in less tolerant desirable species using LMB, alone, where pre-application communities are dominated by more competitive non-native species.

## 4.2. Introduction

Submerged aquatic macrophytes (macrophytes) perform essential functions in lakes (Jeppesen et al., 1997; Scheffer et al., 1993) and support clear waters through many feedback mechanisms (Ibelings et al., 2007; Scheffer et al., 1993). However, macrophyte recovery may take anywhere from 2 – 40+ years (Verdonschot et al., 2011) following reduced catchment phosphorus (P) loads to lakes. This delay in recovery can be caused by P cycling between the lake bed sediments and the overlying waters (i.e. internal loading) (Søndergaard et al., 2003). P-sorbing products such as Lanthanum (La)-modified-bentonite (LMB) have been used to control internal loading following catchment load reduction (Spears et al., 2016). Reduced total phosphorus (TP), soluble reactive phosphorus (SRP) and chlorophyll-*a* (Chl-*a*) concentrations have been found across many lakes in response to LMB application (Copetti et al., 2016; Spears et al., 2016). Despite this, the establishment of macrophytes in LMB treated lakes has been weak with only minimal recovery being reported (Gunn et al., 2014; Spears et al., 2018, 2016; Waajen et al., 2016b, 2016a). Several potential reasons exist for the lack of recovery in these treated lakes including both abiotic and biotic factors (Spears et al., 2016). This recovery bottleneck is not LMB specific and is reported across P reduction studies (Bakker et al., 2013; Lauridsen et al., 2003a; Phillips et al., 2016; Søndergaard et al., 2007). macrophyte recovery following improvements in water quality, in general, has been reported to take years to decades as a result of biological connectivity and physical distribution barriers (Jeppesen et al., 2000; Verdonschot et al., 2011). This timescale is particularly concerning with regard to meeting agreed ecological targets such as the European Water Framework Directive's (WFD) minimum 'good' ecological status for qualifying lakes by 2027 (European Commission, 2000) and, for example, other targets for macrophytes in UK lakes (European Commission, 2000; JNCC, 2015). It is, therefore, important to confirm the reasons behind the reported lack of recovery following LMB treatment in lakes and to confirm whether direct inhibition is a potential constraint (Spears et al., 2016). For example, it is not known if the application of LMB causes stress to macrophytes through P limitation, reduced light availability caused by direct shading effects, or through La toxicity.

Light and nutrient availability are two key factors known to regulate macrophyte distribution and community composition in lakes (Chambers and Kalff, 1987). macrophytes exhibit characteristics that allow adaptation to environmental stresses including low light and low/high nutrients; adaptive features that constitute a

"plant strategy" (Grime, 1979). Grime (1979) defines stress as "the environmental constraints which limit the rate of dry matter production of all or part of the vegetation" whilst disturbance involves "the mechanisms which limit the plant biomass by causing its partial or total destruction". Disturbance is also defined as "any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment" (Pickett and White, 1985). Different species may deviate in their susceptibility to stress which, therefore, may influence community composition (Grime, 1979). Disturbance can also modify community composition and species that are more tolerant to frequent disturbances are considered to be disturbance tolerant (Murphy et al., 1990).

In this respect, a LMB application could be considered as a disturbance as it changes the resources available to macrophytes through reducing P concentrations and, therefore, may cause stress to some species and may limit or increase their growth. Some species that require or tolerate high nutrient concentrations prior to an application may increase shoot and/or root growth post-treatment in search of P in the water column or sediment. This growth may be species-specific depending on individual macrophyte growth strategies, e.g. whether or not they can up-take nutrients from the water column or through the sediments. Extra or diminished root or shoot growth may impact on the overall biomass and could influence macrophyte community composition, favouring only more robust species, following a treatment. A LMB application may also be considered a disturbance in relation to changing the physical environment through limiting light availability. It is unclear if the product smothers leaf surfaces that could impact physiological processes, such as the rate of photosynthesis. Differences may also be seen across water depths, with LMB causing a further stress to macrophytes already growing at depths where reduced light levels prevail.

Toxicity from $La^{3+}$ ions liberated after LMB treatments has been documented (Copetti et al., 2016) and it is unclear if $La^{3+}$ ions may directly impact macrophytes, particularly as applications are not always confined to non-macrophyte growing seasons. Despite the merging of bentonite with La to prevent toxic effects when applied to waterbodies (Haghseresht et al., 2009), elevated filterable La concentrations have been reported in some whole lake studies (Lürling and van Oosterhout, 2013; Meis et al., 2012; Spears et al., 2013b). Many reports have attempted to determine the toxicity of La to freshwater biota and humans (Afsar and Groves, 2009; Clearwater, 2004; D'Haese et al., 2019; Herrmann et al., 2016; Lürling

and Tolman, 2010; NICNAS, 2001; Spears et al., 2018, 2013b), although few studies have assessed the direct effects on macrophyte species (Barry and Meehan, 2000; Copetti et al., 2016). macrophyte species that have been tested show that La can be bioavailable and can be incorporated into tissues (Waajen et al., 2017; Weltje et al., 2002; Xu et al., 2012). La tissue content has been found to depend on the La dose, ion speciation and the focal species (Herrmann et al., 2016; Wolterbeek and Van Der Meer, 1996), with some macrophytes (e.g. *Hydrocharis dubia* (Bl.) Backer) exhibiting negative physiological and cellular effects (Xu et al., 2012). However, the majority of the subject species in these experiments were floating macrophytes, or macrophytes that absorb their nutrients from the water column. Finally, it is important to consider responses in submerged rooted macrophytes, as they may be exposed to relatively high concentrations of LMB and, therefore, potentially higher La concentrations (van Oosterhout et al., 2014).

The objective of this study was to test whether LMB addition impacts macrophyte photosynthesis and physical growth responses under different light levels, where these act as a proxy for different macrophyte growing depths. We hypothesized that macrophyte species would respond differently to LMB application, as determined through their stress tolerance mechanisms. There are currently no guidelines for suitable submerged macrophyte test species for such experiments (Arts et al., 2008), so we selected test species that vary in morphological traits (Table 4.1). We expected species such as *Littorella uniflora* (L.) Asch. (*LU*) and *Najas flexilis* (Willd.) Rostk. & W. L. E. Schmidt *NF*) to be well-adapted to LMB given their higher stress tolerance (Murphy et al., 1990). This is based on being tolerant to a number of traits such as: having a high root:shoot ratio, slow biomass turnover and being tolerant to low light availability etc (Murphy et al., 1990). *Elodea canadensis* Michx. (*EC*), *Myriophyllum spicatum* L. (*MS*) and *Potamogeton perfoliatus* L. (*PP*) were expected to be less tolerant, given their low stress strategy survival mechanisms. *PP* may be the least adapted species from the list of five, whereas *LU* and *NF* may be the best adapted (Table 4.1). We hypothesized that responses to reduced light will be generalised. Species that are more competitive, which have traits, such as, large peak biomass, are canopy forming and have a fast biomass turnover (Grime, 1979), e.g. *EC, MS* and *PP*, may produce longer shoots under stress. We expected P concentrations to be reduced and La concentrations to increase following LMB addition, in-line with the literature (Copetti et al., 2016; Spears et al., 2016, 2013a). However, if macrophytes respond negatively to LMB addition or to reduced light, then

we expected macrophyte senescence and increased P in the water column indicating reduced efficiency of LMB. LMB may disturb the physical and chemical environment for macrophytes and, it was, therefore, expected, that more disturbance tolerant species such as *MS* and *EC* would be more tolerant to this stress. The specific questions addressed were, as follows: (1) using a series of bio-assay experiments, under well-lit and low light conditions (that represent shallow and deeper macrophyte growing depths), do different macrophyte species respond similarly overall to LMB addition, as determined through measuring specific macrophyte response strategies that are known to be sensitive to stress, e.g. *F*v/*F*m, total shoot and root length, wet and dry weight?; (2) are water column P concentrations reduced and La concentrations higher than in un-treated water, as expected following LMB addition?; and (3) is there any evidence of La incorporation into tissues leading to toxicity to macrophytes in LMB treated cores?

**Table 4.1.** Macrophyte test species and their number of survival strategy traits per strategy element (C – competition tolerance, S – stress tolerance, D – disturbance tolerance) taken from Murphy et al., (1990) and their desirability for establishment in United Kingdom waterbodies. Parentheses indicate only one strategy trait in this category. Red = worst adapted, green = best adapted prediction to LMB application.

| Species | Strategy trait | | | Strategy type | Species desirability |
|---|---|---|---|---|---|
| | C | S | D | | |
| *Potamogeton perfoliatus* | 6 | 0 | 2 | CD | Desirable |
| *Myriophyllum spicatum* | 6 | 0 | 3 | CD | Desirable |
| *Elodea canadensis* | 6 | 0 | 3 | CD | Undesirable – non-native invasive |
| *Littorella uniflora* | 0 | 5 | 1 | S (D) | Desirable |
| *Najas flexilis* | 3 | 2 | 2 | CSD | Desirable – nationally rare, protected |

## 4.3. Methods

### 4.3.1. *Macrophyte species suitability, collection and cultivation*

Five different macrophyte species were chosen for inclusion in the experimental assays which covered a range of desirable, invasive and rare submerged rooted species. Selection was also determined by availability and leaf size to allow detection of photosynthesis indicators using *F*v/*F*m measurements.

*Potamogeton* and *Elodea* species are often the first groups of macrophytes to return to lakes following lake remediation measures (Perkins and Underwood, 2002;

Strand and Weisner, 2001), therefore, *PP* and *EC* were chosen to try and understand why they might be the first to respond, e.g. due to their high number of competitive traits. They were also chosen to represent desirable and invasive groups, respectively. Other species were selected to represent desirable macrophytes, namely *LU, MS* and *NF. LU* was selected for its high stress tolerance. *MS* was chosen for its comparability with *EC,* in that they have the same number of strategy traits. *NF* was chosen as a rare UK and EU protected species (Council of the European Union, 1992; HMSO, 1994, 1981) but also due to its inferred higher stress tolerance.

Macrophyte species were collected from a range of different sources. *EC, LU* and *PP*, plants were collected from Loch Leven, Scotland, U.K. *MS* was bought over the internet from a UK distributer. *NF* individuals were collected from Tangy Loch, Scotland, U.K under licence number 123404, provided by Scottish Natural Heritage. Due to problems encountered during collection, only *NF* fragments were used without their seed attached and any subsequent roots. *NF* fragments were still used in the experiment despite individuals of this species being unable to reproduce vegetatively (Hutchinson, 1957). We, therefore, did not expect any additional shoot or root growth, although other above ground responses may still be expressed.

Following collection, macrophyte species were cultured in glasshouses at CEH Edinburgh. Each plant was inserted manually into Loch Leven sediment overlain by water, both of which were collected from the Reed Bower Monitoring Site (Appendix 3., Figure 1). The duration of cultivation in glasshouses was variable for each species due to macrophyte collection times and the number of individuals originally collected. If only small numbers of individuals were collected, more time (*ca.* three months) was needed to allow more individuals to establish in order to select enough suitable specimens for the assays. *NF* was the only species that was not cultured but it was placed in the glasshouses under the same conditions as the other species for five days before experimental conditions began.

### 4.3.2. *Sediment collection and experimental set-up*

The experimental design is detailed visually in Appendix 3., Figure 1. Separate experiments were performed on each of the five macrophyte species from 2016 – 2018 using a fully randomised design. Twenty cores measuring 33.3 cm in length and 6.4 cm diameter were randomly divided into two treatments. Ten of the twenty cores were separated into a light and the other 10 into a dark treatment. Each ten were then

separated into a control and the other 5 into a LMB treatment which resulted in five replicates per treatment. For each experiment, lake water (15 L) and eight sediment cores were collected with an HTH gravity corer (6.4ø, 50 cm length; Pylonex, Umeå, Sweden) from the Loch Leven Reed Bower sampling site (Appendix 3., Figure 1). The sediment cores (about 20 cm sediment depth) and overlying water were stored within a few hours of collection in the dark at 4˚C prior to processing. The following day, water from the cores was extracted and the remaining sediment from the eight cores were homogenised. Approximately 5 cm of homogenised sediment was then placed into twenty bottom-bunged cores. 15 cm of lake water was carefully syphoned on top of the sediment into each core, cling film with a pinhole was then placed over the top of each core to prevent evaporation. The twenty cores were then positioned into a fully randomised block design and placed into an incubator (Panasonic MIR-554-PE) to allow any sediment that was disturbed to settle overnight. The incubator was set to a 14 - hour (light):10 - hour (dark) cycle at 12 ˚C, set to mimic typical spring/summer conditions. The following day, twenty individual macrophytes with similar lengths were taken from stock and were washed in a zip lock bag with 100 ml of distilled water and gently shaken for 60 seconds to remove epiphytes (Zimba and Hopson, 1997). Each individual had a small amount of cotton wool wrapped around the roots to allow anchorage into the sediment. Each individual was then placed into a single core tube and left in the incubator for ten days to acclimatise before experimental treatment commenced. Acclimatisation was necessary to prevent any positive or negative effects seen after immediate placement into new surroundings.

### 4.3.3. *Pre-treatment measurements*

#### 4.3.3.1. *Physico-chemical and chemical measurements*

Following the acclimatisation period, cores were removed from the incubator and a series of physiochemical parameters including conductivity (µS cm), pH and dissolved oxygen (DO) (mg $L^{-1}$) were measured using a HACH multi-parameter meter (HQ30D) 5 cm below the surface of the water. Probes were calibrated against standard pH and conductivity buffer solutions (HACH), prior to any measurements being taken. 45 ml of water was taken 1 – 2 cm above the sediment surface from a plastic tube; 30 ml of which was filtered through a Whatman GF/F filter (pore size 0.7 µm). This and the remaining 15 ml (unfiltered) water were then frozen immediately at -18 ˚C for future analysis of SRP (µg $L^{-1}$) and TP (µg $L^{-1}$), respectively. Each individual plant was

removed along with the cotton wool and submersed in 100 ml of distilled water in a zip-lock bag and gently shaken for 60 seconds to remove any epiphytes present (Zimba and Hopson, 1997). The macrophyte was removed and the water used to rinse the individual was retained and placed in the dark at 4 ˚C until further processing.

#### 4.3.3.2. *Macrophyte Fv/Fm measurements*

Photosystem (PSII) activity was used to assess stress (Maxwell and Johnson, 2000). Each plant was placed into a black petri dish with a small amount of distilled water and the fluorometer probe (AquaPen – P AP-P 100 (Photon Systems Instruments, Drásov, Czech Republic)) was placed over the leaf but no measurements were taken. The dish was then covered with a dark lid and was left to dark adapt for five minutes to maintain a non-stressed state (Maxwell and Johnson, 2000). A non-stressed state is necessary prior to chlorophyll fluorescence measurement by PSII activity, to ensure all PSII reaction centres are open. Following five minutes of dark adaptation, a Quantum Yield (QY) measurement of PSII was taken, which is the equivalent to an *F*v/*F*m measurement. *F*v/*F*m compares the dark-adapted pre-photosynthetic leaf fluorescent state called minimum fluorescence (*F*o) with PSII reaction centres fully open, against the maximum fluorescence (*F*m), maximum photosynthetic activity, with PSII reaction centres closed. *F*v/*F*m is the ratio created by dividing *F*o and *F*m to give variable fluorescence (*F*v). This ratio represents the maximum QY of PSII. *F*o is measured using a light source that is too low to drive photosynthesis followed by an intense flash of light, a saturation pulse, to close all available reaction centres, *Fm*. The *F*v/*F*m measurement is expected to decline with plant stress. This method was trained for each species using a trial specimen from the original stock from the glasshouses. Each species was trialled to assess the most suitable f pulse (weak pulses of light to induce *F*o), F pulse (saturating pulse intensity to induce *F*m) and A pulse percentage (actinic light pulse intensity ambient light). All three species had an optimum f pulse of 30% that equals 0.027 µmol photon $m^{-2}$ per pulse, an F pulse of 50% that equals 1500 µmol photon $m^{-2}$ $s^{-1}$ and an actinic light of 5% that is equivalent to 50 µmol photon $m^{-2}$ $s^{-1}$. These percentages were assessed by not allowing the F pulse to induce the primary quinone acceptor ($Q_A$) reduction and the f pulse to increase sensitivity, increasing QY without reducing non-photochemical quenching (NPQ) as no quenching of the yield is desirable (*pers. comm.,* Perkins, 2016).

Amongst terrestrial plants an *F*v/*F*m value of > 0.78 would be considered optimal health, although baseline *F*v/*F*m values will vary naturally between species.

#### 4.3.3.3. *Other macrophyte measures*

Following the QY measurement, shoot and root lengths (cm) were measured with a ruler for each plant pre-treatment. Each plant was then individually placed into a 4 mm sieve and shaken for two minutes to remove excess water prior to weighing to provide estimates of total wet weight (g) (Bickel and Perrett, 2015). Plant roots were then wrapped in a small amount of fresh cotton wool to allow anchorage and placed back into the corresponding core with minimal disturbance to the sediment. Water extracted earlier from each core was replenished with lake water collected on the same day as the sediment which had been kept in dark conditions at 4˚C since collection. Overlying water was replenished to maintain a depth of 15 cm in each core. The wash weight from each individual were filtered the following day (Whatman GF/F filter, pore size 0.7 µm) and dried at 75˚C for 48 hours prior to differential weighing over a 48-hour period after which no further change in weights was observed in macrophyte wash weight (g).

### 4.3.4. *LMB treatment*

There is uncertainty in calculating an 'effective dose' of LMB to meet water quality targets (Meis et al., 2013). The most common approach is to be dose dependant on the amount of mobile phosphorus ($P_{mobile}$) in the bed sediments of a waterbody but this is only a proxy for estimating dose (Meis et al., 2013). There are many mechanisms controlling P release across a wide range of sediment P pools (Søndergaard et al., 2003). In this experiment we used a dose of 1.6 g of LMB. This LMB dose represents the 75$^{th}$ percentile of 18 treated lakes from applications based on surface area loads listed in Spears et al. (2016). This dose is equivalent to 5.1 tonnes/hectare which is more than an estimated dose of 2.2 T/ ha to bind the potentially releasable estimated P load recorded in 2012 for Loch Leven with 29.7kg of P in the upper 3 cm of sediment (Spears et al., 2012). Here, the 75$^{th}$ percentile was used to ensure that any potential effect of LMB on macrophytes would be recorded, in the context of reported doses from other treated lakes. LMB was first mixed with 20

ml overlying water extracted from the core before being added back to the core in slurry form.

### 4.3.5. *Light and dark treatments*

The light treatment represents macrophytes growing in shallow areas of a water body with reasonable light availability up to a depth of 1 m ( 16 - 100 % of light hitting the lakes surface, reaches the sediment surface in Loch Leven (Spears et al., 2012)). The dark treatment simulated macrophytes growing at depths of ≥ 3.5 m in Loch Leven where light levels are reduced (0.28 - 0.56 µmol $m^{-2}$ $s^{-1}$ (measured 21/06/2016)) with only 1.4% of light reaching the sediment surface (Spears et al., 2012). Light levels were tested prior to the experiment with a LI-COR® light meter (LI-250A, LI-COR® Environmental UK Ltd, Cambridge, UK) to assess light levels in the incubator and in Loch Leven. The incubator (PANASONIC MIR-554-PE) contained fluorescent lighting as standard. The ten light treatment cores (i.e. five control and five LMB) were placed into the incubator with a light availability of ~29.2 µmol $m^{-2}$ $s^{-1}$ and set to a 14 -hour (light):10-hour (dark) cycle at 12˚C (same as the acclimatisation period) (Figure 4.1). The ten dark treatment cores were placed inside a thick black plastic bag sealed at the top (Figure 4.1). Several holes were pierced into the bag to allow gas exchange and minimal light availability. The cores were left for 21 nights and 20 days in experimental conditions.

### 4.3.6. *Post-experimental chemical measurements*

At the end of the 20-day incubation, cores were removed from the incubator for physiochemical parameter measurement, as above. Unfiltered water was removed 1 - 2 cm above the sediment surface for all cores for TP (mg $L^{-1}$). Water was collected also from *PP*, cores only for the determination of total La (TLa; µg $L^{-1}$) calcium (Ca; µg $L^{-1}$), manganese (Mn; µg $L^{-1}$), iron (Fe; µg $L^{-1}$), barium (Ba; µg $L^{-1}$), praseodymium (Pr; µg $L^{-1}$) and neodymium (Nd; µg $L^{-1}$). Only in the case of *PP* were total metals measured in both the control and LMB treated cores to confirm that no La was present in the control cores. Sub-samples of water were filtered (Whatman GF/F filter, pore size 0.7 µm) for SRP (µg $L^{-1}$), ammonium ($NH_4^+$; mg $L^{-1}$), nitrate ($NO_3^-$; mg $L^{-1}$) and dissolved organic carbon (DOC; mg $L^{-1}$). All total and unfiltered water samples were immediately frozen following collection at -18˚C until processing.

**Figure 4.1.** Control and LMB treated cores for *Najas flexilis* (bottom left), light treatment (top left) and dark treatment (top right) placed inside the incubator (bottom right) (© Kate Waters-Hart).

### 4.3.7. *Post-experimental macrophyte measurements*

After water chemistry sampling was complete, macrophytes were then removed from the cores and *F*v/*F*m, shoot length, root length, macrophyte wash weight (g) and wet weight (g) were measured again, as described in section 4.3.3.2 – 4.3.3.3. The effectiveness of the macrophyte wash procedure was confirmed using Scanning Electron Microscope imagery (see chapter 5, Figure 5.2 – 5.5). All water sample analysis methods for P (TP and SRP) (Appendix 2.1.1), $NH_4^+$ and $NO_3$- (Appendix

3.1.1), DOC (Appendix 3.1.2) and metals (Ca, Mn, Fe, Ba, La, Pr and Nd) (Appendix 2.1.2) are detailed in Appendices 2.1 and 3.1.

### 4.3.8. *Statistical analysis*

#### 4.3.8.1. *Macrophyte responses*

For each measured parameter post-treatment values were subtracted from pre-treatment values to give delta values to facilitate statistical analysis. Linear mixed-effects models (LMMs) were used to examine macrophyte responses to LMB addition across species and to determine if the response varied with light treatment. Treatment (control/LMB) and light (light /dark) were included in each model as fixed factors with an interaction term. The macrophyte response variables were; *F*v/*F*m, shoot length (cm), root length (cm), macrophyte wash weight (g), wet weight (g) and dry weight (g). Pre-treatment dry weight values were estimated by dividing post-treatment dry weight (g) by post-treatment wet weight (g) values and multiplying this value by pre-treatment wet weight values. Species within light within treatment was included as a random intercept in each model to account for species specific responses between the different treatments and pseudoreplication of each of the five experiments conducted. Model simplification was used to remove the non-significant interaction term (where applicable) from each model ($P > 0.05$) (Zuur et al., 2009). Optimal models were selected through model simplification using the likelihood ratio test and Akaike Information Criterion (AIC) (Burnham and Anderson, 2002; Zuur et al., 2009). Variance components analysis was performed on final models to assess if there were species-specific responses to each dependent variable. Model validation was conducted on the final models with normality assumptions evaluated by plotting theoretical quantiles versus standardized residuals (Q-Q plots) and heterogeneity assessed by plotting residuals versus fitted values. All dependent variables did not meet normality and homogeneity assumptions of models, therefore, each dependent variable was log or log (+1) transformed and then scaled from 0 – 1 to meet model assumptions. Random effects in final models were also checked for normality by assessing Q-Q plots from each LMM of which all met assumptions.

#### 4.3.8.2. *Macrophyte species-specific responses*

To assess the species-specific responses in more detail, individual macrophyte species were analysed separately for each dependent variable using delta values (post- minus pre-treatment values). Two-Way Analysis of Variance (ANOVA) models were used to assess each dependent variable with an interaction term; *F*v/*F*m, shoot length (cm), root length (cm), wet weight (g), macrophyte wash weight (g), wet weight (g) and dry weight (g). All model assumptions were checked for normality and ensured heterogeneity was met using Q-Q plots and fitted values plotted against residuals. For those dependent variables that did not meet heterogeneity or normality assumptions, dependent variables were log (+1) transformed. If models still did not reach model assumptions following transformation, each factor was analysed separately using the Kruskal-Wallis test with *P* adjustment using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) to account for multiple testing and to avoid a type I error occurring. If interaction terms were significant ($P \leq 0.05$) in models, Tukey's post hoc tests were performed to identify where significant differences lay between treatments. If treatments were assessed using the Kruskal-Wallis test, interactions were assessed using the aligned rank transformation method with *P* adjustment (Leys and Schumann, 2010). If interactions were significant ($P \leq 0.05$) the Dunn test was performed to assess where significant differences existed between treatments. All statistical analysis were performed using the software R, version 3.6.1 (R Development Core Team, 2019) with the additional packages lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017) and ARTool (Kay and Wobbrock, 2019; Wobbrock et al., 2011).

#### 4.3.8.3. *Species – specific percent change*

Post-treatment values for all measured macrophyte responses were subtracted from pre-treatment median values for each treatment and percent change values were calculated for each individual from each treatment. Scores were averaged across the five individuals from each treatment to give one average percent change against pre-treatment median value in order to assess the amount of change each species exhibited for each measured dependent variable.

#### 4.3.8.4. *Physico-chemical, nutrient and metal chemistry responses*

General responses across species and species-specific responses were determined for physio-chemical parameters (pH and DO), nutrients (SRP, TP, $NH_4^+$ and $NO_3$-), DOC and metals (Ca, Mn, Fe and Ba) following the statistical process described in section 4.3.8.1 and 4.3.8.2.

## 4.4. Results

### 4.4.1. *General macrophyte responses to LMB in light and dark treatments*

There was clear evidence of both general and species-specific responses to LMB under different light treatments for all measured response variables (Appendix 3.2: Figures 1a – 1f and Table 1.). No interactions were reported for any variable between the LMB treatments and the different light levels. *Fv/Fm* values across all species generally decreased in LMB treated cores, although not significantly (Table 4.2 and Appendix 3.2: Figure 1a) and values increased generally in the light treatment, but this was not significant. 28.1% of the variation in the *F*v/*F*m model was explained by species-specific responses (Table 4.3 and Figure 4.2a). Shoot length and root length increased in both LMB and light treatments compared to control and dark conditions, but this was not significant (Table 4.2 and Appendix 3.2: Figures 1b and c). Shoot length responses were highly species specific with 64.2% of the variance in the model explained by species-specific responses (Table 4.3 and Figure 4.2b). Root length was also species-specific, with 51.7% of the variation explained by species (Table 4.3 and Figure 4.2c). Macrophyte wet weight significantly increased in LMB treated cores compared to controls (Table 4.2 and Appendix 3.2: Figure 1d) but also with species with 53.2% of variance explained by species-specific responses to treatments (Table 4.3 and Figure 4.2d). macrophyte wash weight showed a highly significant increase in weight in LMB treated cores compared to controls (Table 4.2 and Appendix 3.2: Figure 1e). This was a response observed across all species (Table 4.3 and Figure 4.2e). Dry weight increased significantly in light compared to the dark treatment (Table 4.2 and Appendix 3.2: Figure 1f) with 52.3% of the model variation explained by species (Table 4.3 and Figure 4.2f). Individual species responses were confirmed for many of the measured variables (Figure 4.3) with species either being more negatively or positively impacted than the global response for *F*v/*F*m (0.60 ± 0.07) (Figure 4.3a), shoot length (0.27 ± 0.11) (Figure 4.3b), root length (0.22 ± 0.11)

(Figure 4.3c), wet weight (0.35 ± 0.08) (Figure 4.3d), macrophyte wash weight (0.07 ± 0.04) (Figure 4.3e) and dry weight (0.44 ± 0.08) (Figure 4.3f).

**Table 4.2.** Model coefficients for all fixed effects with standard error for each dependent macrophyte growth variable assessed.

| Response | Fixed effects | Estimate | Standard error | t | *P* |
|---|---|---|---|---|---|
| *Fv/Fm* | Intercept | 0.600 | 0.072 | 8.321 | **<0.0001** |
| | Treatment - LMB | -0.094 | 0.083 | -1.126 | 0.276 |
| | Light - Light | 0.144 | 0.083 | 1.733 | 0.101 |
| Shoot length | Intercept | 0.266 | 0.114 | 2.335 | **<0.05** |
| | Treatment - LMB | 0.092 | 0.131 | 0.702 | 0.492 |
| | Light - Light | 0.112 | 0.131 | 0.854 | 0.405 |
| Root length | Intercept | 0.215 | 0.106 | 2.026 | 0.059 |
| | Treatment - LMB | 0.037 | 0.123 | 0.303 | 0.766 |
| | Light - Light | 0.205 | 0.123 | 1.668 | 0.114 |
| Wet weight | Intercept | 0.345 | 0.080 | 4.322 | **<0.001** |
| | Treatment - LMB | 0.023 | 0.113 | 0.205 | 0.840 |
| | Light - Light | 0.317 | 0.113 | 2.807 | **<0.05** |
| Macrophyte wash weight | Intercept | 0.072 | 0.039 | 1.854 | 0.081 |
| | Treatment - LMB | 0.488 | 0.045 | 10.899 | **<0.0001** |
| | Light - Light | -0.002 | 0.045 | -0.041 | 0.968 |
| Dry weight | Intercept | 0.441 | 0.078 | 5.669 | **<0.0001** |
| | Treatment - LMB | 0.088 | 0.090 | 0.042 | 0.967 |
| | Light - Light | 0.236 | 0.090 | 2.627 | **<0.05** |

**Table 4.3.** Random intercept variance and standard deviation and variance components analysis to assess how much of the variation in the model is explained by species-specific macrophyte responses within treatments.

| Response | Random effects | Variance | Std. Dev. | Variance components analysis (%) |
|---|---|---|---|---|
| *Fv/Fm* | Species:LMB:Light | 0.023 | 0.151 | 28.1 |
| Shoot length | Species:LMB:Light | 0.078 | 0.280 | 64.2 |
| Root length | Species:LMB:Light | 0.063 | 0.252 | 51.7 |
| Wet weight | Species:LMB:Light | 0.026 | 0.162 | 53.2 |
| Macrophyte wash weight | Species:LMB:Light | 0.002 | 0.044 | 4.5 |
| Dry weight | Species:LMB:Light | 0.034 | 0.182 | 52.3 |

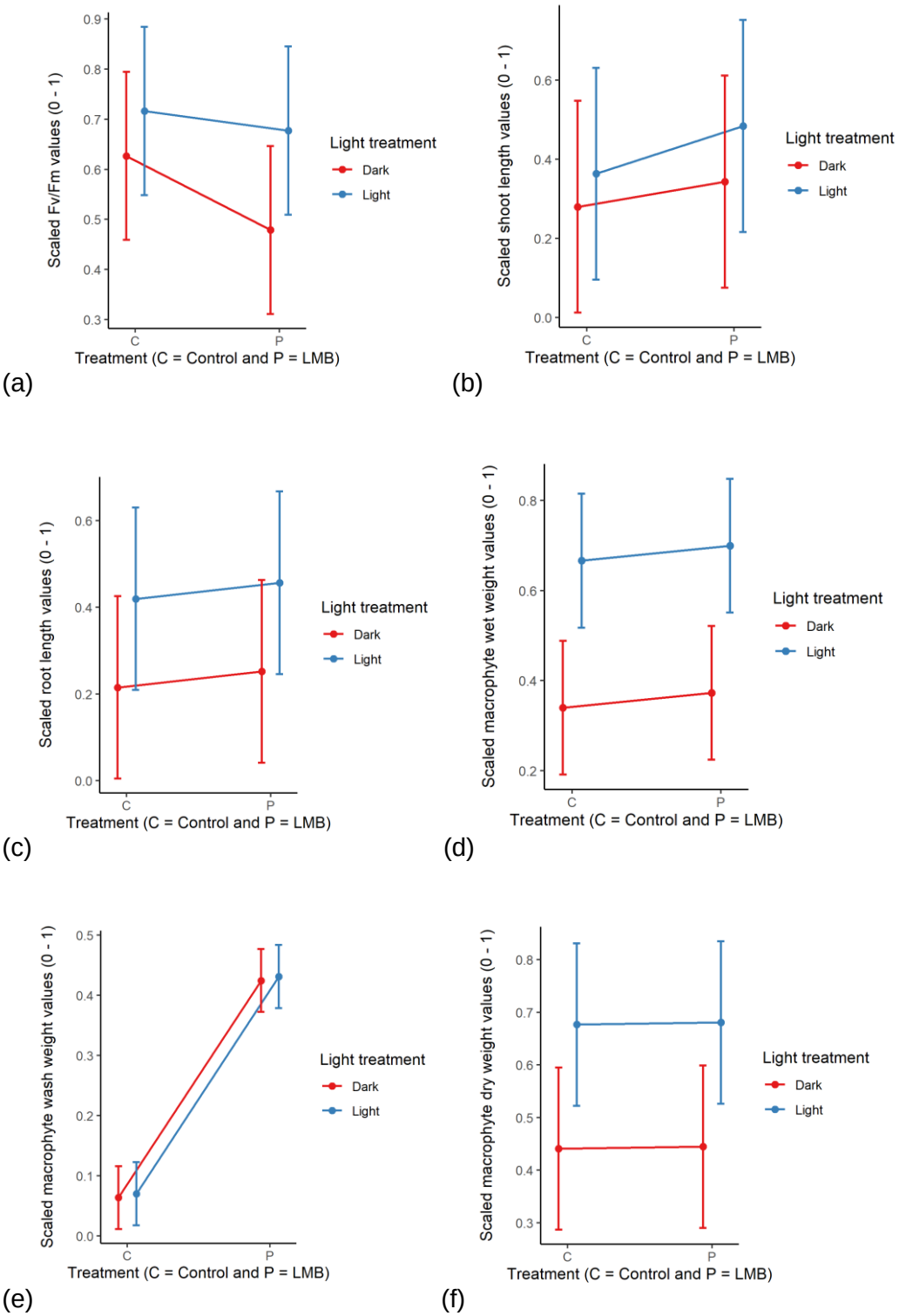



**Figure 4.2.** Interaction plots of the main treatment effects (n=5 for each treatment) with 95% confidence intervals of (a) *Fv/Fm*, (b) shoot length, (c) root length, (d) macrophyte wet weight, (e) macrophyte wash weight dry and (f) macrophyte dry weight.

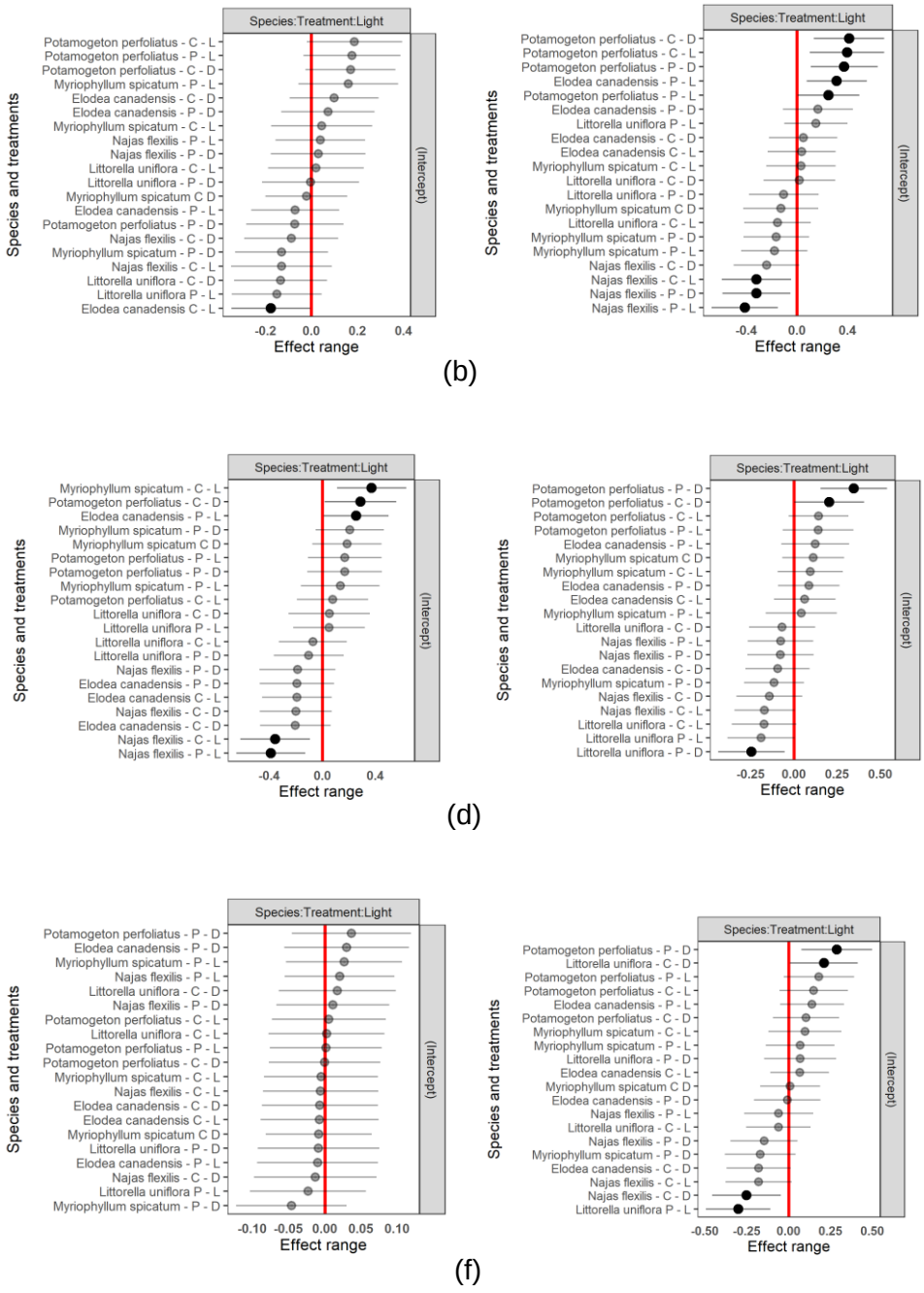



**Figure 4.3.** Median effect estimates (differences between light within treatment within species intercepts and the global model intercept (species combined) of the random effects with 95% confidence intervals for (a) *Fv/Fm*, (b) shoot length, (c) root length, (d) wet weight, (e) macrophyte wash weight and (f) dry weight between light within treatment within species. Black values show species with confidence intervals that do not overlap 0 (median) and are either more negative or positive than the global response of each linear mixed effect model.

### 4.4.2. *Macrophyte species-specific responses to LMB in light and low light levels*

All macrophytes exhibited species-specific responses to the measured dependent variables (Table 4.4, Appendix 3.2: Table 1 and Figures 2 - 6). *PP* exhibited a significant decline in *Fv/Fm* in the LMB treatment and in the dark treatment with an interaction; a more severe decline was seen in the LMB/dark treatment compared to LMB/light as indicated by the post hoc Dunn test (Table 4.4, Appendix 3.2: Figure 2a). macrophyte wash weight expressed a significant increase in weight for individuals in the LMB treatment compared to weight from the controls (Table 4.4, Appendix 3.2: Figure 2e).

*MS* expressed a significant decline in *Fv/Fm* values in the dark compared to the light but not in the LMB treatment (Table 4.4, Appendix 3.2: Figure 3a). There were significantly shorter roots in the dark treatment compared to those exposed to light conditions (Table 4.4, Appendix 3.2: Figure 3c). Wet weight was also significantly lower in the dark treatment compared to the light treatment (Table 4.4 and Appendix 3.2: Figure 3d). macrophyte wash weight increased significantly for individuals in the LMB compared to the control treatment with an interaction expressing an increase in weight in LMB/light, which was higher than the increase in LMB/dark (Appendix 3.2: Figure 3e). Dry weight was significantly lower in the dark treatment compared to the light treatment (Table 4.4, Appendix 3.2; Figure 3f).

*EC* showed a significant increase in shoot length in LMB treated cores compared to controls (Table 4.4, Appendix 3.2: Figure 4b). Root length was shorter in the dark treatment in comparison to the light (Table 4.4, Appendix 3.2: Figure 4c). Wet weight was significantly higher in the LMB treated cores compared to controls but was significantly lower in the dark treatment compared to the light (Table 4.4, Appendix 3.2: Figure 4d). macrophyte wash weight was significantly higher in the LMB treated cores compared to the controls (Table 4.4, Appendix 3.2: Figure 4e). Dry weight was also significantly higher in the LMB treatments compared to the controls, but weight was significantly higher in the light treatment compared to the dark (Table 4.4, Appendix 3.2: Figure 4f).

*LU* expressed a significant interaction with LMB/light having significantly longer shoots than control/light subjects but with no change in shoot length in the LMB/dark and control/dark cores (Table 4.4, Appendix 3.2: Figure 5b). The wet weight of individuals was significantly lower in the dark treatment compared to the light treatment (Table 4.4, Appendix 3.2: Figure 5d). macrophyte wash weight was

significantly higher in the LMB treated cores compared to the un-treated cores (Table 4.4, Appendix 3.2: Figure 5e). Dry weight was significantly lower in the LMB treatment compared to control cores (Table 4.4, Appendix 3.2: Figure 5f).

*NF* had a significantly lower wet weight and dry weight in the dark treatments compared to the light treatment (Table 4.4, Appendix 3.2; Figures 6d and f). macrophyte wash weight significantly increased in the LMB treated cores compared to controls (Table 4.4, Appendix 3.2: Figure 6e). Root and shoot lengths of this species did not change during the experiment.

**Table 4.4.** Individual species responses to measured variables (*Fv/Fm*, shoot length, root length, wet weight, macrophyte wet weight and dry weight for main treatment effects (LMB control) and light treatment (light dark) and an interaction (LMB control*Light dark) using Two-Way ANOVA's and individual Kruskal-Wallis tests with *P* value correction for multiple testing, Aligned rank transformation test for non-parametric interaction testing and Tukey's Post hoc Dunn test (with P value adjustment) for significant interaction terms (non - parametric only).

| **Response** | **LMB Control** | | | **Light Dark** | | | **LMB Control*Light Dark** | | | **Post Hoc** |
|---|---|---|---|---|---|---|---|---|---|---|
| | F chi-squared | *P* | Df | F/ chi-squared | *P* | Df | F/ chi-squared | *P* | Df | *P* |
| ***Potamogeton perfoliatus*** | | | | | | | | | | |
| *Fv/Fm* (**K**) | 7.434 | ↓ | 1 | 4.497 | ↓ | 1 | 12.630 | ↓ | 16 | CD - PL*, CL – PD **, PL - PD* |
| Shoot length (cm) (**K**) | 0.0514 | 0.821 | 1 | 0.571 | 0.821 | 1 | 1.190 | 0.821 | 3 | |
| Root length (cm) | 0.002 | 0.969 | - | 0.465 | 0.505 | - | 1.310 | 0.269 | 16 | |
| Wet weight (g) | 1.634 | 0.219 | - | 3.502 | 0.080 | - | 0.692 | 0.418 | 16 | |
| Mac wash weight (g) | 34.273 | ↑↑↑↑ | - | 0.262 | 0.616 | - | 0.833 | 0.375 | 16 | |
| Dry weight (g) | 1.634 | 0.219 | - | 3.502 | 0.080 | - | 0.692 | 0.418 | 16 | |
| ***Myriophyllum spicatum*** | | | | | | | | | | |
| *Fv/Fm* ◆ | 1.614 | 0.222 | - | 15.593 | ↓↓↓ | - | 2.779 | 0.115 | 16 | |
| Shoot length (cm) (**K**) | 0.143 | 0.706 | 1 | 5.143 | 0.070 | 1 | 5.380 | 0.219 | 3 | |
| Root length (cm) (**K**) | 0.693 | 0.405 | 1 | 5.860 | ↓ | 1 | 7.377 | 0.091 | 3 | |
| Wet weight (g) | 4.327 | 0.054 | - | 37.115 | ↓↓↓↓ | - | 1.626 | 0.221 | 16 | |
| Mac wash weight (g) | 32.709 | ↑↑↑↑ | - | 7.558 | ↓ | - | 4.542 | ↑ | 16 | CD – PL ***, PL – PD *, PL – CL *** |
| Dry weight (g) | 4.327 | 0.054 | - | 37.115 | ↓↓↓↓ | - | 1.626 | 0.221 | 16 | |
| ***Elodea canadensis*** | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Fv/Fm* | 0.072 | 0.792 | - | 1.894 | 0.188 | - | 0.299 | 0.592 | 16 | |
| Shoot length (cm) | 12.077 | ↑↑ | - | 4.400 | 0.052 | - | 0.793 | 0.387 | 16 | |
| Root length (cm) ◆ | 4.163 | 0.058 | - | 12.092 | ↓↓ | - | 4.163 | 0.058 | 16 | |
| Wet weight (g) | 5.889 | ↑ | - | 43.360 | ↓↓↓↓ | - | 2.038 | 0.173 | 16 | |
| Mac wash weight (g) ◆ | 75.687 | ↑↑↑↑ | - | 2.524 | 0.132 | - | 2.754 | 0.117 | 16 | |
| Dry weight (g) | 5.502 | ↑ | - | 47.010 | ↓↓↓↓ | - | 1.293 | 0.272 | 16 | |
| ***Littorella uniflora*** | | | | | | | | | | |
| *Fv/Fm* | 1.333 | 0.265 | - | 1.399 | 0.254 | - | 4.462 | 0.051 | 16 | |
| Shoot length (cm) | 4.162 | 0.058 | - | 1.753 | 0.204 | - | 5.396 | ↑ | 16 | PL – CL * |
| Root length (cm) ◆ | 0.022 | 0.883 | - | 4.040 | 0.062 | - | 1.936 | 0.183 | 16 | |
| Wet weight (g) | 0.955 | 0.343 | - | 13.319 | ↓↓ | - | 1.411 | 0.252 | 16 | |
| Mac wash weight (g) | 9.360 | ↑↑ | - | 0.642 | 0.435 | - | 0.121 | 0.733 | 16 | |
| Dry weight (g) | 5.253 | ↓ | - | 2.502 | 0.133 | - | 0.314 | 0.583 | 16 | |
| ***Najas flexilis*** | | | | | | | | | | |
| *Fv/Fm* | 0.788 | 0.388 | - | 0.606 | 0.448 | - | 0.260 | 0.617 | 16 | |
| Wet weight (g) ◆ | 4.017 | 0.062 | - | 26.795 | ↓↓↓↓ | - | 0.016 | 0.902 | 16 | |
| Mac wash weight (g) | 32.930 | ↑↑↑↑ | - | 0.063 | 0.806 | - | 0.008 | **0.932** | 16 | |
| Dry weight (g) ◆ | 4.017 | 0.062 | - | 26.795 | ↓↓↓↓ | - | 0.016 | 0.902 | 16 | |

**K** – Non-parametric Kruskal Wallis test

◆ - Logged response variable

Significant main effect and direction, increase or decrease- ↑ ↓ : **≤0.05,** ↑ ↑ ↓↓ : **≤0.01,** ↑↑↑ ↓↓↓: **≤0.001,** ↑↑↑↑ ↓↓↓↓ **: ≤0.0001**

Significant Tukey's *Post Hoc* Dunn test - *: <0.05, **: <0.01, ***:<0.001 for listed groups CL (control – light), CD (control – dark), PL – (LMB – light), PD – (LMB – dark)

Red highlight: an undesirable response given the species, green highlight: a desirable response given the species

### 4.4.3. *Macrophyte species-specific percent change*

Some species exhibited stronger responses than others (Table 4.5). *EC* was the worst affected in terms of a general decline in *Fv/Fm* values, relative to all other species, with a -297% decline in the LMB/dark treatment compared to initial conditions. However, the controls also saw large declines by up -149% in both the light and dark treatments which masked the apparent LMB effect statistically. *NF* and *LU* were the least impacted by LMB with respect to *Fv/Fm* values. *PP* was the most impacted by LMB addition, declining by -69% in LMB/dark and by -21% in LMB/light. *MS* was more negatively impacted by the lack of light than the addition of LMB declining in the dark treatments by -68% in the LMB/dark and -56% in the control dark.

Shoot length increased the most in the LMB/light treatment for *EC*, increasing by an average of +580% compared to pre-treatment median values. Shoot length also increased in the LMB/dark by +172% compared to a decline of -78% in the control/ dark treatment. *MS* also showed an increase in shoot length in the light treatment, increasing by +208% in LMB/light and by +260% control/light. *EC* expressed the highest increase in root length out of all species compared to pre-treatment values by an increase of +820% in the LMB/light treatment whilst only increasing by +234% in the control/light. There was no root growth in the dark treatments for this species. Most species expressed root length increases. The only declines in root length were by *PP* in the LMB/dark and by *LU* in the LMB/dark, declining by -74% and -104%, respectively.

The largest increase in wet weight was reported for *PP* in the LMB/light treatment (+60%), secondly by the LMB/dark treatment (+44%) and thirdly in the control/light treatment (+36%). *MS* wet weight declined by -39% and -30% in the LMB/dark and control/dark treatments, respectively. All other species exhibited more minor fluctuations in macrophyte wet weight change (< 25% increase or a < -15% decrease).

The highest increase in macrophyte wash weight was recorded for *PP* LMB/dark (+5.6%) and LMB/light (+4.3%). The only decline in wet weight was minor at < -1% for *PP* control/dark. For all the other macrophytes an increase of up to +2.2% was observed in the LMB treatment whilst on average < +0.2% increase was observed for the control treatment.

For dry weight, the highest increase was observed for *PP* in the LMB/light treatment (+4.9%), followed by *MS* in the LMB/light (+2.4%). *LU* experienced an

overall decline in dry weight for all treatments but with higher declines in the LMB/light treatment with a -16% decline.

**Table 4.5.** Species-specific average percent change across five individuals from pre-treatment median values for each treatment (lanthanum-modified bentonite (LMB)/light, LMB/dark, control/light and control/dark)).

| ***Potamogeton perfoliatus*** | ***Fv/Fm*** | **Shoot length** | **Root length** | **Wet weight** | **Mac wash weight** | **Dry weight** |
|---|---|---|---|---|---|---|
| LMB/light | ↓ -21% | ↑ 5% | ↑ 104% | ↑ 60% | ↑ 4.3% | ↑ 4.9% |
| LMB/dark | ↓ -69% | ↓ -6% | ↓ -74% | ↑ 44% | ↑ 5.6% | ↑ 1.6% |
| Control/light | ↓ -3% | ↑ 156% | ↑ 50% | ↑ 36% | ↑ 0.2% | ↑ 2.1% |
| Control/dark | ↓ -19% | ↑ 20% | ↑ 60% | ↑ 10% | ↓ -0.04% | ↑ 1.1% |
| ***Myriophyllum spicatum*** | | | | | | |
| LMB/light | ↓ -44% | ↑ 208% | ↑ 432% | ↑ 23% | ↑ 2.2% | ↑ 2.4% |
| LMB/dark | ↓ -68% | ↑ 16% | ↑ 232% | ↓ -39% | ↑ 0.9% | ↓ -4.0% |
| Control/light | ↓ -46% | ↑ 260% | ↑ 704% | ↑ 18% | ↑ 0.1% | ↑ 0.8% |
| Control/dark | ↓ -56% | ↓ -62% | ↑ 268% | ↓ -30% | ↑ 0.001% | ↓ -3.1% |
| ***Elodea canadensis*** | | | | | | |
| LMB/light | ↓ -2% | ↑ 580% | ↑ 820% | ↑ 24% | ↑ 2% | ↑ 0.4% |
| LMB/dark | ↓ -297% | ↑ 172% | → 0% | ↑ 1% | ↑ 2% | ↑ 0.5% |
| Control/light | ↓ -149% | ↑ 14% | ↑ 234% | ↑ 14% | ↑ 0.05% | ↑ 0.9% |
| Control/dark | ↓ -149% | ↓ -78% | → 0% | ↓ -10% | ↑ 0.003% | ↓ -0.5% |
| ***Littorella uniflora*** | | | | | | |
| LMB/light | ↓ -1.4% | ↑ 64% | ↑ 294% | ↑ 2% | ↑ 0.1% | ↓ -16.0% |
| LMB/dark | ↓ -0.8% | ↑ 114% | ↓ -104% | ↓ -1% | ↑ 0.2% | ↓ -11.8% |
| Control/light | ↓ -0.8% | ↑ 114% | ↑ 344% | ↑ 8% | ↑ 0.01% | ↓ -13.3% |
| Control/dark | ↓ -1% | ↑ 126% | ↑ 204% | ↑ 13% | ↑ 0.03% | ↓ -14.9% |
| ***Najas flexilis*** | | | | | | |
| LMB/light | ↑ 0.2% | → 0% | → 0% | ↑ 8% | ↑ 1.6% | ↑ 0.5%) |
| LMB/dark | ↓ -2.2% | → 0% | → 0% | ↓ -1% | ↑ 1.6% | ↓ -0.0002% |
| Control/light | ↓-1.2% | → 0% | → 0% | ↑ 5% | ↑ 0.1% | ↑ 0.2% |
| Control/dark | ↓ -3.4% | → 0% | → 0% | ↓ -14% | ↑ 0.01% | ↓ -0.8% |

| Percent change (%) |
|---|
| +100+ |
| +76 - 100 |
| +51 - 75 |
| +26 - 50 |
| +11 – 25 |
| +1 – 10 |
| 0 |
| -1 - 10 |
| -11 – -25 |
| -26 - -50 |
| -51 - -75 |
| -76 -100 |
| -100+ |

### 4.4.4. *Overall ecological responses to LMB addition under different light levels*

Some macrophyte species expressed a desirable ecological outcome following LMB treatment whilst others expressed an undesirable outcome looking generally across all six measured response variables (Table 4.6). The combined species responses indicate that LMB addition did not negatively impact growth responses at a community level in the light. However, the LMB dark treatment, had a negative impact on the overall combined species growth responses (Table 4.6).

**Table 4.6.** Overall response for individual species across treatments (lanthanum-modified bentonite (LMB)/light, LMB/dark, control/light and control/dark) based on the average response across all six measured variables.

| Species | LMB/light | LMB/dark | Control/light | Control/dark |
|---|---|---|---|---|
| *Potamogeton perfoliatus* | ↑ | → | ↑ | ↑ |
| *Myriophyllum spicatum* | ↑ | → | ↑ | → |
| *Elodea canadensis* | ↑ | ↑ | ↑ | ↓ |
| *Littorella uniflora* | ↑ | ↓ | ↑ | ↑ |
| *Najas flexilis* | ↑ | ↓ | ↓ | ↓ |
| Combined species response | ↑ | ↓ | ↑ | → |

↓: Value generally decreased across all six measured responses

→: Values both equally increased and decreased across all six measured responses

↑: Value generally increased across all six measured responses

↑→↓: an ecologically negative response based on the desirability of the species

↑: an ecologically positive response based on the desirability of the species

### 4.4.5. *General and species-specific responses for phosphorus and lanthanum*

TP concentrations in the experimental cores prior to treatment had a mean of 61.1 – 63.14 µg $L^{-1}$ (Appendix 3.2: Table 3). Post-treatment concentrations ranged from 69.7 – 265.14 µg $L^{-1}$ with concentrations significantly lower in the LMB treatment compared to controls and significantly lower in the light treatment compared to the dark (Appendix 3.2: Table 4 and, Figures 7a and 8a). Species-specific responses only accounted for 3.2% of response variability (Appendix 3.2: Table 5) with certain species exhibiting more positive or negative TP concentrations compared to the global modelled response estimate (0.59 ± 0.044). There were variable species-

specific responses for TP (Appendix 3.2: Tables 6 and 7, and Figure 9); TP was significantly higher in the dark treatment for all species (Appendix 3.2: Tables 6 and 7, and Figure 9).

SRP concentrations across all cores prior to experimental conditions had a mean of 35.1 – 36.8 µg $L^{-1}$ across the four treatments (Appendix 3.2: Table 3). Post-treatment, mean concentrations ranged from 41.6 – 84.7 µg $L^{-1}$ across treatments, with SRP concentrations significantly lower in the LMB treatment compared to the controls and also significantly lower in the light treatment compared to the dark (Appendix 3.2: Tables 3 and 4, and Figures 7b and 8b). Individual species responses only accounted for 4.8% of response variability (Appendix 3.2: Table 5) with species either being more negatively or positively impacted than the global response estimate for SRP (0.67 ± 0.051). Species-specific responses were variable (Appendix 3.2: Tables 6 and 7, and Figure 10); SRP was significantly lower in the LMB treatment for *PP* but with a significant interaction; LMB/dark concentrations were lower than LMB/light (Appendix 3.2: Table 7 and Figure 10a). SRP concentrations for *MS* and *EC* were significantly lower in the light treatment compared to the dark (Appendix 3.2: Table 7, and Figures 10b and c). SRP concentrations for *LU* were significantly lower in the light treatment compared with the dark, with a significant interaction with lower concentrations in control/light compared to control/dark and between LMB/light and LMB/dark (Appendix 3.2: Table 7 and Figure 10d). Concentrations for SRP for *NF* did not experience any significant effects despite SRP concentrations declining in the LMB/dark treatment (Appendix 3.2; Table 7 and Figure 10e).

Total La concentrations in the LMB treatments ranged from 0.3 – 314 µg $L^{-1}$ in the LMB/light treatment with a mean of 95.1 ± 68.6 µg $L^{-1}$ and from 0.2 - 193 µg $L^{-1}$ in the LMB/dark treatment with a mean of 100.3 ± 47.3 µg $L^{-1}$ (Appendix 3.2: Table 3) which was also species–specific (Appendix 3.2: Table 6). Minimum and maximum concentrations varied considerably for each species (Table 4.7). To check assumptions that no La was present in the *PP* controls, both control and LMB treatments were assessed for total La. Mean concentrations in the controls were found to be below the level of detection (LOD) (< 0.012 µg $L^{-1}$) with significantly lower concentrations reported in control cores relative to LMB treated cores ($P$ = < 0.05) (Appendix 3.2: Table 7). All other general and species-specific physico-chemical (conductivity, pH and DO) and chemical analysis results for $NH_4^+$, $NO_3^-$, DOC, and for *PP* total Ca, Fe, Mn, Ba, Nd and Pr are reported in Appendix 3.2.

**Table 4.7.** Minimum and maximum total lanthanum-modified bentonite (LMB) concentrations (µg $L^{-1}$) in the light and dark LMB treatments for each macrophyte species.

| Species | Light | | Dark | |
|---|---|---|---|---|
| | Min | Max | Min | Max |
| *Potamogeton perfoliatus* | 44.4 | 314.2 | 124.8 | 152.7 |
| *Myriophyllum spicatum* | 0.30 | 211.4 | 0.20 | 192.9 |
| *Elodea canadensis* | 71.9 | 115.2 | 84.6 | 110.4 |
| *Littorella uniflora* | 41.5 | 118.7 | 58.1 | 178.8 |
| *Najas flexilis* | 57.7 | 204.2 | 66.3 | 108.4 |

## 4.5. Discussion

### 4.5.1. *Macrophyte general responses under LMB and reduced light stress*

There were no clear general negative or positive impacts from the LMB application (Table 4.6) across the species. The LMMs clearly demonstrated that many measured responses were highly species-specific, such as shoot length, root length, wet weight and dry weight, with several species within treatments expressing more negative or positive effects than the overall LMM global model responses, indicating dominance of species-specific stressor responses. As to be expected, the light treatment did significantly increase macrophyte wet weight and dry weight compared to dark conditions across the five macrophyte species, with species generally responding negatively to reduced light levels, in agreement with our original hypothesis.

We expected the light treatment to be a significant influence on more of the responses, particularly *Fv/Fm* values, as light plays a crucial role in photosynthesis. We also expected light to be more important across all macrophyte species in determining shoot length, as some species in the dark might have increased their shoot length in search of light. However, overall, this was not the case; separate species responses were observed but, rather than elongation, this may have manifested as new shoots, which was, unfortunately, not recorded (though should be captured in the dry matter response). The number of new shoots and roots would have been important extra growth measurements to document, particularly as some species expressed high numbers in the LMB treatments in comparison with the controls (e.g. Figure 4.1). However, it would have been difficult to account for this for

each individual species given the definition of new shoot/root growth e.g. *MS* grew new shoots but as an extension to its existing shoots (Figure 4.4) whilst *PP* grew new shoots through a separate shoot (Figure 4.4). New shoot growth was easy to observe in LMB-treated cores where the applied product was visible on most individuals whilst newly sprouted shoots were clearly not coated in the product. However, this response would have been difficult to measure in controls.

The only significant impact reported for LMB across all species was an increase in macrophyte wash weight, which was significantly higher in LMB cores than the controls. However, macrophyte wash weight could not be differentiated between epiphyte load and the LMB product in LMB treated cores as in all cases filter papers were visibly loaded with the product which remained on the surface of individuals at the end of experiment, despite standard epiphyte washing methods (Zimba and Hopson, 1997).. The product was also still visible after standard drying practices as a powdery residue on macrophyte tissue. It was, therefore, inappropriate to undertake metal analysis on the dried material to investigate the bioavailability of La across species or the potential for La toxicity.


**Figure 4.4.** New shoot growth in LMB/dark treated cores for *Potamogeton perfoliatus* (top left), *Myriophyllum spicatum* LMB/light (top right) and new shoot and root stolon growth in LMB/light treated cores for *Elodea canadensis* (bottom) (© Kate Waters-Hart).

### 4.5.2. *Species-specific responses strategies to LMB and reduced light stress*

#### 4.5.2.1. *Light vs reduced light*

All measured macrophyte growth responses were highly species-specific with some responding positively to LMB addition and reduced light availability but others negatively. Individual responses to the dark treatment were as expected with no increase in measured responses relative to the light treatment. *LU* and *NF* were the most tolerant to dark conditions, going against our original predictions. However, these species commonly occur in lakes at depths > 3m. In terms of *F*v/*F*m, the species most impacted by the lack of light were *MS and PP.* Their *Fv/Fm* values significantly declined in the dark, with *MS* exhibiting the greater negative effect. *PP* and *MS* have been known to grow in both shallow and deep waters (Aiken et al., 1979; Kautsky, 1988; Nichols and Shaw, 1986) so this was a surprising result considering lower light availability at deeper depths and their competitive strategies (Murphy et al., 1990) such as, strong apical growth which helps to counter light limitation. *PP* is considered to be a stress-tolerant species (Kautsky, 1988), however, stolons were observed in the dark treatment for several individuals which indicates the species was under low – high stress (Wiegleb and Brux, 1991). *PP* also had several extra shoots sprouting in the dark treatment (Figure 4.4) which was also a likely response to stress; this behaviour is known to one of its competitive strategies (Murphy et al., 1990). *PP* individuals sought light as reflected in significantly increased wet weights, particularly for the LMB/dark treatment (+44%) whereas the control/dark did not increase as much (+10%) (Table 4.5). This response implies that *PP* was below its light compensation point (Middelboe and Markager, 1997) but even more so in the LMB/dark, indicating that LMB is clearly causing an additional stress, here. Wet weight was significantly lower in the dark treatment for all species as to be expected except for *PP* that had a significant increase in new shoots which, explained this result (Figure 4.4).

*MS* is also considered to be stress-tolerant to low light levels, with a light compensation point of 1-2% of full surface irradiance (Grace and Wetzel, 1978). However, it can only perform photosynthesis rapidly under optimal light conditions for a short period of time (Grace and Wetzel, 1978). Contrastingly, it has also been noted that *MS* needs 39 µmol $m^{-2}$ $s^{-1}$, which is 10 µmol $m^{-2}$ $s^{-1}$ above the light levels we used, and *MS* might, therefore, may have been stressed even in the light treatment. The *Fv/Fm* values were 35.34% lower in the LMB/dark compared to LMB/light and 17.9% lower in the control/dark compared to control/light indicating that LMB caused a further impact in addition to light. It is possible that the length of daylight chosen for this study

(14 -hour (light):10-hour (dark)) might have been the optimal length for some of the test species but not for others. Macrophyte metabolism is triggered by daylight length for marine macrophytes (Schaffelke and Luning, 1994) and it is likely this is the same for freshwater macrophytes.

The rate of change for some species across the incubation period was, again, species-specific. *EC* was negatively impacted in terms of *Fv/Fm* but not in terms of other growth indicators in response to dark and LMB treatments. Although *EC* can grow across a wide depth range it is relatively light-demanding (Bowmer et al., 1995), with a light compensation point expected to be approximately 15% of full sunlight intensity (Nichols and Shaw, 1986). It is most likely these species are experiencing multiple stresses, especially under dark conditions where lower DO, higher $NH_4^+$ and higher metal concentrations were reported (Appendix 3.2; Tables 3 – 7). $NH_4^+$ concentrations were significantly higher in the dark treatments for *PP*, *MS* and *EC* with some individuals experiencing concentrations of > 0.5 mg $L^{-1}$ which are levels known to cause physiological stress in plants (Cao et al., 2007; Smolders et al., 2000; Van Katwijk et al., 1997) through accumulation into chloroplasts leading to reduced rates of photosynthesis and physiological damage to leaves (Puritch and Barker, 1967).

Root length was significantly shorter for *MS* and *EC* in the dark treatments compared to the light. Increased total Fe concentrations can cause physiological impacts to both leaves and roots (Immers et al., 2013). In some individuals, particularly *MS*, black bases of stems and roots were visible in the control/dark treatments (Figure 4.5) which can indicate physical symptoms of direct Fe toxicity (Wheeler and Cook, 1985). This could imply that high Fe concentrations around *MS* roots induced root die-off as indicated by the significantly reduced root length for this species in the control/dark treatment. There were obvious signs of the conversion of Fe(III) to Fe(II) ions in some of the cores, e.g. *MS* (Figure 4.5). There was also a significant increase in concentrations of total Fe and Mn in the dark treatments, which further implies the potential for Fe toxicity. Mean total Fe concentration was >3x higher in the LMB/dark treatment compared to the LMB/light treatment and almost 2.5x higher than the control/dark for *PP.* These high total Fe concentrations could have contributed to Fe toxicity, given the concentrations are within the potentially toxic range (≥ 200 µg $L^{-1}$) (Batty and Younger, 2003), and is, therefore, a potential reason why individuals in the LMB/dark treatment were more impacted than LMB/light.

Reduced DO concentrations were observed for all species in the dark

treatments which implies precipitation of Fe and Mn within the water column. Additionally, there were significantly higher TP and SRP concentrations in the dark treatments compared with light treatments. This was possibly due to disassociation from Fe and Mn complexes, in addition to senescence of plants. Wet weight was significantly lower for *MS*, *EC* and *LU* and for dry weight for *MS*, *EC* and *NF* in the dark treatments. This was to be expected given the lack of light available. Decreases in wet weight and dry weight all reflect an overall decline in biomass which confirms these species were in a stressed state predominantly as a result of low light (Grime, 1979).

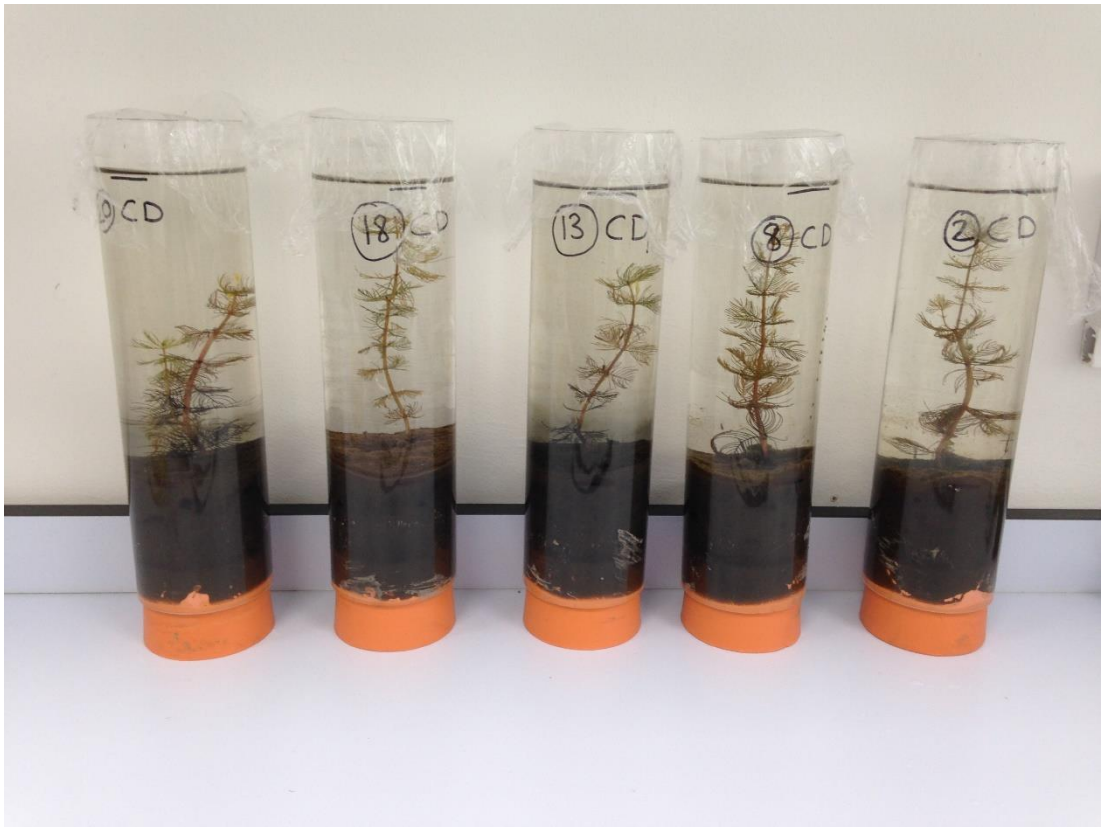



**Figure 4.5.** Darkened base of stem and black basal leaves visible on some individuals of *Myriophyllum spicatum* in the control dark treatment where iron concentrations were significantly raised in comparison to other treatments (© Kate Waters-Hart).

### 4.5.3. *LMB vs control*

The only macrophyte species to experience a significant decline in *Fv/Fm* values in response to LMB addition was *PP*. *PP* also had the heaviest macrophyte wash weights in both the LMB/light and LMB/dark treatments which is possibly due to the curvature of its leaves which creates areas for the product to lodge, more so than other species tested. We might have expected *MS* to accumulate more product initially due to its larger leaf surface area but as LMB could fall through the leaves more easily than *PP* this result is logical. *PP* was clearly in a stressed state following LMB addition and the suspended particle size of LMB could have reduced the light penetrating the leaves and lowered the rate of photosynthesis through shading. However, LMB/dark *Fv/Fm* values were lower than LMB/light values, confirming that LMB could cause a shading effect in the LMB/light but in the LMB/dark there were additional reasons for its decline. *MS* and *EC* also had reduced *Fv/Fm* compared to median pre-treatment values following LMB addition, although this was not significant statistically. It is possible that the product remaining on leaf surfaces may have lowered *Fv/Fm* readings through interference (see chapter 5).

One commonly reported mechanism of toxicity is the inhibition of biological processes such as photosynthesis and mitochondrial electron transport (Babu et al., 2005). LMB application has been shown to increase $NH_4^+$ concentrations to receiving waters post- treatment (de Magalhães et al., 2019; Reitzel et al., 2012; van Oosterhout and Lürling, 2012) and could potentially explain the higher declines in *F*v/*F*m values in the LMB treatment. Necrosis of leaves and decreased photosynthetic rates have been reported as $NH_4^+$ toxicity symptoms for *EC* and other macrophytes (Dendène et al., 1993; Zaman and Asaeda, 2013). Higher $NH_4^+$ concentrations and reduced light conditions have also significantly impacted *MS* in other studies (Cao et al., 2011) and for other *Potamogeton* spp. (Cao et al., 2004). *PP*, *MS* and *EC* all experienced brown discolouration of leaves in the dark treatments. This was particularly obvious in the controls where no LMB product hampered observations of leaf colour. It is, therefore, unlikely that this was an LMB specific response and most likely the result of light limitation. However, $NH_4^+$ concentrations were significantly higher in the LMB treatments compared to controls. The combinations of the LMB product smothering the leaves, the lack of light and significantly increased $NH_4^+$ concentrations could, collectively, have caused a more pronounced decline in *Fv/Fm* in LMB/dark compared to LMB/light, particularly for *PP*. It is difficult to conclude that higher $NH_4^+$ concentrations were caused by LMB itself or if LMB caused negative

effects to the plants which then led to elevated $NH_4^+$ levels in the water. In the mentioned studies where $NH_4^+$ has increased post-application, macrophytes were absent. A core experiment using LMB without macrophytes has also reported elevated $NH_4^+$ concentrations post-application which were reported to be caused by the temporary suppression of nitrification/denitrification under aerobic conditions, which lead to significantly elevated $NH_4^+$- N concentrations (Gibbs and Özkundakci, 2011).

*EC* exhibited a significant increase in shoot length that was noticeable in the LMB treatments (Figure 4.4). *Elodea* spp. have often been reported to increase in abundance after LMB applications (Gunn et al., 2014; Waajen et al., 2016a) and have increased after other in-lake methods such as $FeCl_3$ addition (Immers, 2014). It is also recognised as a pioneer coloniser following lake remediation (Ozimek et al., 1990). However, these colonisation reports are commonly attributed to responses to improved water clarity and not as a stress response (Murphy et al., 1990; Barrat-Segretain et al., 2002). Therefore, its colonising abilities should not be solely assumed to be a response to clearer water, but could also be a response to stress, as indicated by our results.

*LU* shoot length only increased in the LMB/light treatment but not in the LMB/dark treatment. *LU* is a high stress-tolerant species (Robe and Griffiths, 1998) and has been reported to increase its shoot: root ratio in response to stress (Kolář, 2014) with shoot length varying depending on the type of stress (Robe and Griffiths, 1998). The majority of studies relating to stress for *LU* consider water level fluctuations as the stressor. It is, therefore, difficult to compare these studies with the effects of nutrient reduction through LMB addition. However, our results clearly indicate that *LU* was stressed in the LMB/light treatment; the reason for which remains unclear.

*EC* was the only species to increase in wet weight in response to the LMB treatments which makes sense due to the significant increase in shoot length recorded. *EC* also had a significant increase in dry weight as a result of shoot lengths and probably also shoot multiplication. These significant increases in weight for *EC* must be interpreted carefully as there was still LMB product on many individuals following standard epiphyte washing and this residue remained after drying. Therefore, any additional wet weight cannot be fully accredited to increased biomass and could be due in part to the weight of the applied product remaining on leaves. Contrastingly, *LU* had a significantly lower dry weight in LMB treatments compared to controls. This was unusual given the significant increase in shoot length in response

to the LMB/light treatment. As shoot length did not increase in the LMB/dark treatment, the dry weight was still lower compared to controls even after the significant shoot growth. A decrease in LMB dry weight for *LU* could be the result of a toxic effect as seen in other floating macrophyte species (Snowden and Wheeler, 1995), but this needs further investigation.

All macrophyte species exhibited a significant increase in macrophyte wash weight in LMB treatments compared to controls. *LU* was the least impacted species, probably due to its waxy, slender tubular leaves making it harder for the product to remain attached. Although it is difficult to differentiate between epiphyte load and the LMB product in the combined wash weight collected, the product was always a notable component of the wash material. *MS* had a higher wash weight in the LMB/light than the LMB/dark treatment. This could be explained by there being more epiphytes present on plants than in the dark treatment, yet no other species mirrored this response. In natural lake conditions, the product is less likely to remain on the surface of macrophyte leaves as long as it did in this experiment. It is likely that turbulence commonly experienced *in situ* as a result of wind and wave movements or grazing and other disturbances by fish and water birds, would dislodge the product although no direct assessments of this have been reported in the literature.

### 4.5.4. *Phosphorus inactivation and lanthanum concentrations*

#### 4.5.4.1. *Phosphorus inactivation*

Overall, across all species, TP and SRP concentrations were significantly lower in the LMB treatments, which confirms our original hypothesis. However, across macrophyte species the impact of LMB on TP and SRP concentrations varied. For all species, TP concentrations were significantly reduced in the LMB treatment, but the majority of this reduction was observed in the dark treatment in comparison with the light treatment. For all species, higher TP concentrations in the LMB/light treatment compared with the control/light treatment was observed. For most species, although LMB lowered SRP concentrations, they were not significantly lower than controls. For *MS*, *EC*, *LU* and *NF* there was no significant effect of LMB treatment on availability of SRP in core water in the LMB/light. The TP and SRP concentrations at the outset of the incubation were relatively low compared to the concentrations observed in many treated lakes, prior to LMB application (Spears et al., 2016). So LMB effectiveness in our study should be considered relative to other core studies (Reitzel et al., 2012). Our efficiency estimates indicate that TP and SRP reductions in the LMB/dark

treatment compared to the control/dark treatment were 123.2 µg $L^{-1}$ and 32.2 µg $L^{-1}$, respectively. Based on our results, it is unclear if adding LMB to waters with relatively low P concentrations may actually increase P concentrations in the water column given the increase in mean TP (+24 µg $L^{-1}$) and SRP (+5.4 µg $L^{-1}$) concentrations seen collectively across species, specifically in the light treatment where P concentrations were generally lower than dark conditions but this requires further testing.

A comparable study by Spears et al. (2008) reported water column TP and SRP concentrations in light and dark treated cores from the same sediment and water collection site in Loch Leven of 47 µg $L^{-1}$ (light) and 67 µg $L^{-1}$ (dark) for TP and 24 µg $L^{-1}$ (light) and 41 µg $L^{-1}$ (dark) for SRP, respectively. The pre-experimental concentrations (62 – 63 µg $L^{-1}$ for TP and 35 – 37 µg $L^{-1}$ for SRP) and control/light post-treatment concentrations from our experiment (70 µg $L^{-1}$ for TP and 37 - 42 µg $L^{-1}$ for SRP) are roughly comparable to these concentrations. It is clear, however, from our results that macrophytes can alter P concentrations and that these effects are species-specific. The differences in SRP concentration in the water column of the cores could also be due to individual species nutrient requirements from the sediment or the water column.

The differences in growth response between the macrophyte species did not seem to rely on P availability and are, therefore, likely to be the outcome of direct and indirect effects of LMB. We cannot discount P limitation in the surface sediments for species that rely on uptake of P via roots, but as no species showed a significant increase in root growth in the LMB treatments this seems unlikely. Where there were higher SRP concentrations in LMB treated cores compared to controls this could potentially be due to macrophyte senescence. macrophytes can act as a nutrient pump, sequestering dissolved P from the sediment and releasing it into the water column via leaves (Carpenter, 1981). Unfortunately, our experimental design could not establish whether this was an important pathway, but this should be assessed further.

#### 4.5.4.2. *Lanthanum concentrations*

Mean total La concentrations in the water column were high across all LMB treated species, although we also report species-specific effects on total La concentrations. Mean total La concentrations were high with slightly higher mean concentrations in the LMB/dark compared to LMB/light treatment overall. This confirms our original

hypothesis that concentrations would be elevated following LMB application. Higher total La concentrations in the dark could be due to a diminished capacity for macrophytes to retain particles whilst under stress.

The highest total La concentration was 314.2 µg $L^{-1}$ recorded for *PP* LMB/light which is within the range reported in surface and bottom waters one month post-treatment (Spears et al., 2013a). The variation in concentrations within and between treatments could be attributed to the effects of plant physiology on settling rates of particles. It should also be noted, however, that it was difficult to collect water samples without disturbing macrophyte leaves and so it is possible that the product was re-introduced to the water column as an experimental artefact. The shape, serration, roughness and flexural rigidity of leaves from the different species is likely to have attributed to how easily disturbed the product was during sampling (Albayrak et al., 2012). Leaf shape is considered the most important factor determining flow-leaf interactions with pinnate shaped leaves experiencing higher drag force than other leaf shapes (Albayrak et al., 2012) and could by why *PP*, *MS* and *NF* experienced the higher mean La concentrations compared to the other species as they would be more easily disturbed during sampling.

#### 4.5.4.3. *Lanthanum bioaccumulation and toxicity potential*

La tissue content or La toxicity was, unfortunately, not assessed in our experiments due to the LMB product sticking to the surface of leaves posing a potential contamination issue. Even though the direct effects of toxicity were not tested, it may be that La tolerance across the species used in these experiments is expressed through the various measured responses as a proxy to stress, e.g. *PP* expressed a decline in *Fv/Fm, EC* shoot length increased, and *LU* had a lower dry weight and increased shoot length (LMB/light only) in response to a LMB treatment. It is not known if these individual responses are a direct impact of the product or a result of higher concentrations of La in the water and sediment of treated cores. Filtered La concentrations were not assessed for these experiments but if filtered samples were taken they would have most likely contained La-colloids which cannot solely be regarded as $La^{3+}$ ions due to filter size used in our experiment, and many others (Reitzel and Jensen, 2018). Even a finer filter would not have been able to determine $La^{3+}$ ions without the use of finer filtering techniques and centrifugation (Reitzel and Jensen, 2018), which are time-consuming and costly.

$La^{3+}$ ions are considered to carry the greatest risks biologically (Das et al., 1988; Spears et al., 2013a) and we cannot rule out La toxicity as a cause of stress in our experiments. However, Spears et al. (2013a) reported that $La^{3+}$ concentrations decreased with increasing alkalinity through speciation modelling, and considering that Loch Leven is a high alkalinity lake (Salgado et al., 2010), it is unlikely there would be high concentrations of $La^{3+}$ ions present. Evidence on LMB and La toxicity to aquatic organisms and, more specifically macrophytes, remains poor (Copetti et al., 2016; Herrmann et al., 2016). The uptake of $LaCl_3$ into the cell walls of macrophytes has been reported for some species and has been related to oxidative stress and disturbed mineral uptake leading to degenerative processes at high concentrations which is species specific (1.39 mg $L^{-1}$ for *Lemna minor* L. and 0.28 mg $L^{-1}$ for *Hydrocharis dubia*) (Ippolito et al., 2010; Xu et al., 2012). Recent work has highlighted that *Nymphaea alba*, *Phragmites australis*, *Scirpus lacustris*, *Typha latifolia* and *Elodea nuttallii* all bioaccumulated La post-LMB application to Lake Rauwbraken, The Netherlands (van Oosterhout et al., 2019). Concentrations were 6 -130 times higher for floating leaved and emergent macrophytes which ranged from 22.6 - 136 mg La kg $^{-1}$ DW. For *Elodea nuttallii*, concentrations were 235 – 389 times higher, ranging from 1764 – 2925 mg La $kg^{-1}$ DW four months post-application. van Oosterhout et al., (2019) reported that macrophytes were not hampered as they expanded post-treatment. However, given our results, the expanse of macrophytes reported by van Oosterhout et al., (2019) post-treatment could be a result of individual species stress strategies in response to the treatment. Further work in this area is necessary particularly given the species-specific responses to LMB we observed. Speciation modelling has been used amongst other LMB studies (D'Haese et al., 2019; Lürling et al., 2014; Spears et al., 2013a; van Oosterhout et al., 2014; Weltje et al., 2002) and provides a cheaper alternative to estimate potential $La^{3+}$ ion concentrations and could be used to further assess these risks.

### 4.5.5. *Implications of LMB addition on macrophyte communities*

Adding the equivalent of 5.1 T/ ha of LMB did not result in a consistent negative response across the five test macrophyte species. However, as individual species exhibited negative responses across some of the indicators, under different light conditions, we cannot conclude that undesirable changes in community composition at the whole lake scale will not occur. For example, the conditions produced following

LMB application may favour the growth of undesirable species through competitive selection. This may, in turn, hamper the germination from the seed bank of more desirable species. For example, the species responses reported in Chapter 2 indicated that more desirable species such as charophytes or *Potamogeton* spp. may in fact have limited germination success in the presence of *EC* in spring following an LMB application.

*EC* was the only species to exhibit an overall positive response to LMB/light across the six measured responses, which equates to a negative ecological response. All tested macrophytes growth declined in LMB/dark conditions across the measured responses, which means this was a positive ecological response for *EC,* as it grew less. We, therefore, conclude that at deeper, and, therefore, darker macrophyte growing depths LMB may impose additional stress on all our macrophyte species. *EC* relies less on rooting into sediments to uptake P from the sediment than other macrophyte test species as it can also uptake P via shoots, with leaves being the main uptake route (Robach et al., 1995; Madsen & Cedergreen., 2002). Its adaptable nutrient uptake strategy enables access to nutrients from both the sediment and from the water column, which probably explains why this species is more tolerant to rapid declines in dissolved P concentrations as it can uptake from either source. Where conservation of desirable species is the restoration target, it, therefore, might be unwise, to apply LMB to a waterbody with an macrophyte community dominated by *EC* as the resultant effect may be to promote *EC* dominance, especially in shallow well-lit waters. The *EC* response reported here may indicate similar responses in other undesirable species including *Elodea nuttallii* (Planch.) H. St. John. *PP,* in particular, suffered growth declines following LMB addition, with *Fv/Fm* declining markedly in light and dark conditions (Appendix 3.2: Figure 2a), possibly due to the amount of product remaining on the leaves. At the lake-scale, LMB could have detrimental impacts for this species and potentially other broad-leaved species (e.g. *Potamogeton lucens* L.), although the impacts of water turbulence on product retention remain unclear. Some macrophyte species (e.g. *LU*) may be able to tolerate LMB applications in shallow water but not deep water where smothering by LMB particles may act to enhance shading.

## 4.6. Conclusions

Based on the results of this study it can be concluded that:

- The addition of LMB and reduced light caused additional stress to some of the macrophyte test species used in this experiment, but species responded in-line with their strategy traits.
- Phosphorus concentrations were significantly lower in LMB treated cores compared to controls but was highly species-specific.
- Variable total La concentrations in the water column existed for each macrophyte which could be related to the ability of the product to remain on leaf surfaces before being re-suspended into the water column if disturbed.
- Care should be taken when applying LMB to systems where desirable and less-stress-tolerant species coexist
- Lake managers should consider carefully when applying LMB to waterbodies where macrophyte communities are dominated by *EC,* as undesirable responses may been seen

# 5. Chapter 5: General discussion and conclusion

The discussion brings together the key findings from Chapters 2 – 4 to address the objectives listed in Chapter 1. The main results from each chapter are discussed, followed by a discussion of three general themes that appeared amongst all three experimental chapters. Firstly, the limitations of aquatic macrophyte (macrophyte) monitoring data and standard methodologies are presented. The second theme is focused on macrophyte recovery bottlenecks and thirdly, the unintended consequences when using Lanthanum (La) - modified bentonite (LMB) which could be contributing to the lack of desirable macrophyte community responses following treatment, as well as reduced LMB efficiency. Knowledge gaps are discussed throughout this chapter. The wider implications of the study are examined in the context of using LMB to promote desirable macrophyte recovery to meet ecological targets. The key outstanding questions are discussed, and the conclusions presented.

## 5.1. Macrophyte recovery following LMB applications

Knowledge of macrophyte recovery following LMB additions is very limited. This is also the case more generally for lake restoration studies (Coops and Doef, 1996; Jeppesen et al., 2005). The majority of studies assessing LMB use in lakes focus on quantifying chemical recovery with macrophyte assessments being based on short-term data of a few years post application which, as argued below, is insufficient to assess full community responses. As a consequence, the existing body of research has largely focused on single case-studies with only three studies reporting macrophyte community responses following LMB addition at the lake scale (Gunn et al., 2014; Waajen et al., 2016a, 2016b) and only one multi-lake LMB study (Spears et al., 2016). This is despite the fact that LMB has been applied to over 200 waterbodies globally (Copetti et al., 2016). Collectively, the published studies on LMB macrophyte recovery only focus on the short-term (≤ 2 years) changes following a treatment. Chapter 2 combined and assessed this body of evidence in combination with unpublished longer-term monitoring data from Lake Rauwbraken and Crome's Broad n and south basins along with long-term data for untreated control lakes (Alderfen Broad, Upton Great Broad, Whitlingham Little Broad and Witlingham Great Broad) for comparison. This allowed the assessment of macrophyte recovery across multiple lakes both in the short (≤ 2 years) and long-term period post – application (≥ 2 years), providing the most in-depth assessment yet of macrophyte responses across twelve

treated lakes across countries. Chapter 2 confirmed that macrophyte recovery timescales following LMB addition were lake–specific with little changes reported in community composition up to ten years post-application, with most sites dominated by *Elodea canadensis* pre- and post-treatment. Species gains were dominated by Characeae species.

These results support the findings of the individual lake case studies which report *Elodea* species and charophytes as typically being the first to colonise waterbodies following the application of phosphorus (P) control materials to lakes and reservoirs (Bishop and Richardson, 2018; Gunn et al., 2014; Immers et al., 2015; Perkins and Underwood, 2002; Waajen et al., 2016a). There is discussion further in this chapter why these species dominate pre- and post- application and why charophytes may be the first new or pioneer species to appear post-application. The findings from Chapter 2 confirmed that treated lakes with data are not currently meeting ecological targets, including good ecological status under the WFD or favourable condition targets for Sites of Special Scientific Interest (SSSI), despite P being reduced to concentrations that should favour increases in macrophyte extent and diversity (Jeppesen et al., 2000; Spears et al., 2016). Results revealed that dominance by pioneers could impede the establishment of more desirable species through competition for light, space and nutrients. Additionally, the colonisation potential from both the seedbank and external dispersal vectors could also cause ecological responses to fall below their potential.

## 5.2. The potential for macrophyte recovery from the seedbank using LMB

Studies of macrophyte seed banks are rare (Bakker et al., 2013) and no studies have considered the effects of LMB on seed banks previously. This knowledge gap was addressed in Chapter 3 and the conclusions from the experimental responses show that LMB did not restrict macrophyte growth from the seed bank. This result is contrary to the suggestion that LMB may inhibit macrophyte recovery through the formation of a physical barrier or burial of propagules deeper in the sediment, thereby preventing germination (Hilt et al., 2006). However, it does pose the question as to why species do not rapidly appear at the lake-scale when treated with LMB? Biotic constraints are still concerns following lake interventions, with fish, birds and invasive species all being causes for low germination success (De Winton and Clayton, 1996; Green et al., 2002; Lauridsen et al., 1993, 2003a; Pollux, 2011; Søndergaard et al., 1996a,

2000). Invasive species such as *Elodea canadensis*, which was reported to be the most commonly occurring species pre- and post-LMB applications across lakes in Chapter 2, have been documented to significantly lower seed number and species richness of desirable species in seed banks. (De Winton and Clayton, 1996). The lower abundance of desirable species and higher abundance of invasive species, inhibits *in-situ* seed production of desirable species which affects community compositions in following seasons (De Winton and Clayton, 1996; Irfanullah and Moss, 2004).

Inhospitable abiotic conditions following LMB treatments may also limit macrophyte recovery (Lürling and van Oosterhout, 2013), and the results from Chapter 3 indicate that LMB may have insufficiencies when controlling soluble reactive phosphorus (SRP) concentrations when there is a high bioturbation rate, as discussed in Section 5.6. A lack of standard methodologies (further discussed in section 5.4.2) for assessing seed bank recovery, including viability of historic, or buried seed banks and propagule dormancy requires attention in future studies. An assessment of seed bank viability should be conducted prior to future lake restoration interventions, such as LMB application, where macrophyte recovery is a key objective. Where, it appears that a desirable species will not recover through the contemporary seed banks, translocation (species transplantation work) may be explored to support recovery (Knopik and Newman, 2018).

## 5.3. Species-specific responses to LMB

Very little information exists on how different macrophyte species respond to nutrient reduction (Bakker et al., 2013; Lauridsen et al., 2003a; Phillips et al., 2016; Søndergaard et al., 2007). Chapter 4 revealed the macrophyte test species responded very specifically, expressing differences in their stress mechanisms to LMB and reduced light. All five species responded positively in the LMB/light treatment on average across the measured responses. However, as *Elodea canadensis* was the only undesirable species out of these five, this was not classed ecologically as a positive response due to its undesirability. When making comparisons to lake-scale applications, *Elodea canadensis* and *Elodea nuttallii* have also been reported to increase in coverage post-treatment at the whole lake scale (Gunn et al., 2014; Waajen et al., 2016a). Gunn et al., (2014) found *Elodea canadensis* to increase in the coverage at Loch Flemington, UK from 30 – 40%, to

approx. 80% after treatment. It also increased in maximum macrophyte growing depth from 1.4 – 2.6 m pre-application in 2009 to 2.3 – 2.9 m in 2011 post-application. Waajen et al., (2016a) found *Elodea nuttallii* to increase in coverage by 776 $m^2$ two months after a 'flock and lock' treatment (iron (III); Flock and LMB; Lock) in lake De Kuil, The Netherlands. Increase in expanse at the lake-scale has been attributed to higher water clarity reported post-application but our results suggest it could also be due to a competitive trait of the species, as reported in Chapter 3, and elsewhere (Murphy et al., 1990). *Elodea canadensis* can impact on desirable species establishment (Bishop et al., 2019; De Winton and Clayton, 1996) and could be a reason why community compositions did not change post-treatment as indicated with our multi-lake observations from Chapter 2.

Other experimental studies have reported similar stress responses for *Elodea canadensis*. For example, *Elodea canadensis* exhibited stronger increases in primary production when compared to *Myriophyllum spicatum* and *Najas flexilis* in response to increasing salt (chloride) contamination and sediment disturbance (turbidity) (Stoler et al., 2018). Stoler et al. (2018) also reported similar observations to the results in Chapter 4, in that species responses (net primary productivity (NPP), gross primary productivity (GPP) and respiration) were highly specific. All macrophyte species tested in Chapter 4 exhibited a negative response to the LMB/dark treatment. Due to very little evidence examining macrophyte communities at depth in LMB treated lakes it is not possible to compare the findings to lake-scale observations. The results are, however, similar to those of Stoler et al. (2018). When comparing the 'dark' responses with Stoler's high turbidity responses for *Myriophyllum spicatum*, *Najas flexilis* and *Elodea canadensis* it is apparent that growth responses indicated a decline for the former two species but an increase for the latter in response to decreased light.

It has been speculated that P - capping agents may smother macrophytes following applications (de Winton et al 2013; Hickey and Gibbs 2009; Douglas et al., 2016). Chapter 4 confirmed that the LMB product was retained on leaf surfaces at the end of experimentation. A study using alum attributed smothering through shading as a cause of reduced growth in *Chara hispida*, in addition to lowered pH and a toxic influence (Rybak and Joniak, 2018). Rybak and Joniak., (2018) also stated that it is highly likely that different charophyte species would react differently to applications based on variations in morphological traits. Findings from Albayrak et al., (2012) suggest species with pinnate leaves would probably be able to dislodge product more easily at the lake-scale than species with elliptic or rectangular shaped leaves.

Pinnate shaped leaves are less tolerant of higher drag force due to a more complex leaf geometry. Given the results from the Albayrak et al., (2012) study, the leaf shape may be the reason why some species retained more product than others in Chapter 4. From the bioassay experiments, *Littorella uniflora* and *Myriophyllum spicatum* had the lowest macrophyte wash weights compared to the other macrophyte species in the LMB treatments. *Littorella uniflora* has pointed, rigid leaves with a round profile and *Myriophyllum spicatum* has fan-like pinnate leaves. The differences of leaf shape, texture and flexural rigidity are all likely to be factors that control the dislodgement of the product at the lake-scale. The effects of LMB through smothering of leaf surfaces and further reducing light availability was concluded as the most likely explanation for species decline in Chapter 4, which is discussed further in section 5.6.

## 5.4. Limitations of macrophyte monitoring data and standard methodologies

### 5.4.1. *Paucity of monitoring data and inconsistent methodologies to assess macrophytes at the lake-scale*

The availability of pre-and post- monitoring data could be a key limiting factor in understanding macrophyte recovery timescales and community compositions in response to LMB treatments, or in response to any lake remediation measure. It was clear that the insufficiency of macrophyte data hindered any robust statistical assessment of recovery across the treated lakes from Chapter 2 with a lack of both pre- and post-treatment data. These available data are insufficient to confirm long-term positive or negative responses in macrophytes following LMB application. Chapter 2 concluded that those treated lakes with sufficient data to allow analysis (n=2) did not meet ‘good’ ecological status, as defined by the WFD, following LMB application.

Different macrophyte assessment methods are in use across European member states, with few of these methods being published for wider use (Penning et al., 2008). Consequently, intercalibration methods are presently operating to compare statuses across countries (Poikane et al., 2018). These exercises can be time-consuming, and information can sometimes be lost during these processes. The lack of standardised monitoring programmes across lakes and countries inhibits comparison and general conclusions of effectiveness at the larger scale for LMB and other restoration methods. The development of standardised monitoring protocols capable of producing comparable data for international use are badly needed.

macrophytes are a severely under monitored aquatic group, yet great importance is placed upon them in order to make national and European assessment of ecological quality in freshwaters. A simple monitoring protocol should be put into practice such as the Common Standards Monitoring (CSM) (JNCC, 2015) method which is widely used in the UK and does not include any specialized survey techniques such as diving based surveys or snorkelling surveys to assess macrophyte diversity and abundance.

### 5.4.2. *Variations in germination methodologies*

Standard methodologies for assessing germination success of macrophytes from submerged seed banks are lacking (Mcfarland and Shafer, 2011). Most seed bank methodologies have been created for terrestrial, wetland or riparian habitats (Bakker et al., 2013; Leck and Graveline, 1979) and so adaptations of these have generally been made to conduct experiments with lake bed or riverine seed banks. The number of germination studies looking specifically at submerged macrophytes are sparse (Bakker et al., 2013) with only five studies using lake sediments. These studies have used a range of different water depths including 2 – 3 cm water depth above the sediment surface (Boedeltje et al., 2003), 5 cm (Harwell and Havens, 2003), 14 cm (Strand and Weisner, 2001), ~50 cm (De Winton et al., 2000) and 1 L of water added to 1.5 L containers (Ozimek, 2006) to assess macrophyte germination. Similar variation is apparent in the container size, whether additional substrates were added to allow adequate germination depths, in the addition of sediment with propagules, the water source, cold-stratification and/or drying and re-wetting, and experimental duration (Mcfarland and Shafer, 2011).

The comparability amongst apparent species-specific germination cues based on these different methods is therefore poor. It is critical to know specific requirements of species for germination where sites are isolated and so reliant on contemporary viable seed banks for macrophyte recovery. The method used in Chapter 3 allows determination of the likely community response from germination of the contemporary seed bank and may be used, with limitations, to assess lake-scale recovery potential. Our method also offers the most realistic scale of sediment to water depth ratio in comparison to other studies, although larger mesocosm trials would offer a more representative intermediate scale. The use of mesocosms may address the reported issue that small scale seed bank germination trials can often indicate very different community responses when compared to whole lake responses (Casanova, 2015).

Although, this approach may be costly and time extensive, it is however likely that existing seedbank potential may need to be assessed more in the future given the response of macrophyte recovery and predictions of macrophyte recovery timescale from Chapter 2. The use of paleoecology may be the first step to assess the types of species that may be present historically (Alderton et al., 2017; Salgado et al., 2010; Sayer et al., 2010a). Additional seed viability studies may also be needed to assess the potential ecologically active community composition before conducting larger-scale trials.

High variability between smaller scale replicate germination trials can be caused by high heterogeneity of propagule distribution in sediments (Hammerstrom and Kenworthy, 2003), seasonal variations in plant abundances (Thompson and Grime, 1979), transient seed longevity, and the domineering presence of propagating species are likely factors (Bakker et al., 2013). It is also possible that high variability amongst experimental replicates are unrealistic when compared to field-scale conditions, as a result of experimental design. For example, the exposure of seedbanks to artificial environmental conditions may not sufficiently mimic natural conditions limiting the cues for germination of dormant seeds and propagules that would otherwise occur in the lake (Nishihiro et al., 2004). However, these cues remain largely unidentified limiting improvements in experimental design.

### 5.4.3. *Variations in lab-scale experiments assessing macrophyte responses*

The use of submerged macrophytes in bioassay style experiments has not been widely reported in the literature. Again, no standard methodologies exist for assessing the impact of phytotoxicity and various stressors on submerged macrophyte growth (Lewis, 1995; Mohan and Hosetti, 1999). The toxicity assessments that have been reported have been designed to assess macrophyte responses to suspended solids, heavy metals and nutrient removal potential (Mohan and Hosetti, 1999). It is even rarer for subject species to be submerged macrophytes, with most studies focussing on floating macrophytes, particularly *Lemna* spp. (Babu et al., 2005; Feiler et al., 2006; Ippolito et al., 2010; Wang, 1991, 1988; Weltje et al., 2002).

Of the examples that exist in the literature for macrophytes only one unpublished thesis is available which examines the growth of *Elodea nuttallii* in the presence of LMB and other P - binding materials (Chrzanowski, n.d.), and one other study reports on an assessment of macrophyte responses to iron (Fe) addition for P

control (Immers, 2014); both studies focus on Lake Terra Nova, a peat lake in the Netherlands. The former study reports that, *Elodea nuttallii* growth (total biomass, root biomass, shoot biomass, shoot:root ratio, root length and shoot length) did not differ between LMB and untreated cores after 4 weeks (Chrzanowski, n.d.). The latter study indicates that *Elodea nuttallii* had no significant responses (root biomass, shoot biomass, total biomass, total biomass increase, shoot:root ratio and relative growth rate) in the presence of Fe, although *Potamogeton pectinatus* growth was lower following Fe treatment after 84 days exposure.

Macrophyte response indicators vary across the few studies reporting on macrophyte responses to P - binding materials. Common macrophyte response indicators include dry weight, root:shoot ratios, shoot length, root length, net primary production and gross primary production. Commonly, studies only measure a sub-set of these indicators, which limits the ability to detect responses in a full range of morphological traits. The indicators used in Chapter 4 employed a range of response indicators in an attempt to produce a net effect measure, or response metric. These indicators were found to produce, at times, conflicting positive and/or negative growth responses. It was apparent that *F*v/*F*m was a useful approach for assessing subtle photophysiological responses prior to changes in more physical indicators, as highlighted by Babu et al. (2005). macrophyte responses are difficult to measure, particularly if multiple stressors are operating (Stoler et al., 2018), as it can be challenging to prise apart individual causes to the response seen. The results from the bioassay experiments in Chapter 4 demonstrate this. Multiple indicator responses should be measured when testing subject species to potential pollutants to have confidence in detecting the specific stress traits that different species exhibit.

Additionally, some chosen response indicators used in bioassay-style experiments are not complimentary to the morphological traits of subject macrophyte species. For example, testing biomass or shoot length of slow growing species over a short-term experiment may provide an inaccurate response to stress if an increase/decrease in shoot length is not one of the test species strategy traits to exhibit when stressed. It is important to understand the traits different species exhibit in response to stress for these style experiments and use indicator response measures based on this.

It was clear that visually, some of the macrophyte species were under stress but these signs did not manifest in our measured response indicators. Simple visual assessment measures may be an added beneficial response measurement to use to

assess toxicity/stress that are less time consuming, yet very informative. There is a need for multiple indicators to produce a response metric (e.g. as presented in Chapter 4) across bioassay studies to assess macrophyte responses more robustly. It is however, important to understand what each chosen measured response is relaying and what it is responding to.

No standard macrophyte test species are currently in use for bioassay experiments (Arts et al., 2008) and it is important to consider the variation in responses across indicators among different ecotypes (Stoler et al., 2018). As such, results from single experiments could potentially provide misleading response data when considering responses across lakes where different macrophyte ecotypes may exhibit variable tolerances to common stressors. For example, light tolerance thresholds for photosynthesis. Where available in the literature, our subject species in Chapter 4 indicated a high degree of variability (Table 5.1.). This may be due to variation in test conditions and in the use of different ecotypes making comparison with our own study difficult. For future assessments of macrophyte recovery potential, it is important to select representative test species as opposed to selecting ecotypes that are not local, but perhaps easily sourced or grown.

**Table 5.1.** Light requirements of subject species from Chapter 4.

| Species | Light requirements | Reference |
|---|---|---|
| *Potamogeton perfoliatus* | 6 -8 µmol $m^{-2}$ $s^{-1}$ (*Potamogeton polygonifolius*), 416+ µmol $m^{-2}$ $s^{-1}$ (*Potamogeton pectinatus*) | (Hoostmans and Vermaat, 1991; Van Den Berg et al., 1998a) |
| *Myriophyllum spicatum* | 39 µmol $m^{-2}$ $s^{-1}$ or LCP of 1 - 2% of surface light | (Grace and Wetzel, 1978; Madsen et al., 1991; Nichols and Shaw, 1986) |
| *Elodea canadensis* | 3.5 – 10 µmol $m^{-2}s^{-1}$ or 15% daylight | (DeGroote and Kennedy, 1977; Hough, 1979; Madsen et al., 1991; Madsen and Sand-Jensen, 1994) |
| *Littorella uniflora* | 11.6 µmol $m^{-2}s^{-1}$ | Sand-Jensen and Borum, (1991) |
| *Najas flexilis* | 0.53 – 7.32 LEC | Wingfield et al., (2006) |

LCP – Light compensation point

LEC – Light extinction coefficient

## 5.5. Recovery bottlenecks

The results reported in Chapter 2 indicated that macrophyte responses to LMB addition across the 12 treated lakes were dominated by a single species, *Elodea canadensis*. This is a particularly good pioneering species and over-winters in waterbodies to remain *in situ* until the following growing season. Its ability to ramify more in summer and autumn increases its competitive advantage the following season. *Elodea canadensis* has a wide nutrient tolerance range (Preston and Croft, 2001; Trémolières, 2004) which, perhaps, helps to explain why it is able to dominate LMB treated waterbodies pre- and post- application. As such, the presence of invasive non-native or pioneering species that over-winter may prevent the re-establishment of more desirable species following LMB applications. This is in agreement with growth response data provided in Chapter 4. Chapter 2 also emphasized that recovery may be heavily influenced by the connectivity of each waterbody to other external propagule sources and the presence of a viable seed bank. A simple assessment of the distance to the nearest similar sized waterbody or waterway may give an indication of recolonisation potential of macrophytes from external sources, as indicated for treated lakes in Chapter 2 (Table 2.1). Tools such as the UK Lakes Portal (https://eip.ceh.ac.uk/apps/lakes/) can be used to assess this for UK lakes providing information on macrophyte species for certain waterbodies. These data may be used, for example, to predict species distribution in locally connected water bodies to inform potential for species ingress.

As mentioned in section 5.4.2, the viability of seed banks and longevity of desirable macrophyte species to contribute to the re-colonisation of waterbodies following restoration efforts is limited in the literature. It is possible that the effectiveness of regeneration from seed banks following restoration efforts is limited by the longevity of desirable species. For example, expecting *Najas flexilis* to return to waterbodies after an LMB application where it has been lost through nutrient pollution (e.g. Loch Flemington (Gunn et al., 2014)), may not be possible due to it being rare in the landscape but also to its low persistence in the seed bank, with dormancy estimates of < 0.5 years (Bakker et al., 2013; Kleyer et al., 2008). Examples of dormancy periods for *Najas flexilis* and other species are provided (Table 5.2).

Charophytes are the most numerous taxa in lake seed banks (Grillas et al., 1993), and have the highest longevity in terms of dormant viability (Bonis and Grillas, 2002; De Winton et al., 2000). However, species-specific macrophyte longevity timescales are widely unknown, although efforts have been made in the last decade

to produce estimates of longevity (Bakker et al., 2013; Kleyer et al., 2008). This work indicates that most desirable species have short viability periods and could, therefore, be lost from the contemporary seed bank within one year of unfavourable conditions. As such, re-establishment may require transplantation of live material to establish the seedbank. It has become evident that variations in methodologies and differences in abiotic conditions used in germination experiments may not be sufficient to force species-specific germination cues and therefore non-standardized methodologies could be to blame for misunderstandings in species-specific germination requirements and variations in seed viability estimates.

**Table 5.2.** Longevity of submerged macrophyte propagule/seed/fragment estimates from literature for our bioassay experiment in chapter 4 and germinated species from the germination experiment in Chapter 3

| **Species** | **Reproduction strategy** | **Mean/range of viable propagule longevity (years)** | **Reference** |
|---|---|---|---|
| *Potamogeton perfoliatus* | Rhizome/seed | < 1 | Kleyer et al., (2008); Bakker et al., (2013) |
| *Myriophyllum spicatum* | Fragments | < 1 | Kleyer et al., (2008); Bakker et al., (2013) |
| *Elodea canadensis* | Fragments | < 1 | Kleyer et al., (2008); Bakker et al., (2013) |
| *Littorella uniflora* | Rhizome/seed | > 30+ | Kolář, (2014) |
| *Najas flexilis* | Seed | < 1 | Kleyer et al., (2008); Bakker et al., (2013) |
| *Chara/Nitella* spp. | Oospores | 0 – 300+ | Bonis and Grillas, (2002); Wade and Edwards, (1980); Stobbe et al., (2014) |
| *Potamogeton pectinatus* | Seed/rhizome | 0.07 | Kleyer et al., (2008); Bakker et al., (2013) |
| *Potamogeton obtusifolius* | Seed/rhizome | - | - |

- indicates no data available but for other *Potamogeton* spp. propagules can be viable for 150 years (Alderton et al., 2017)

### 5.5.1. *Climate change*

The predicted impacts of climate change on freshwaters (Jeppesen et al., 2017; Strayer and Dudgeon, 2013) are expected to favour non-native invasive species. Chapter 4 demonstrated that the only species exhibiting an undesirable response in LMB/light was *Elodea canadensis*. Increased temperatures may put pressure on macrophyte community compositions and invasive pioneer species may, therefore, increase their competitive advantage, under favourable environmental conditions (Bakker et al., 2013). Climate change could also alter germination cues, including temperature fluctuation.

Climate change may interfere with ecological recovery in other ways; producing more extreme weather events including storms and flooding incidents (IPCC, 2014, 2007). These extreme events may disrupt macrophyte recovery, for example through disturbance of bed sediments in winter and spring (Spears and Jones, 2010) and through the delivery of high nutrient loading in summer (Mooij et al., 2005). Submerged macrophytes also depend on a spring clear-water phase to establish and extreme weather conditions during winter and spring may determine the relative success of phytoplankton growth (Phillips et al., 2016). This could lead to more extended periods of algal dominance which will reduce light levels and constrain macrophyte communities to fewer remaining species (Phillips et al., 2016). These conditions may, again, favour robust and fast growing invasive species, for example, *Elodea nuttallii* has been known to favour high trophic levels and can exist across wide nutrient ranges (Ozimek et al., 1993). The control and eradication of non-native submersed macrophytes is notoriously difficult to achieve following their establishment and eradication is often considered impossible after colonisation (Willby, 2007). The control methods used generally depends on the subject species and can vary in terms of cost, logistics and mode of action, i.e. physical control through shading, or chemical control using herbicides (Oreska and Aldridge, 2011). Effectiveness of control depends on treatment in the early phases of colonisation and so rapid detection on ingress is essential (Dawson and Warman, 1987).

## 5.6. Unintended consequences of LMB application

### 5.6.1. *Elevated phosphorus responses*

Findings from Chapter 4 revealed that SRP concentrations increased in the LMB/light treatment for some species in comparison to pre-application concentrations and to

controls post-experimentation. In Chapter 3, the LMB treated containers also had higher SRP concentrations in comparison to un-treated containers. The reason for this is unknown but it may have something to do with the low SRP concentrations of the receiving waters. Generally, at the lake-scale, SRP concentrations are higher than the pre-experimental concentrations measured in the cores in Chapter 4 (36 µg $L^{-1}$ (light), 35 µg $L^{-1}$ (dark)). The pre-experimental SRP concentrations were within the target range of concentrations that are commonly reported post-LMB application, at both the laboratory and field-scale.

Reports of LMB trials indicate post-application SRP concentrations in controlled laboratory experiments range between < 5 µg $L^{-1}$ – 47 µg $L^{-1}$ (Reitzel et al., 2012; Spears et al., 2013b; Wang et al., 2017) compared to those reported in field-scale applications where SRP concentrations range from 5 µg $L^{-1}$ – 95 µg $L^{-1}$ post-treatment (Epe et al., 2017; Gunn et al., 2014; Lürling and Faassen, 2012b; Lürling and van Oosterhout, 2012; Spears et al., 2016, 2018). The concentrations reported following LMB application for Chapter 3 (22 – 36 µg $L^{-1}$) remained within the controlled laboratory reported ranges from other studies but concentrations from Chapter 4 (47 µg $L^{-1}$ (light), 52 µg $L^{-1}$ (dark)) were more similar to the lake-scale range reported in the literature. The reduction of SRP as a result of LMB application, appears to decrease with increasing scale (from lab to lake-scale) or with the complexity of the experimental system.

There is a clear need to identify the reasons why LMB treated cores had higher SRP concentrations after application and why concentrations were high. Reasons/hypotheses reported in the literature for weaker than expected P reductions through field trials have been attributed to:

- Iron-P cycling, releasing P into the water column (Yasseri and Epe, 2016)
- Interference of La – P binding due to high concentrations of humic substances such as dissolved organic carbon (DOC) which compete P for La binding sites (Copetti et al., 2016; Lürling et al., 2014; Lürling and Faassen, 2012b; Spears et al., 2016)
- Continued sources of P via inflows or release from sediments deeper in the sediment profile (Lürling et al., 2014)
- Uneven coverage of LMB to applied sediments (Lürling et al., 2014)

- Time since application as P-binding appears more effective as time passes, e.g. Gunn et al., (2014)
- Benthic fish disturbance, causing re-suspension of P-capping layer (Huser et al., 2016a)

All these could be lowering the P-binding capacity at the lake scale. However, some of these are not feasible explanations for the elevated P concentrations in the bioassay experiments which were more similar to lake-scale concentrations post-treatment. The most likely reasons for high P concentrations from lake trials are discussed in the context of the results from the bioassay experiments.

In Chapter 4, there were elevated Fe and manganese Mn concentrations in both LMB light and dark treatments and control dark conditions post-experimentation. In addition, significantly lower dissolved oxygen concentrations were recorded in dark treatments across all species which could have contributed to higher P concentrations in LMB treated cores. The DOC concentrations in the bioassay experiments in Chapter 4 were low (4 – 4.8 mg $L^{-1}$) across treatments but were significantly lower than controls ($p$ = <0.001) and so there may have been some competition for La-binding sites. As sediment fractionation methods were not conducted pre- and post-experimentation due to the method design it cannot be concluded if P was released from deeper down the sediment profile. The time of the core experiment was short (30 days) and full binding capacity of P to La might not have taken place, particularly as La particles need to come into contact with SRP, as mixing has been found to increase La-P binding (Lürling et al., 2014). The bioassay experiments were roughly the same length (30 days) as a study by Reitzel et al., (2012) (35 days) which reported the exact same SRP concentrations in their LMB treatment (47 µg $L^{-1}$) compared to the LMB/light treated cores post-experimentation from Chapter 4 (47 µg $L^{-1}$). The SRP concentrations from the germination experiment (Chapter 3) were also within a similar range (22 – 36 µg $L^{-1}$). Reitzel et al., (2012) stated high SRP concentrations compared to controls could be due to sediment deposition on top of the LMB layer or the formation of a biofilm at the sediment surface which restricted exchange of solutes between the sediment and overlying waters.

It appears from the bioassay experiments that SRP concentrations were reduced (LMB/dark) and increased (LMB/light) to within a specific concentration range for each species (Chapter 4, Appendix 3.2., Table 6, Figure 9 (TP) and Figure 10 (SRP). It could therefore be possible that macrophytes are mediating the chemistry,

but P-uptake/release capacity of macrophytes were not addressed in this study. This does not however, relate to the elevated SRP concentrations for the Reitzel et al., (2012) study as no macrophytes were present. It therefore poses the question, could LMB actually increase P concentrations when applied to receiving waters where P concentrations are already low? This would require further investigations to assess but at the lake-scale LMB is not applied to low P waterbodies and so it could be that LMB is simply working to within its limit given the different processes operating in the lab system. It is also possible that the SRP samples contained particulate forms of P as the filter sizes used (0.7 µm) may have been too large to accredit the sample as just SRP. This may have also slightly elevated the SRP concentrations in the germination trial and the bioassay experiments.

The results from Chapter 3 and 4 revealed there could be additional causes for higher SRP concentrations post-LMB addition at the lake-scale in comparison to concentrations reported from lab trials, these are highlighted and discussed in the proceeding sections.

### 5.6.2. *Elevated phosphorus concentration through the presence of macrophytes and other mechanisms*

Results from Chapter 3 and 4 revealed that the presence of macrophytes and other mechanisms may have reduced the efficiency of LMB P-binding. There are several possible reasons/hypotheses for this as follows:

- LMB suppressed benthic algae abundance (Chapter 3) potentially leading to reduced P-uptake through a lower algal abundance leading to higher water column P concentrations
- High bioturbation rate (Chapter 3) could be delivering sediment rich in P from deeper down the sediment profile to expose it at the surface water interface (Phillips et al., 2015)
- Senescence of macrophytes could be increasing P in LMB treated cores (Welch and Kelly, 1990)
- macrophytes could be acting as nutrient pumps, taking sediment up through roots and releasing into the water column (Carpenter, 1981)

- Species-specific P-uptake capacity could explain differences in P concentrations for each tested species (Christiansen et al., 2016)
- Retention of the product on leaf surfaces not reaching the bed sediments may have occurred, causing uneven application of LMB to the sediments (Lürling et al., 2014) which may have reduced P-uptake kinetics
- High denitrification (Chapter 4), could have caused lower DO concentrations which led to increased Fe and Mn and P in the water column of the bioassay experiments
- Product on leaf surfaces could be preventing SRP uptake through each species leaves which could be why there were species-specific P concentrations

Those highlighted from Chapters are further discussed.

### 5.6.3. *Reduced benthic algae following LMB addition*

Chapter 3 revealed that LMB significantly reduced benthic algae percent volume inhabited (PVI) but reduction was species-specific with LMB significantly reducing the growth of *Spirogyra* spp. over other filamentous algae. Containers treated with LMB had higher SRP concentrations compared to un-treated containers where algal growth had higher PVI scores. Benthic algae are very effective at P-uptake, reducing $PO_4^{3-}$- P from 11.6 mg $L^{-1}$ to 6.1 – 7.7 mg $L^{-1}$ in one day from horticultural wastewater (Liu et al., 2016). P up-take capacity is also reported to be species specific ranging between 3.8 – 5.0 mg $PO_4^{3-}$- P $L^{-1}$ $d^{-1}$ between monocultures of certain algae (n= 3 species) and between 3.8 – 5.2 mg $PO_4^{3-}$ - P $L^{-1}$ $d^{-1}$ for communities (n=2) (Liu et al., 2016). *Spirogyra* algae are able to remove 4 – 5 µg $L^{-1}$ SRP after 4.5 – 12.9 days growth in flowing waters (Adey et al., 1993). The significance of this is that benthic algae could outcompete phytoplankton P-uptake at low P concentrations and potentially change communities. Chlorophyte species number has been reported to increase following LMB applications elsewhere (Bishop and Richardson, 2018) and our results from Chapter 3 is in agreement with this, at the lab-scale. The presence of benthic algae could also potentially impact macrophyte establishment from seed banks as benthic algae has led to macrophyte reduced growth elsewhere (Irfanullah and Moss, 2004).

### 5.6.4. *Bioturbation*

Benthic invertebrates are ecosystem engineers (Hölker et al., 2015) and in shallow lakes occur at densities of between 70 – 11,000 individuals / $m^2$ (Armitage et al., 1995; Mousavi, 2002). Quantifying the nutrient flux of nitrogen (N) and P produced by common lake benthic invertebrate such as chironomids is complex due to bioturbation of sediments, aeration and excretion; many of these processes interact and are therefore difficult to quantify, particularly at the lake scale (Hölker et al., 2015). There are currently no studies looking at the interactions between LMB, benthic macroinvertebrate nutrient fluxes and benthic primary producers, such as algae. The results from chapter 3 pose three key questions relating to LMB efficiency and interference from bioturbation: (1) how efficient is LMB at reducing P when applied to systems with high bioturbation rates? (2) how does LMB control benthic algal growth? and (3) with increased bioturbation, how does a higher benthic algal biomass impact macrophyte growth? This role of benthic invertebrates may be contributing to a re-occurring cycle of P into the water column following LMB applications (Figure 5.1) which may limit macrophyte recovery and needs further investigation.

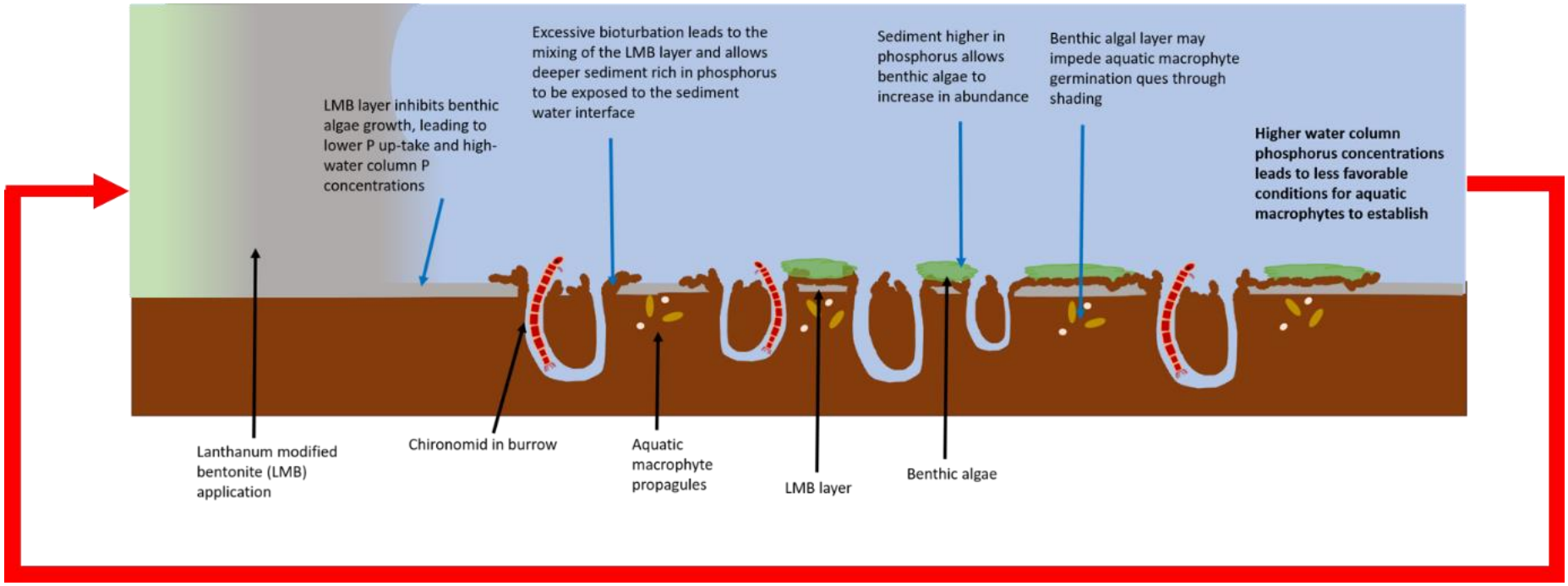



**Figure. 5.1.** Hypothesis of the role of lake benthic organisms (e.g. chironomids) which may limit macrophyte recovery through continued phosphorus delivery to overlying water post-lanthanum modified bentonite application (© Kate Waters-Hart).

### 5.6.5. *LMB impact on already established macrophytes*

Chapter 4 indicated that significant amounts of the LMB product remained on the surface of leaves following standard epiphyte washing for all species. LMB may have caused a shading effect on macrophytes through smothering of leaves. The average particle size of LMB is 22 µm with a range of 2.11 – 46.15 µm (Ross et al., 2008). Based on the macrophyte wash weight from Chapter 4, it is evident that all macrophyte species were exposed to high loads of suspended solids (SS) resulting in significant accumulation on above ground plant structures. The highest loads retained on leaf surfaces measured here (57 mg /100ml), i.e. for *Potamogeton perfoliatus* (Table 5.3), was 1.4 times greater than a reported load (40 mg $L^{-1}$) that resulted in a 13 - 50% decline in primary productivity in macrophytes (Bilotta and Brazier, 2008). The lowest leaf load reported for *Littorella uniflora* (1 mg /100ml) was just below the 8 mg $L^{-1}$ reported value to cause a decline in primary productivity by 3 – 13% (Bilotta and Brazier, 2008). The declines of macrophyte coverage by 80% and 50% in North Halfmoon Lake and Lofty Lake, respectively, have been speculatively caused by increased sedimentation on leaf surfaces through single calcium hydroxide ($Ca(OH)_2$) treatments of 74 and 107 mg $L^{-1}$, respectively (Reedyk et al., 2001) but the authors state the mechanisms for the declines and community shifts post-treatment are unknown.

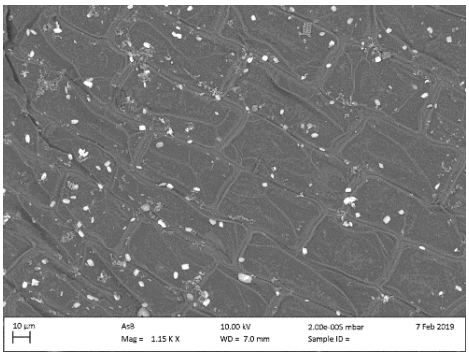
**Table 5.3.** Total suspended solid (SS) load retained on specimens from macrophyte wash procedures from species included in Chapter 4.

| **Species** | **Average total plant wash load (TSS – mg/100ml)** | **LMB SS load to core (mg $L^{-1}$)** |
|---|---|---|
| *Potamogeton perfoliatus* | 57 | 0.33 |
| *Myriophyllum spicatum* | 9 | 0.33 |
| *Elodea canadensis* | 20 | 0.33 |
| *Littorella uniflora* | 1 | 0.33 |
| *Najas flexilis* | 20 | 0.33 |

LMB loading at the lake-scale has been estimated to be in the range of 0.62 – 46.0 mg $L^{-1}$ (Spears et al., 2013a). The loads of LMB from the experiment in Chapter 4 (0.33 mg $L^{-1}$) are within the range reported in the literature. However, waterborne particles are known to settle on macrophytes in the field with macrophytes able to

capture 10 - 50% of suspended sediment particles in wetlands (Huang et al., 2008). However, it is unknown if the LMB product can be dislodged after an application. The macrophyte wash weight from Chapter 4 revealed species-specific SS loads retained macrophyte structures but it is difficult to assess if this may have been retained through the presence of epiphytes or through morphological structuring and texture of leaf surfaces.

To assess whether different leaf surface textures may attract an increased LMB load, Scanning Electron Microscope (SEM) X-ray analysis on specimens from the bioassay experiments was conducted, following the washing and drying procedure detailed in Chapter 4. This analysis indicated that LMB particles were still present on both *Potamogeton perfoliatus* (Figure 5.2 and Figure 5.3) and *Elodea canadensis* leaves, even after washing (Figure 5.4 and Figure 5.5). There was no evidence of epiphytes in the SEM images. However, this implies the *Fv/Fm* readings may have potentially been negatively impacted by the product remaining during post-experimental measurements. It could be a possible reason why *Fv/Fm* values were lower as less of the surface area of the leaf may have been included during the reading.

a) 

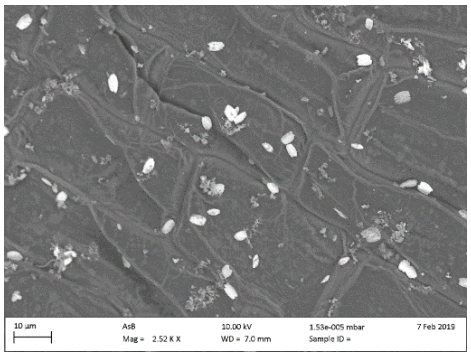
b) 


**Figure 5.2.** *Potamogeton perfoliatus* random leaf from the core experiments from chapter 4 from a Lanthanum (La) - modified bentonite treated core in light conditions at 1.15 kX magnification (a) and an area within this image at 2.52 kX magnification (b). Contrasting white areas represent La-rich particles, AsB - angle selective backscatter, kV – kilovolt, mbar – millibar.

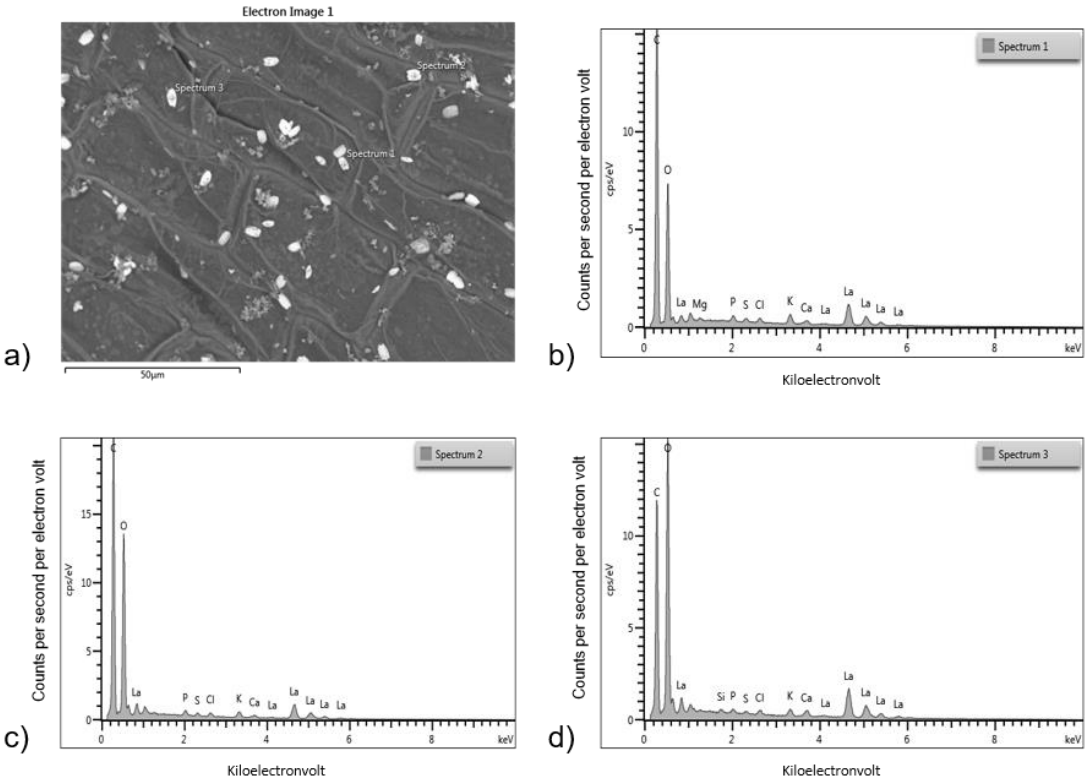



**Figure 5.3.** *Potamogeton perfoliatus* leaf from Figure 5.1(b) highlighting three spectrums for backscatter imaging using X-ray analysis to assess La - rich particles on the leaf surface (a) within these three highlighted areas (spectrum 1 (b), spectrum 2 (c) and spectrum 3 (d)). Contrasting white areas represent La**-**rich particles.

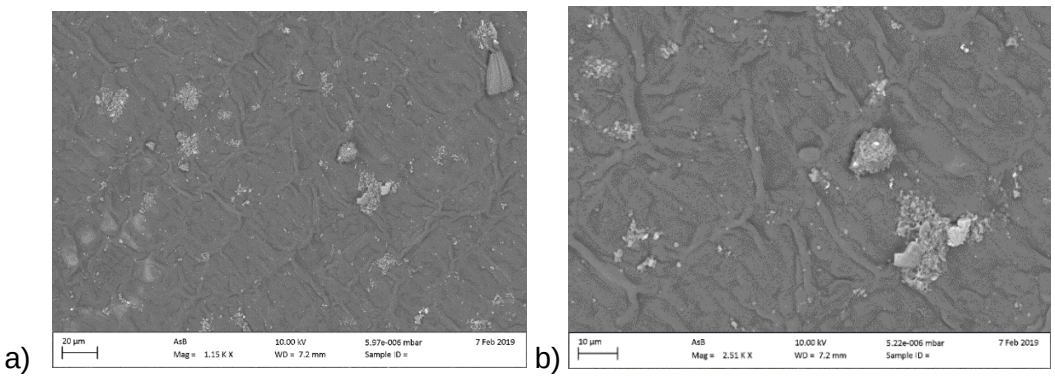



**Figure 5.4.** *Elodea canadensis* random leaf from the core experiments from chapter 4 from a Lanthanum (La) - modified bentonite treated core in light conditions at 1.15 kX magnification (a) and an area within this image at 2.51 kX magnification (b). Contrasting white areas represent La**-**rich particles, AsB - angle selective backscatter, kV – kilovolt, mbar – millibar.

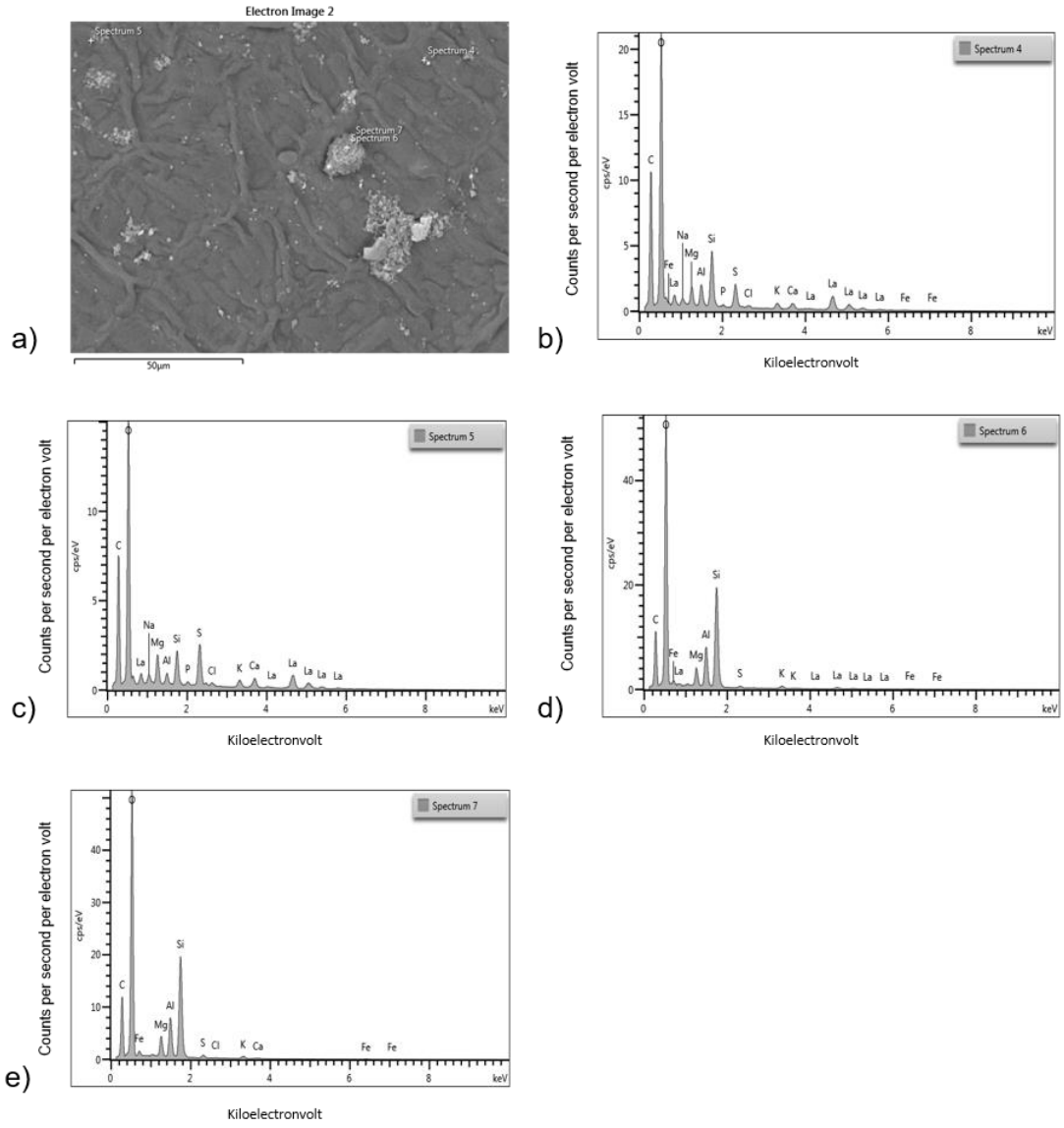



**Figure 5.5.** *Elodea canadensis* leaf from Figure 5.3(b) highlighting four spectra for backscatter imaging using X-ray analysis to assess La - rich particles on the leaf surface (a) within these four highlighted areas (spectrum 4 (b), spectrum 5 (c), spectrum 6 (d) and spectrum 7 (e)). Contrasting white areas represent La-rich particles.

The functional role of LMB on leaf surfaces is unclear. However, it is possible that the product could perform a similar functional role as that reported for epiphytes. This includes light limitation of macrophytes (Sand-Jensen and Søndergaard, 1981) and direct SRP uptake from the water column (Pelton et al., 1998). In dense macrophyte stands it is possible that retention of LMB on above ground structures could reduce

the intended load to the bed sediments. The potential for this reduced bed load to result in reduced control of internal loading is not assessed here but could be one reason why SRP reductions appear to decrease with increasing scale, as described in Section 5.6.1 above. This could be assessed through laboratory experiments by adding surface loads and by injecting LMB directly onto the surface sediments to assess LMB performance.

The efficiency of LMB whilst in the presence of macrophyte species clearly needs investigating. Specific species P-uptake strategies could be impeded through product remaining on leaf surfaces. The retention of La-rich particles on leaf surfaces at the lake-scale should be addressed to ascertain if the applied product does remain *in-situ* and provide an epiphyte-like role through shading. Experimental and modelling approaches, such as those used for emergent vegetation (Huang et al., 2008) and riverine macrophytes (Albayrak et al., 2012), could be used to assess how water movement and velocity may dislodge LMB particles from macrophyte surfaces. To assess if LMB performs an epiphyte-like role, a similar experiment conducted by Albayrak et al., (2012) using artificial macrophytes maybe useful to assess how LMB competes with epiphytes for P-uptake.

## 5.7. Wider implications for lake management and macrophyte conservation

LMB may not be effective at rapidly forcing desirable macrophyte species recolonisation (Chapter 2). Evidence has been presented in this thesis to confirm that macrophyte species can germinate in the presence of LMB (chapter 3), although responses may be species-specific (chapter 4). Rapid macrophyte recovery may be confounded through low seed bank viability (Chapters 2 and 3) and the presence of dominant and stress-tolerant non-native macrophyte species (Chapters 2 and 4). It is, therefore, unlikely that LMB use, alone, will result in macrophyte recovery to meet conservation or ecological quality targets, for example, as set by the Water Framework Directive or Habitats Directive in Europe.

It is becoming more apparent that additional measures will be necessary across many lakes to support macrophyte recovery. Additional measures may include active transplantation of desirable macrophyte species to help speed-up recovery further. This may be particularly relevant for rare species, such as *Najas flexilis* in the UK (Bishop et al., 2018), which may not re-establish following periods of contemporary absence without intervention (Bakker et al., 2013).

Chapter 3 revealed that bioturbation may impede the functional role of LMB to applied waters. Evidence from the germination trial suggest that using LMB to treat biomanipulated lakes or ones that are prone to fish kills may not be a sensible option as the rate of bioturbation may increase following fish removal due to increased abundance of chironomids and oligochaetes (Phillips et al., 2015). Higher abundances may reduce the effectiveness of P uptake by LMB.

Chapter 3 also revealed that LMB was able to reduce benthic algae growing in shallow waters. This may aid the establishment of desirable species (Irfanullah and Moss, 2004) through allowing macrophytes to germinate or establish without competing with algae for light, space and nutrients.

The expected functional role of LMB also needs careful consideration when applying to lakes with macrophytes already present. It may impact both the efficiency of the application but also impact certain macrophyte species growing in both shallow and deeper waters which may lead to changes in community compositions and lean towards more stress-tolerant communities which are well-adapted to change, which are mostly non-native invasive species. This may incur extra management measures and associated control costs.

The key processes of lake restoration summarised from this thesis are detailed in Figure 5.5, which highlights the complexity of management intervention to restore desirable macrophytes that are no longer viable in seedbanks, or where quick recovery is required to meet legislative ecological deadlines. There may be several steps needed to provide a more supportive environment for macrophyte domination. The reduction of external nutrient loads and measures to control internal load provides more favourable conditions for macrophyte establishment. If there is increased P interference from benthic organisms, such as chironomids and oligochaetes, control over high numbers may be more favourable to allow colonisation from active seedbanks. If there is limited recovery potential from seedbanks, then macrophyte transplantation maybe required. The manipulation of other macrophytes may also be necessary, e.g. invasive species control.

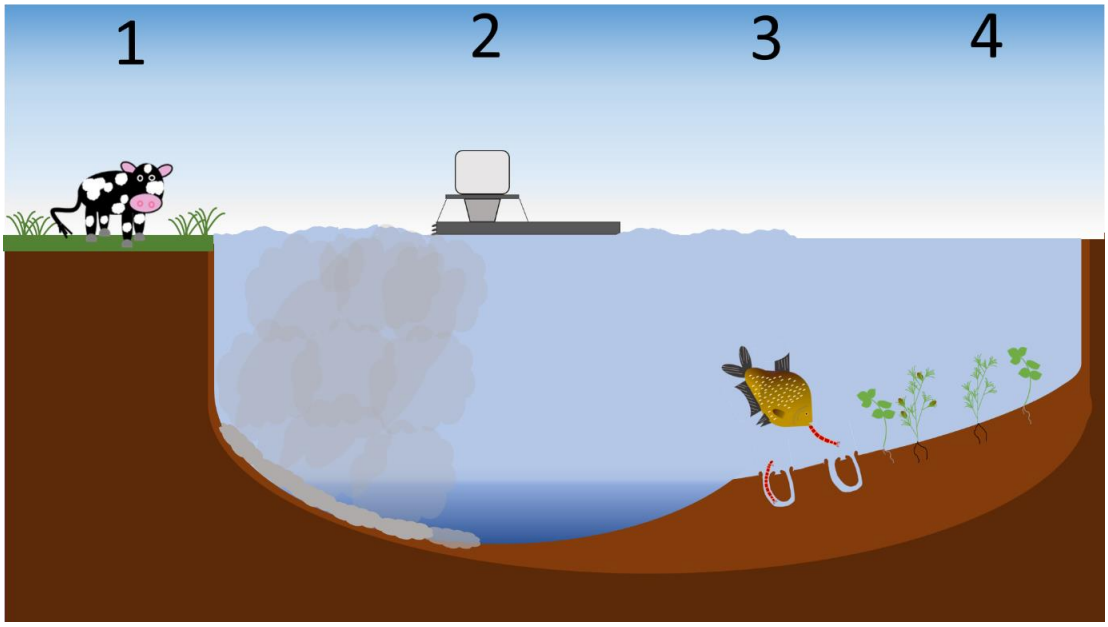



**Figure 5.5.** Diagram outlining the processes of lake restoration (1) reduce catchment load entering waterbodies, (2) control the internal load to reduce internal loading, (3) foodweb reconstruction to control bioturbation and (4) desirable macrophyte transplantation to restore contemporary seed banks (© Kate Waters-Hart).

## 5.8. Outstanding knowledge gaps

This research has provided important insights into the mechanisms that may prevent desirable macrophyte recovery in LMB treated lakes. Key knowledge gaps have been presented throughout this chapter and so are not repeated here. There are, however, a number of key questions remaining not already identified that needs further research. Firstly, the interspecific competition between macrophytes once applied with LMB needs further investigation to see how communities might act at the lake-scale. Competition for nutrients may exhibit intensified responses seen from the results in Chapter 4.

Secondly, knowledge gaps remain on assessing the potential toxicity of $La^{3+}$ through a LMB application to submerged macrophytes as this is still absent from the literature (Copetti et al., 2016). This is a particularly important area to be investigated as this may hamper meeting conservation targets for particular macrophyte species given some of the negative impacts observed for other macrophytes (Xu et al., 2012).

The bioaccumulation of La into other aquatic biota such as crayfish (van Oosterhout et al., 2014) fish, chironomids and to *Elodea canadensis* has been

reported up to five years after application (for fish) (Waajen et al., 2017) with no negative impacts. *Nymphaea alba*, *Phragmites australis*, *Scirpus lacustris*, *Typha latifolia* and *Elodea nuttallii* all bioaccumulated La four months post-LMB application to Lake Rauwbraken, The Netherlands (van Oosterhout et al., 2019). van Oosterhout et al., (2019) stated that La can be passed through food chains and means that macrophytes can be vectors for La. There are no studies assessing bioaccumulation in macrophytes long-term or following repeated LMB applications to assess negative or positive impacts. It is also not known if desirable macrophytes can bioaccumulate La and if they react similarly to undesirable species such as *Elodea canadensis* and *Elodea nuttallii* that have been tested, but further trials are needed.

## 5.9. Conclusions

This study has provided some important insights into the recovery of macrophytes following LMB application. Through the analysis of long-term field studies, it can be concluded that:

- macrophytes do not recover quickly following LMB application to 12 water bodies up to ten years following treatment
- Lakes under the remit of legislation are currently not meeting ecological targets three – five years post-LMB application
- At the lake-scale macrophyte communities remain dominated by *Elodea canadensis* pre- and post- LMB addition with new colonisations mainly by charophyte species
- It is clear that macrophyte recovery could be confounded by lake isolation from other waterbodies, low seed bank viability and pre-emption by pioneering macrophyte species or communities

Through a 21-week germination trial using lake bed sediments from a eutrophic lake, to assess if the application of LMB impeded germination from the 'ecologically' active seedbank, it was concluded that:

- LMB does not confound macrophyte recovery through the formation of a 'barrier' from application

- macrophyte species richness and biomass did not vary compared to untreated sediments
- LMB however significantly reduced benthic algae which was species-specific; significantly reducing *Spirogyra* algae compared to filamentous algae

The assessment of the direct and indirect effects of an LMB application in light and low light conditions to already growing submerged desirable, undesirable and rare species concluded that:

- macrophyte species responded as expected in-line with their strategy traits
- All species expressed a positive growth response to LMB/light treatment whilst *Elodea canadensis* was the only species to be seen as having an ecologically undesirable response as a result of its positive growth
- All species expressed reduced growth responses to LMB/dark conditions

The overarching conclusion for this thesis is that lake restoration for macrophyte conservation is very complex and requires the control of multiple processes, either simultaneously or consecutively depending on the desired timelines of recovery. Restoration for macrophytes will likely become even more complex and costly going forward into the future as a consequence of climate change.

## 6. References


Abernethy, V.J., Willby, N.J., 1999. Changes along a disturbance gradient in the density and composition of propagule banks in floodplain aquatic habitats. Plant Ecol. 140, 177–190. https://doi.org/10.1023/A:1009779411686

Adey, W., Luckett, C., Jensen, K., 1993. Phosphorus removal from natural waters using controlled algal production. Restor. Ecol. 1, 29–39.

Afsar, A., Groves, S., 2009. Comparison of P-inactivation efficacy and ecotoxicity of Alum and Phoslock®. PWS Rep. Number IR.

Aiken, S.G., Newroth, R., Wile, I., 1979. The biology of canadian weeds: *Myriophyllum spicatum L*. Can. J. Plant Sci. 59, 201–215.

Alahuhta, J., Kosten, S., Akasaka, M., Auderset, D., Azzella, M.M., Bolpagni, R., Bove, C.P., Chambers, P.A., Chappuis, E., Clayton, J., de Winton, M., Ecke, F., Gacia, E., Gecheva, G., Grillas, P., Hauxwell, J., Hellsten, S., Hjort, J., Hoyer, M. V., Ilg, C., Kolada, A., Kuoppala, M., Lauridsen, T., Li, E.H., Lukács, B.A., Mjelde, M., Mikulyuk, A., Mormul, R.P., Nishihiro, J., Oertli, B., Rhazi, L., Rhazi, M., Sass, L., Schranz, C., Søndergaard, M., Yamanouchi, T., Yu, Q., Wang, H., Willby, N., Zhang, X.K., Heino, J., 2017. Global variation in the beta diversity of lake macrophytes is driven by environmental heterogeneity rather than latitude. J. Biogeogr. 44, 1758–1769. https://doi.org/10.1111/jbi.12978

Albayrak, I., Nikora, V., Miler, O., O'Hare, M., 2012. Flow-plant interactions at a leaf scale: Effects of leaf shape, serration, roughness and flexural rigidity. Aquat. Sci. 74, 267–286. https://doi.org/10.1007/s00027-011-0220-9

Alderton, E., Sayer, C.D., Davies, R., Lambert, S.J., Axmacher, J.C., 2017. Buried alive: Aquatic plants survive in 'ghost ponds' under agricultural fields. Biol. Conserv. 212, 105–110. https://doi.org/10.1016/j.biocon.2017.06.004

Álvarez-Manzaneda, I., Baun, A., Cruz-Pizarro, L., de Vicente, I., 2019. Ecotoxicity screening of novel phosphorus adsorbents used for lake restoration. Chemosphere 222, 469–478. https://doi.org/10.1016/j.chemosphere.2019.01.103

Angeler, D.G., Goedkoop, W., 2010. Biological responses to liming in boreal lakes: An assessment using plankton, macroinvertebrate and fish communities. J. Appl. Ecol. 47, 478–486. https://doi.org/10.1111/j.1365-2664.2010.01794.x

Annadotter, H., Cronberg, G., Aagren, R., Lundstedt, B., Nilsson, P.-Å., Ströbeck, S., 1999. Multiple techniques for lake restoration. Hydrobiologia 395–396, 77–85. https://doi.org/Doi 10.1023/A:1017011132649

Ares, A., duckett, J. G., Pressel, S., 2014. Asexual reproduction and protonemal development *in vitro* in *Fontinalis antipyretica* Hedw. Journal of Bryology. 36(2), 122 - 133. https://doi.org/10.1179/1743282014Y.0000000099

Armitage, P.., Pinder, L.C. V, Cranston, P.., 1995. The Chironomidae: Biology and ecology of non-biting midges. Chapman and Hall, London, UK.

Arts, G.H.P., Belgers, J.D.M., Hoekzema, C.H., Thissen, J.T.N.M., 2008. Sensitivity of submersed freshwater macrophytes and endpoints in laboratory toxicity tests. Environ. Pollut. 153, 199–206. https://doi.org/10.1016/j.envpol.2007.07.019

Ayres, K.R., Sayer, C.D., Skeate, E.R., Perrow, M.R., 2008. Palaeolimnology as a tool to inform shallow lake management: An example from Upton Great Broad, Norfolk, UK. Biodivers. Conserv. 17, 2153–2168. https://doi.org/10.1007/s10531-007-9223-1

Babu, T.S., Tripuranthakam, S., Greenberg, B.M., 2005. Biochemical responses of the aquatic higher plant Lemna gibba to a mixture of copper and 1,2-dihydroxyanthraquinone: Synergistic toxicity via reactive oxygen species. Environ. Toxicol. Chem. 24, 3030–3036. https://doi.org/10.1897/05-073R.1

Bakker, E.S., Sarneel, J.M., Gulati, R.D., Liu, Z., van Donk, E., 2013. Restoring macrophyte diversity in shallow temperate lakes: Biotic versus abiotic constraints. Hydrobiologia 710, 23–37. https://doi.org/10.1007/s10750-012-1142-9

Bakker, E.S., Van Donk, E., Immers, A.K., 2016. Lake restoration by in-lake iron addition: a synopsis of iron impact on aquatic organisms and shallow lake ecosystems. Aquat. Ecol. 50, 121–135. https://doi.org/10.1007/s10452-015-9552-1

Balian, E. V., Segers, H., Lévèque, C., Martens, K., 2008. The Freshwater Animal Diversity Assessment: An overview of the results. Hydrobiologia 595, 627–637. https://doi.org/10.1007/s10750-007-9246-3

Barrat-Segretain, M.H., Elger, A., Sagnes, P., Puijalon, S., 2002. Comparison of three life-history traits of invasive *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch.) H. St. John. Aquat. Bot. 74, 299–313. https://doi.org/10.1016/S0304-3770(02)00106-7

Barry, M.J., Meehan, B.J., 2000. The acute and chronic toxicity of lanthanum to Daphnia carinata. Chemosphere 41, 1669–1674. https://doi.org/10.1016/S0045-6535(00)00091-6

Baskin, C.., Baskin, J.., 1998. Seeds: Ecology, biogeography and evolution of dormancy and germination. Acadenmic Press, San Diego.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/doi:10.18637/jss.v067.i01

Batty, L.C., Younger, P.L., 2003. Effects of external iron concentration upon seedling growth and uptake of fe and phosphate by the common reed, Phragmites australis (Cav.) Trin ex. Steudel. Ann. Bot. 92, 801–806. https://doi.org/10.1093/aob/mcg205

Behets, G.J., Mubiana, K.V., Lamberts, L., Finsterle, K., Traill, N., Blust, R., D'Haese, P.C., 2019. Use of lanthanum for water treatment A matter of concern? Chemosphere 239, 124780. https://doi.org/10.1016/j.chemosphere.2019.124780

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bennion, H., Simpson, G.L., Goldsmith, B.J., 2015. Assessing degradation and recovery pathways in lakes impacted by eutrophication using the sediment record. Front. Ecol. Evol. 3. https://doi.org/10.3389/fevo.2015.00094

Bickel, T., Perrett, C., 2015. Precise determination of aquatic plant wet mass using a salad spinner. Can. J. Fish. Aquat. Sci. 73, 1–4.

Bilotta, G.S., Brazier, R.E., 2008. Understanding the influence of suspended solids on water quality and aquatic biota. Water Res. 42, 2849–2861. https://doi.org/10.1016/j.watres.2008.03.018

Bishop, I.J., Bennion, H., Patmore, I.R., Sayer, C.D., 2018. How effective are plant macrofossils as a proxy for macrophyte presence? The case of *Najas flexilis* in Scotland. J. Paleolimnol. 60, 153–165. https://doi.org/10.1007/s10933-017-9988-5

Bishop, I.J., Bennion, H., Sayer, C.D., Patmore, I.R., Yang, H., 2019. Filling the "data gap": Using paleoecology to investigate the decline of *Najas flexilis* (a rare aquatic plant) . Geo Geogr. Environ. 6, 1–18. https://doi.org/10.1002/geo2.81

Bishop, W.M., McNabb, T., Cormican, I., Willis, B.E., Hyde, S., 2014. Operational evaluation of Phoslock phosphorus locking technology in Laguna Niguel Lake, California. Water. Air. Soil Pollut. 225. https://doi.org/10.1007/s11270-014-2018-6

Bishop, W.M., Richardson, R.J., 2018. Influence of Phoslock® on legacy phosphorus, nutrient ratios, and algal assemblage composition in hypereutrophic water resources. Environ. Sci. Pollut. Res. 25, 4544–4557. https://doi.org/10.1007/s11356-017-0832-2

Bjerring, R., Windolf, J., Kronvang, B., Sorensen, P.., Timmermann, A., Kjeldgaard, A., Larsen, S.., Thodsen, H., Bogestrand, J., 2014. Estimations of Loading to Lakes, Danish Centre for Environment (DCE) Report No. 36.

Blindow, I., 1992. Decline of charophytes during eutrophication: comparison with angiosperms. Freshw. Biol. 28, 9–14. https://doi.org/10.1111/j.1365-2427.1992.tb00557.x

Blindow, I., Hargeby, A., Andersson, G., 2002. Seasonal changes of mechanisms maintaining clear water in a shallow lake with abundant Chara vegetation. Aquat. Bot. 72, 315–334. https://doi.org/10.1016/S0304-3770(01)00208-X

Blindow, I., Hargeby, A., Hilt, S., 2014. Facilitation of clear-water conditions in shallow lakes by macrophytes: differences between charophyte and angiosperm dominance. Hydrobiologia 737, 99–110. https://doi.org/10.1007/s10750-013-1687-2

Boedeltje, 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. https://doi.org/10.1016/S0304-3770(03)00078-0

Boelee, N.C., Temmink, H., Janssen, M., Buisman, C.J.N., Wijffels, R.H., 2011. Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms. Water Res. 45, 5925–5933. https://doi.org/10.1016/j.watres.2011.08.044

Boers, P., Van der Does, J., Quaak, M., Van der Vhigt, J., 1994. Phosphorus fixation with iron (III) chloride: a new method to combat internal phosphorus loading in shallow lakes?. The Release Of Dissolved Phosphorus From Lake Sediments.

Bonis, A., Grillas, P., 2002. Deposition, germination and spatio-temporal patterns of charophyte propagule banks: A review. Aquat. Bot. 72, 235–248. https://doi.org/10.1016/S0304-3770(01)00203-0

Bonis, A., Lepart, J., 1994. Vertical structure of seed banks and the impact of depth of burial on recruitment in two temporary marshes. Vegetatio 112, 127–139. https://doi.org/10.1007/BF00044687

Bormans, M., Mars, B., Janc, D., 2016. Controlling internal phosphorus loading in lakes by physical methods to reduce cyanobacterial blooms : a review 407–422. https://doi.org/10.1007/s10452-015-9564-x

Born, S.M., 1979. Lake rehabilitation: A status report. Environ. Manage. 3, 145–153. https://doi.org/10.1007/BF01867026

Bornette, G., Puijalon, S., 2011. Response of aquatic plants to abiotic factors: A review. Aquat. Sci. 73, 1–14. https://doi.org/10.1007/s00027-010-0162-7

Bortone, G., Arevalo, E., Deibel, I., Detzner, H.-D., Propris, L., Elskens, F., Giordano, A., Hakstege, P., Hamer, K., Harmsen, J., Hauge, A., Palumbo, L., Veen, J., 2004. Synthesis of the sednet work package 4 outcomes. J. Soils Sediments 4, 225–232. https://doi.org/10.1007/BF02991117

Bowmer, K.H., Jacobs, S.W.L., Sainty, G.R., 1995. Identification , Biology and Management of Elodea canadensis , Hydrocharitaceae. J. Aquat. Plant Manag. 33, 13–19.

Bray, J.R., Curtis, J.T., 1957. An Ordination of the Upland Forest Communities of Southern Wisconsin. Ecol. Monogr. 27, 325–349. https://doi.org/10.2307/1942268

Brownlie, W.J., Woods, H.J., Waters, K.E., Moore, A.L., Bruce, A.M., Olszewska,

J.P., Ives, S.C., 2017. Freshwater science for the benefit of society: a perspective from early career researchers. Inl. Waters 7, 227–235. https://doi.org/10.1080/20442041.2017.1326793

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information - Theoretic Approach. Springer-Verlag New York. https://doi.org/10.1007/b97636

Cao, T., Ni, L., Xie, P., 2004. Acute biochemical responses of a submersed macrophyte, potamogeton crispus l., to high ammonium in an aquarium experiment. J. Freshw. Ecol. 19, 279–284. https://doi.org/10.1080/02705060.2004.9664542

Cao, T., Ni, L., Xie, P., Xu, J., Zhang, M., 2011. Effects of moderate ammonium enrichment on three submersed macrophytes under contrasting light availability. Freshw. Biol. 56, 1620–1629. https://doi.org/10.1111/j.1365-2427.2011.02601.x

Cao, T., Xie, P., Ni, L., Wu, A., Zhang, M., Wu, S., Smolders, A.J.P., 2007. The role of NH+4 toxicity in the decline of the submersed macrophyte Vallisneria natans in lakes of the Yangtze River basin, China. Mar. Freshw. Res. 58, 581–587. https://doi.org/10.1071/MF06090

Capers, R.S., 2003. Macrophyte colonization in a freshwater tidal wetland (Lyme, CT, USA). Aquat. Bot. 77, 325–338. https://doi.org/10.1016/j.aquabot.2003.08.001

Capers, R.S., Selsky, R., Bugbee, G.J., White, J.C., 2009. Species richness of both native and invasive aquatic plants influenced by environmental conditions and human activity. Botany 87, 306–314. https://doi.org/10.1139/B08-144

Carpenter, S.R., 2008. Phosphorus control is critical to mitigating eutrophication. Proc. Natl. Acad. Sci. 105, 11039–11040. https://doi.org/10.1073/pnas.0806112105

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Carpenter_et_al-1998-Ecological_Applications. Ecology 8, 559–568. https://doi.org/10.1890/1051-0761(1998)008[0559:NPOSWW]2.0.CO;2

Carpenter, S.R., Cottingham, K.L., 1997. Resilience and restoration of lakes. Ecol.

Soc. 1, 1–14. https://doi.org/10.1093/intimm/dxu005

Carpenter, S.R., Lodge, D.M., 1986. Effects of submersed macrophytes on ecosystem processes. Aquat. Bot. 26, 341–370. https://doi.org/10.1016/0304-3770(86)90031-8

Carvalho, L., Kirika, A., 2003. Changes in shallow lake functioning: response to climate change and nutrient reduction. Hydrobiologia 506–509, 789–796. https://doi.org/10.1093/nq/s12-IV.87.326-b

Carvalho, L., Maberly, S., May, L., Hughes, R.M., Brazier, R., Heathwaite, L., Elliott, A., Willby, N., Dils, R., Phillips, G., 2005. Risk Assessment Methodology for Determining Nutrient Impacts in Surface Freshwater Bodies. Ecology 220.

Carvalho, L., Mackay, E.B., Cardoso, A.C., Baattrup-Pedersen, A., Birk, S., Blackstock, K.L., Borics, G., Borja, A., Feld, C.K., Ferreira, M.T., Globevnik, L., Grizzetti, B., Hendry, S., Hering, D., Kelly, M., Langaas, S., Meissner, K., Panagopoulos, Y., Penning, E., Rouillard, J., Sabater, S., Schmedtje, U., Spears, B.M., Venohr, M., van de Bund, W., Solheim, A.L., 2019. Protecting and restoring Europe's waters: An analysis of the future development needs of the Water Framework Directive. Sci. Total Environ. 658, 1228–1238. https://doi.org/10.1016/j.scitotenv.2018.12.255

Casanova, M.T., 2015. The seed bank as a mechanism for resilience and connectivity in a seasonal unregulated river. Aquat. Bot. 124, 63–69. https://doi.org/10.1016/j.aquabot.2015.03.008

Chaffin, J.D., Kane, D.D., 2010. Burrowing mayfly (Ephemeroptera: Ephemeridae: Hexagenia spp.) bioturbation and bioirrigation: A source of internal phosphorus loading in Lake Erie. J. Great Lakes Res. 36, 57–63. https://doi.org/10.1016/j.jglr.2009.09.003

Chambers, P.A.., Kalff, J., 1987. Light and Nutrients in the Control of Aquatic Plant Community Structure . I . In Situ Experiments. J. Ecol 75, 611–619.

Chambers, P.A., Lacoul, P., Murphy, K.J., Thomaz, S.M., 2008. Global diversity of aquatic macrophytes in freshwater. Hydrobiologia 595, 9–26. https://doi.org/DOI 10.1007/s10750-007-9154-6

Christiansen, N.H., Andersen, F.O., Jensen, H.S., 2016. Phosphate uptake kinetics

for four species of submerged freshwater macrophytes measured by a 33P phosphate radioisotope technique. Aquat. Bot. 128, 58–67. https://doi.org/10.1016/j.aquabot.2015.10.002

Chrzanowski, C., n.d. Effects of gradual chemical additions on phosphorus mobilization and macrophyte growth in peat lake Terra Nova. Wageningen University, Wageningen, The Netherlands.

Clean Water Act, 1972. Federal Water Pollution Control Act, 2002. 33 U.S.C. § 1251 et seq.

Clearwater, S.J., 2004. Chronic exposure of midge larvae to Phoslock. NIWA, Australia, pp 25.

Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E., 2009. Ecology - Controlling eutrophication: Nitrogen and phosphorus. Science (80-. ). 323, 1014–1015. https://doi.org/10.1126/science.1167755

Cooke, G.D., Welch, E.B., Martin, A.B., Fulmer, D.G., Hyde, J.B., Schrieve, G.D., 1993. Effectiveness of Al, Ca, and Fe salts for control of internal phosphorus loading in shallow and deep lakes. Hydrobiologia 253, 323–335. https://doi.org/10.1007/BF00050758

Cooke, G.D., Welch, E.B., Peterson, S.A., Nichols, S.A., 2005. Restoration and management of lakes and reservoirs, 3rd edition. ed, Biological Conservation. CRC.

Coops, H., Doef, R.W., 1996. Submerged vegetation development in two shallow, eutrophic lakes. Hydrobiologia 340, 115–120. https://doi.org/10.1007/BF00012742

Coops, H., Kerkum, F.C.M., Van Den Berg, M.S., Van Splunder, I., 2007. Submerged macrophyte vegetation and the European Water Framework Directive: Assessment of status and trends in shallow, alkaline lakes in the Netherlands. Hydrobiologia 584, 395–402. https://doi.org/10.1007/s10750-007-0586-9

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., Yasseri, S., Lürling, M.,

2016. Eutrophication management in surface waters using lanthanum modified bentonite: A review. Water Res. 97, 162–174. https://doi.org/10.1016/j.watres.2015.11.056

Costanza, R., Arge, R., Groot, R. De, Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., Neill, R.V.O., Paruelo, J., Raskin, R.G., Sutton, P., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 1–8.

Council Directive, 1991. Council Directive 91/271/EEC concerning urban waste water treatment., Official Journal of the European Communities. Official Journal of the European Communities 135/40.

Council of the European Union, 1992. Council Directive 92/43/EC of 21 May 1992 on the Conservation of Natural Habitats and Wild Fauna and Flora.

Crosa, G., Yasseri, S., Nowak, K.-E., Canziani, A., Roella, V., Zaccara, S., 2013. Recovery of Lake Varese: reducing trophic status through internal P load capping. Fundam. Appl. Limnol. / Arch. für Hydrobiol. 183, 49–61. https://doi.org/10.1127/1863-9135/2013/0427

D'Haese, P.C., Douglas, G., Verhulst, A., Neven, E., Behets, G.J., Vervaet, B.A., Finsterle, K., Lürling, M., Spears, B., 2019. Human health risk associated with the management of phosphorus in freshwaters using lanthanum and aluminium. Chemosphere 220, 286–299. https://doi.org/10.1016/j.chemosphere.2018.12.093

Das, T., Sharma, A., Talukder, G., 1988. Effects of lanthanum in cellualr systems. A review. Biol. Trace Elem. Res. 18, 201–228.

Davies, G.M., Gray, A., 2015. Don't let spurious accusations of pseudoreplication limit our ability to learn from natural experiments (and other messy kinds of ecological monitoring). Ecol. Evol. 5, 5295–5304. https://doi.org/10.1002/ece3.1782

Dawson, F., Warman, E., 1987. *Crassula helmsii* (T. Kirk) Cockayne: Is it an Aggressive Alien Aquatic Plant in Britain? Biol. Conserv. 42, 247–272.

de Magalhães, L., Noyma, N.P., Furtado, L.L., Drummond, E., Leite, V.B.G., Mucci, M., van Oosterhout, F., Huszar, V.L. de M., Lürling, M., Marinho, M.M., 2019. Managing Eutrophication in a Tropical Brackish Water Lagoon: Testing

Lanthanum-Modified Clay and Coagulant for Internal Load Reduction and Cyanobacteria Bloom Removal. Estuaries and Coasts 42, 390–402. https://doi.org/10.1007/s12237-018-0474-8

De Winton, M.D., Clayton, J.S., 1996. The impact of invasive submerged weed species on seed banks in lake sediments. Aquat. Bot. 53, 31–45. https://doi.org/10.1016/0304-3770(95)01010-6

De Winton, M.D., Clayton, J.S., Champion, P.D., 2000. Seedling emergence from seed banks of 15 New Zealand lakes with contrasting vegetation histories. Aquat. Bot. 66, 181–194. https://doi.org/10.1016/S0304-3770(99)00074-1

DeGroote, D., Kennedy, R.A., 1977. Photosynthesis In *Elodea canadensis* Michx. Plant Physiol. 59, 1133–1135. https://doi.org/10.7868/s0869565217190264

Dendène, M.A., Rolland, T., Trémolières, M., Carbiener, R., 1993. Effect of ammonium ions on the net photosynthesis of three species of *Elodea*. Aquat. Bot. 46, 301–315. https://doi.org/10.1016/0304-3770(93)90010-T

Does, J., Verstraelen, P., Boers, P., Roestel, J., Roijackers, R., Moser, G., 1992. Lake restoration with and without dredging of phosphorus-enriched upper sediment layers. Hydrobiologia 233, 197–210.

Douglas, G.B., 2002. US Patent 6350383: remediation material and remediation process for sediments.

Douglas, G. B., Hamilton, D.P., Robb, M.S., Pan, G., Spears, B.M., Lürling, M., 2016. Guiding principles for the development and application of solid-phase phosphorus adsorbents for freshwater ecosystems. Aquat. Ecol. 50, 385–405. https://doi.org/10.1007/s10452-016-9575-2

Douglas, Grant B, Lürling, M., Spears, B.M., 2016. Assessment of changes in potential nutrient limitation in an impounded river after application of lanthanum-modified bentonite. Water Res. 97, 47–54.

Douglas, G.B., Robb, M.S., Coad, D., Ford, P., 2004. A review of solid phase absorbents for the removal of phosphorus from natural and waste waters. In Valsami-Jones, E. (ed), Phosphorus in Environment Technology - Removal, Recovery, Applications. IEA Publishing, London, pp. 291–320.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J.,

Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. Camb. Philos. Soc. 81, 163–82. https://doi.org/10.1017/S1464793105006950

Dudley, B.., Gunn, I.D.M.., Carvalho, L.., Proctor, I.., O'Hare, M.T.., Murphy, K.J.., Milligan, A., 2012. Changes in aquatic macrophyte communities in Loch Leven: evidence of recovery from eutrophication? Hydrobiologia 681, 49–57. https://doi.org/10.1002/2013WR015167.Received

Dudley, B., Dunbar, M., Penning, E., Kolada, A., Hellsten, S., Oggioni, A., Bertrin, V., Ecke, F., Søndergaard, M., 2013. Measurements of uncertainty in macrophyte metrics used to assess European lake water quality. Hydrobiologia 704, 179–191. https://doi.org/10.1007/s10750-012-1338-z

Egertson, C.J., Kopaska, J.A., Downing, J.A., 2004. A century of change in macrophyte abundance and composition in response to agricultural eutrophication. Hydrobiologia 524, 145–156. https://doi.org/10.1023/B:HYDR.0000036129.40386.ce

Eigemann, F., Mischke, U., Hupfer, M., Schaumburg, J., Hilt, S., 2016. Biological indicators track differential responses of pelagic and littoral areas to nutrient load reductions in German lakes. Ecol. Indic. 61, 905–910. https://doi.org/10.1016/j.ecolind.2015.10.045

Eisenreich, S., Bannerman, R.T., Armstrong, D.E., 1975. A Simplified Phosphorus Analysis Technique. Environ. Lett. 9, 43–53.

Ekholm, P., Malve, O., Kirkkala, T. 1997. Internal and external loading as regulators of nutrient concentrations in the agriculturally loaded Lake Phyajarvi (southwest Finland). Hydrobiolgia. 345, 3 - 14.

Environment Agency, n.d. Catchment Data Explorer. Environment Agency [WWW Document]. URL http://environment.data.gov.uk/catchment-planning/WaterBody/ (accessed 11.13.18).

Epe, T.S., Finsterle, K., Yasseri, S., 2017. Nine years of phosphorus management with lanthanum modified bentonite (Phoslock) in a eutrophic, shallow swimming lake in Germany. Lake Reserv. Manag. 33, 119–129. https://doi.org/10.1080/10402381.2016.1263693

European Commission, 2019. European Community Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora: Fourth Report by the United Kingdom under Article 17.

European Commission, 2011. Our life insurance , our natural capital : an EU biodiversity strategy to 2020. Brussels (Belgium).

European Commission, 2009. EU Biodiversity strategy 2020. https://doi.org/10.2779/39229

European Commission, 2000. Council Directive 2000/60/EC of the European Parliment and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy.

European Environment Agency, 2018. European waters - assessmet of status and pressures 2018, EEA report no 7/2018.

Feiler, U., Krebs, F., Heininger, P., 2006. Aquatic plant bioassays used in the assessment of water quality in German rivers. Hydrobiologia 570, 67–71. https://doi.org/10.1007/s10750-006-0163-7

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., Holling, C.S., 2004. Regime Shifts , Resilience , in Ecosystem Management. Annu. Rev. Ecol. Evol. Syst. 35, 557–581. https://doi.org/10.2307/annurev.ecolsys.35.021103.30000021

Forsberg, C., 1966. Sterile Germination Requirements of Seeds of Some Water Plants. Physiol. Plant. 19, 1105–1109. https://doi.org/10.1111/j.1399-3054.1966.tb07103.x

Fukuhara, H., and Sakamoto, M. 1987 Enhancement of inorganic nitrogen and phosphate release from lake sediment by tubific worms and chironomid larvae. Oikos. 48, 312 -320

Foy, R.H., Fitzsimons, A.G., 1987. Phosphorus inactivation in a eutrophic lake by the direct addition of ferric aluminum sulfate - changes in phytoplankton populations. Freshw. Biol. 17, 1–13. https://doi.org/10.1111/j.1365-2427.1987.tb01024.x

Gensemer, R.W., Playle, R.C., 1999. The bioavailability and toxicity of aluminum in aquatic environments. Crit. Rev. Environ. Sci. Technol. 29, 315–450.

https://doi.org/10.1080/10643389991259245

Gibbs, M., Özkundakci, D., 2011. Effects of a modified zeolite on P and N processes and fluxes across the lake sediment-water interface using core incubations. Hydrobiologia 661, 21–35. https://doi.org/10.1007/s10750-009-0071-8

Gonzalez Sagrario, M. a., Jeppesen, E., Goma, J., Søndergaard, M., Jensen, J.P., Lauridsen, T., Landkildehus, F., 2005. Does high nitrogen loading prevent clear-water conditions in shallow lakes at moderately high phosphorus concentrations? Freshw. Biol. 50, 27–41. https://doi.org/10.1111/j.1365-2427.2004.01290.x

Grace, J.B., Wetzel, R.G., 1978. The Production Biology of Eurasian Watermilfoil (*Myriophyllum spicatum* L.): A Review. J. Aquat. Plant Manag. 16, 1–11.

Green, A.J., Figuerola, J., Sánchez, M.I., 2002. Implications of waterbird ecology for the dispersal of aquatic organisms. Acta Oecologica 23, 177–189. https://doi.org/10.1016/S1146-609X(02)01149-9

Grillas, P., Garcia-Murillo, P., Geertz-Hansen, O., Marbá, N., Montes, C., Duarte, C.M., Tan Ham, L., Grossmann, A., 1993. Submerged macrophyte seed bank in a Mediterranean temporary marsh: abundance and relationship with established vegetation. Oecologia 94, 1–6. https://doi.org/10.1007/BF00317293

Grime, J., 1979. Plant Strategies, Vegetation Processes, and Ecosystem Properties. John Wiley & Sons, Ltd.

Grime, J.P., 1977. Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. Am. Nat. 111, 1169–1194. https://doi.org/10.1080/00224065.1996.11979642

Growns, I., Rourke, M., Gilligan, D., 2013. Toward river health assessment using species distributional modeling. Ecol. Indic. 29, 138–144. https://doi.org/10.1016/j.ecolind.2012.12.024

Gulati, R.D., Pires, L.M.D., Van Donk, E., 2008. Lake restoration studies: Failures, bottlenecks and prospects of new ecotechnological measures. Limnologica 38, 233–248. https://doi.org/10.1016/j.limno.2008.05.008

Gulati, R.D., Van Donk, E., 2002. Lakes in the Netherlands, their origin, eutrophication and restoration: State-of-the-art review. Hydrobiologia 478, 73–

106. https://doi.org/10.1023/A:1021092427559

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. https://doi.org/10.1007/s10750-013-1765-5

Gurnell, A., Thompson, K., Goodson, J., Moggridge, H., 2008. Propagule deposition along river margins: Linking hydrology and ecology. J. Ecol. 96, 553–565. https://doi.org/10.1111/j.1365-2745.2008.01358.x

Haag, R.W., 1983. Emergence of seedlings of aquatic macrophytes from lake sediments. Can. J. Bot. 61, 148–156.

Haghseresht, F., 2006. A revolution in phosphorus removal. PWS, Rep. number PS-06. 21pp 21.

Haghseresht, F., Wang, S., Do, D.D., 2009. A novel lanthanum-modified bentonite, Phoslock, for phosphate removal from wastewaters. Appl. Clay Sci. 46, 369–375. https://doi.org/10.1016/j.clay.2009.09.009

Hammerstrom, K.K., Kenworthy, W.J., 2003. A new method for estimation of Halophila decipiens Ostenfeld seed banks using density separation. Aquat. Bot. 76, 79–86. https://doi.org/10.1016/S0304-3770(03)00018-4

Hansel-Welch, N., Butler, M.G., Carlson, T.J., Hanson, M.A., 2003. Changes in macrophyte community structure in Lake Christina (Minnesota), a large shallow lake, following biomanipulation. Aquat. Bot. 75, 323–337. https://doi.org/10.1016/S0304-3770(03)00002-0

Harris, B.E., 2014. Broads Annual Water Plant Monitoring Report 2014 1–43.

Harwell, M.C., Havens, K.E., 2003. Experimental studies on the recovery potential of submerged aquatic vegetation after flooding and desiccation in a large subtropical lake. Aquat. Bot. 77, 135–151. https://doi.org/10.1016/S0304-3770(03)00101-3

Hay, F., Probert, R., Dawson, M., 2008. Laboratory germination of seeds from 10 British species of Potamogeton. Aquat. Bot. 88, 353–357. https://doi.org/10.1016/j.aquabot.2007.12.010

He, F., Zarfl, C., Bremerich, V., David, J.N.W., Hogan, Z., Kalinkat, G., Tockner, K.,

Jähnig, S.C., 2019. The global decline of freshwater megafauna. Glob. Chang. Biol. 1–10. https://doi.org/10.1111/gcb.14753

Herrmann, H., Nolde, J., Berger, S., Heise, S., 2016. Aquatic ecotoxicity of lanthanum - A review and an attempt to derive water and sediment quality criteria. Ecotoxicol. Environ. Saf. 124, 213–238. https://doi.org/10.1016/j.ecoenv.2015.09.033

Hickey, C.W., Gibbs, M.M., 2009. Lake sediment phosphorus release management — Decision support and risk assessment framework.

Hilt, S., Alirangues Nuñez, M.M., Bakker, E.S., Blindow, I., Davidson, T.A., Gillefalk, M., Hansson, L.-A., Janse, J.H., Janssen, A.B.G., Jeppesen, E., Kabus, T., Kelly, A., Köhler, J., Lauridsen, T.L., Mooij, W.M., Noordhuis, R., Phillips, G., Rücker, J., Schuster, H.-H., Søndergaard, M., Teurlincx, S., van de Weyer, K., van Donk, E., Waterstraat, A., Willby, N., Sayer, C.D., 2018. Response of Submerged Macrophyte Communities to External and Internal Restoration Measures in North Temperate Shallow Lakes. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.00194

Hilt, S., Gross, E.M., 2008. Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? Basic Appl. Ecol. 9, 422–432. https://doi.org/10.1016/j.baae.2007.04.003

Hilt, S., Gross, E.M., Hupfer, M., Morscheid, H., Mählmann, J., Melzer, A., Poltz, J., Sandrock, S., Scharf, E.M., Schneider, S., van de Weyer, K., 2006. Restoration of submerged vegetation in shallow eutrophic lakes - A guideline and state of the art in Germany. Limnologica 36, 155–171. https://doi.org/10.1016/j.limno.2006.06.001

Hilt, S., Köhler, J., Adrian, R., Monaghan, M.T., Sayer, C.D., 2013. Clear, crashing, turbid and back - long-term changes in macrophyte assemblages in a shallow lake. Freshw. Biol. 58, 2027–2036. https://doi.org/10.1111/fwb.12188

Hilt, S., Van De Weyer, K., Köhler, A., Chorus, I., 2010. Submerged Macrophyte Responses to Reduced Phosphorus Concentrations in Two Peri-Urban Lakes. Restor. Ecol. 18, 452–461. https://doi.org/10.1111/j.1526-100X.2009.00577.x

HMSO, 1994. The UK Biodiversity Action Plan.

HMSO, 1981. The Wildlife & Countryside Act. HMSO, London.

Hölker, F., Vanni, M., C, M., Grossart, H.-P., Stief, P., Adrian, R., Lorke, A., Dellwig, O., Brand, A., Hupfer, M., Mooij, W., Nützmann, G., Lewandowski, J., 2015. Tube-dwelling invertebrates: tiny ecosystem engineers have large effects in lake ecosystems. Concepts Synth. 85, 333–351.

Hoostmans, M.J.M., Vermaat, J.E., 1991. Macrophytes, a key to understanding changes caused by eutrophication in shallow freshwater ecosystems 412.

Horppila, J. and Nurminen, L., 2003. Effects of submerged macrophytes on sediment resuspension and internal phosphorus loading in Lake Hiidenvesi (Southern Finalnd). Water Research. 37, 4468 - 4474. https:// 10.1016/S0043-1354(03)00405-6

Hough, R.A., 1979. Photosynthesis, respiration, and organic carbon release in *Elodea canadensis* michx. Aquat. Bot. 7, 1–11. https://doi.org/10.1016/0304-3770(79)90002-0

Huang, Y.H., Saiers, J.E., Harvey, J.W., Noe, G.B., Mylon, S., 2008. Advection, dispersion, and filtration of fine particles within emergent vegetation of the Florida Everglades. Water Resour. Res. 44, 1–13. https://doi.org/10.1029/2007WR006290

Hupfer, M., Glöss, S., Schmieder, P., Grossart, H.P., 2008. Methods for detection and quantification of polyphosphate and polyphosphate accumulating microorganisms in aquatic sediments. Int. Rev. Hydrobiol. 93, 1–30. https://doi.org/10.1002/iroh.200610935

Hupfer, M., Lewandowski, J., 2008. Oxygen controls the phosphorus release from lake sediments - A long-lasting paradigm in limnology. Int. Rev. Hydrobiol. 93, 415–432. https://doi.org/10.1002/iroh.200711054

Huser, B.J., Egemose, S., Harper, H., Hupfer, M., Jensen, H., Pilgrim, K.M., Reitzel, K., Rydin, E., Futter, M., 2016a. Longevity and effectiveness of aluminum addition to reduce sediment phosphorus release and restore lake water quality. Water Res. 97, 122–132. https://doi.org/10.1016/j.watres.2015.06.051

Huser, B.J., Futter, M., Lee, J.T., Perniel, M., 2016b. In-lake measures for phosphorus control: The most feasible and cost-effective solution for long-term

management of water quality in urban lakes. Water Res. 97, 142–152. https://doi.org/10.1016/j.watres.2015.07.036

Hutchinson, G., 1957. A Treatise on Limnology. John Wiley and Sons, New York.

Ibelings, B.W., Portielje, R., Lammens, E.H.R.R., Noordhuis, R., Van Den Berg, M.S., Joosse, W., Meijer, M.L., 2007. Resilience of alternative stable states during the recovery of shallow lakes from eutrophication: Lake Veluwe as a case study. Ecosystems 10, 4–16. https://doi.org/10.1007/s10021-006-9009-4

Immers, A., Vendrig, K., Ibelings, B., Van Donk, E., Ter Heerdt, G.N., Geurts, J.J., Bakker, E., 2014. Iron addition as a measure to restore water quality: implications for macrophyte growth. Aquat. Bot. 116, 44–52.

Immers, A., 2014. Preventing or predicting cyanobacterial blooms iron addition as a whole lake restoration tool. Utrecht University, Utrecht, The Netherlands.

Immers, A.K., Bakker, E.S., Van Donk, E., Ter Heerdt, G.N.J., Geurts, J.J.M., Declerck, S.A.J., 2015. Fighting internal phosphorus loading: An evaluation of the large scale application of gradual Fe-addition to a shallow peat lake. Ecol. Eng. 83, 78–89. https://doi.org/10.1016/j.ecoleng.2015.05.034

Immers, A.K., van der Sande, M.T., van der Zande, R.M., Geurts, J.J.M., van Donk, E., Bakker, E.S., 2013. Iron addition as a shallow lake restoration measure: Impacts on charophyte growth. Hydrobiologia 710, 241–251. https://doi.org/10.1007/s10750-011-0995-7

IPCC, 2014. Intergovernmental Panel on Climate Change. Climate change 2014: impacts, adaptation, and vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the IPCC. In: Field CB, Barros VR, Dokken DJ, Mach KJ, Mastrandrea MD, Bilir TE, Cha.

IPCC, 2007. Climate change 2007: synthesis report. Intergovernmental Panel on Climate Change, Geneva, Switzerland. (Available from: http:// www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr.pdf), Intergovernmental Panel on Climate Change. https://doi.org/10.1038/446727a

Ippolito, M.P., Fasciano, C., D'Aquino, L., Morgana, M., Tommasi, F., 2010. Responses of antioxidant systems after exposition to rare earths and their role in chilling stress in common duckweed (*Lemna minor* L.): A defensive weapon

or a boomerang? Arch. Environ. Contam. Toxicol. 58, 42–52. https://doi.org/10.1007/s00244-009-9340-9

Irfanullah, H.M., Moss, B., 2004. Factors influencing the return of submerged plants to a clear-water, shallow temperate lake. Aquat. Bot. 80, 177–191. https://doi.org/10.1016/j.aquabot.2004.07.010

Jensen, H.S., and Andersen, F.Ø., 1992. Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. Limnol. Oceanogr. 37, 577–589. https://doi.org/10.4319/lo.1992.37.3.0577

Jensen, H.S., Reitzel, K., Egemose, S., 2015. Evaluation of aluminum treatment efficiency on water quality and internal phosphorus cycling in six Danish lakes. Hydrobiologia 751, 189–199. https://doi.org/10.1007/s10750-015-2186-4

Jeppesen, E., Jensen, J., Kristensen, P., Søndergaard, M., Mortensen, E., Sortkjaer, O., Olrik, K., 1990. Fish manipulation as a lake restoration tool in shallow, eutrophic, temperate lakes 2: threshold levels, long-term stability and conclusions. Hydrobiologia 200, 219–227.

Jeppesen, E., Jensen, J.P., Søndergaard, M., Lauridsen, T., Landkildehus, F., 2000. Trophic structure, species richness and biodiversity in Danish lakes: Changes along a phosphorus gradient. Freshw. Biol. 45, 201–218. https://doi.org/10.1046/j.1365-2427.2000.00675.x

Jeppesen, E., Jensen, J.P., Søndergaard, M., Lauridsen, T., Pedersen, L.J., Jensen, L., 1997. Top-down control in freshwater lakes : the role of nutrient state , submerged macrophytes and water depth. Hydrobiologia 342, 151–164. https://doi.org/10.1023/a:1017046130329

Jeppesen, E., Kristensen, P., Jensen, J.P., Sondergaard, M., Mortensen, E., Lauridsen, T., 1991. Recovery resilience following a reduction in external phosphorus loading of shallow, eutrophic danish lakes: duration, regulating factors and methods for overcoming resilience. Mem. Ist. Ital. Idrobbol. 48, 127–148.

Jeppesen, E., Meerhoff, M., Jacobsen, B., Hansen, R., Søndergaard, M., Jensen, J., Lauridsen, T., Mazzeo, N., Branco, C.W., 2007a. Restoration of shallow lakes by nutrient control and biomanipulation - The successful strategy varies with lake size and climate. Hydrobiologia 581, 269–285.

https://doi.org/10.1007/s10750-006-0507-3

Jeppesen, E., Søndergaard, M., Jensen, J.P., Havens, K.E., 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. Freshw. Biol. 50, 1747–1771. https://doi.org/10.1111/j.1365-2427.2005.01415.x

Jeppesen, E., Søndergaard, M., Kronvang, B., Jensen, J.P., Svendsen, L.M., Lauridsen, T.L., 1999. Lake and catchment management in Denmark. Hydrobiologia 419–432. https://doi.org/10.1023/A:1017071602549

Jeppesen, E., Søndergaard, M., Liu, Z., 2017. Lake restoration and management in a climate change perspective: An introduction. Water (Switzerland) 9, 1–8. https://doi.org/10.3390/w9020122

Jeppesen, E., Søndergaard, M., Meerhoff, M., Lauridsen, T.L., Jensen, J.P., 2007b. Shallow lake restoration by nutrient loading reduction - Some recent findings and challenges ahead. Hydrobiologia 584, 239–252. https://doi.org/10.1007/s10750-007-0596-7

JNCC, 2015. Common Standards Monitoring Guidance for Freshwater Lakes. Peterborough. https://doi.org/10.1016/b978-0-444-63768-0.00339-5

Jones, C., Welch, E. 1990. Internal mixing phosphorus and dilution shallow related loading in a dendriti, shallow prarie lake. Research Journal of the Water Poll. Control Fed. 92, 847 - 852

Kautsky, L., 1988. Life Strategies of Aquatic Soft Bottom Macrophytes 53, 126–135.

Kay, M., Wobbrock, J., 2019. The Aligned Rank Transformation for Nonparametric Factorial ANOVAS. https://doi.org/10.5281/zenodo.594511

Kelly, A., 2008. Lake Restoration Strategy for The Broads. Broads Authority, Norwich.

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. Mar. Freshw. Ecosyst. 8, 669–684. https://doi.org/10.1002/(SICI)1099-0755(199809/10)8:5<669::AID-AQC307>3.0.CO;2-2

Kimber, A., Korschgen, C.E., Van Der Valk, A.G., 1995. The distribution of *Vallisneria americana* seeds and seedling light requirements in the Upper Mississippi River. Can. J. Bot. 73, 1966–1973. https://doi.org/10.1139/b95-210

Kleeberg, A., Herzog, C., Hupfer, M., 2013. Redox sensitivity of iron in phosphorus binding does not impede lake restoration. Water Res. 47, 1491–1502. https://doi.org/10.1016/j.watres.2012.12.014

Kleeberg, A., Kohl, J.G., 1999. Assessment of the long-term effectiveness of sediment dredging to reduce benthic phosphorus release in shallow Lake Muggelsee (Germany). Hydrobiologia 394, 153–161. https://doi.org/10.1023/A:1003680425229

Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P., Thompson, K., Sonnenschein, M., Poschlod, P., Van Groenendael, J.M., Klimeš, L., Klimešová, J., Klotz, S., Rusch, G.M., Hermy, M., Adriaens, D., Boedeltje, G., Bossuyt, B., Dannemann, A., Endels, P., Götzenberger, L., Hodgson, J.G., Jackel, A.K., Kühn, I., Kunzmann, D., Ozinga, W.A., Römermann, C., Stadler, M., Schlegelmilch, J., Steendam, H.J., Tackenberg, O., Wilmann, B., Cornelissen, J.H.C., Eriksson, O., Garnier, E., Peco, B., 2008. The LEDA Traitbase: A database of life-history traits of the Northwest European flora. J. Ecol. 96, 1266–1274. https://doi.org/10.1111/j.1365-2745.2008.01430.x

Knopik, J.M., Newman, R.M., 2018. Transplanting aquatic macrophytes to restore the littoral community of a eutrophic lake after the removal of common carp. Lake Reserv. Manag. 34, 365–375. https://doi.org/10.1080/10402381.2018.1477885

Kohler, A., 1978. Methoden der Kartierung von Flora und Vegetation von Süßwasserbiotopen 10, 73–85.

Kolář, J., 2014. *Littorella uniflora* (L.) Ascherson: A Review. Sci. Agric. Bohem. 45, 147–154. https://doi.org/10.2478/sab-2014-0101

Körner, S., Nicklisch, A., 2002. Allelopathic Growth Inhibition Of Selected Phytoplankton Species By Submerged Macrophytes. J. Phycol. 38, 862–871.

Kuznetsova, A., P. B, Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82, 1–26. https://doi.org/https://doi.org/10.18637/jss.v082.i13

Lambert, S.J. 2007. The Environmental Range and Tolerance Limits of British Stoneworts (Charophytes).University of East Anglia.pp 463

Lambert, S.J., Davy, A.J., 2011. Water quality as a threat to aquatic plants: Discriminating between the effects of nitrate, phosphate, boron and heavy metals on charophytes. New Phytol. 189, 1051–1059. https://doi.org/10.1111/j.1469-8137.2010.03543.x

Lauridsen, T.L., Jensen, J.P., Jeppesen, E., Søndergaard, M., 2003a. Response of submerged macrophytes in Danish lakes to nutrient loading reductions and biomanipulation. Hydrobiologia 506–509, 641–649. https://doi.org/10.1023/B:HYDR.0000008633.17385.70

Lauridsen, T.L., Jeppesen, E., Andersen, F.Ø., 1993. Colonization of submerged macrophytes in shallow fish manipulated Lake Væng: impact of sediment composition and waterfowl grazing. Aquat. Bot. 46, 1–15. https://doi.org/10.1016/0304-3770(93)90061-Z

Lauridsen, T.L., Jeppesen, E., Declerck, S.A.J., De Meester, L., Conde-Porcuna, J.M., Rommens, W., Brucet, S., 2015. The importance of environmental variables for submerged macrophyte community assemblage and coverage in shallow lakes: differences between northern and southern Europe. Hydrobiologia 744, 49–61. https://doi.org/10.1007/s10750-014-2055-6

Lauridsen, T.L., Jeppesen, E., Søndergaard, M., 1994. Colonization and succession of submerged macrophytes in shallow Lake Væng during the first five years following fish manipulation. Hydrobiologia 275–276, 233–242. https://doi.org/10.1007/BF00026714

Lauridsen, T.L., Sandsten, H., Møller, P.H., 2003b. The restoration of a shallow lake by introducing Potamogeton spp.: The impact of waterfowl grazing. Lakes Reserv. Res. Manag. 8, 177–188. https://doi.org/10.1111/j.1440-1770.2003.00224.x

Law, A., Baker, A., Sayer, C., Foster, G., Gunn, I.D.M., Taylor, P., Pattison, Z., Blaikie, J., Willby, N.J., 2019. The effectiveness of aquatic plants as surrogates

for wider biodiversity in standing fresh waters. Freshw. Biol. 64, 1664–1675. https://doi.org/10.1111/fwb.13369

Leck, M.A., Graveline, K.J., 1979. The seed bank of a freshwater tidal marsh. Am. j. Bot 66, 1006–1015.

Lewandowski, J., and Hupfer, M. 2005. Impact of macrozoobenthos on two-dimensional small-scale heterogeneity of pore water phosphorus concentrations: In-situ study . Limnol. Ocenaog. 50, 1106 - 1118.

Lewandowski, J., Laskov, C., Hupfer, M., 2007. The relationship between *Chironomus plumosus* burrows and the spatial distribution of pore-water phosphate, iron and ammonium in lake sediments. Freshw. Biol. 52, 331–343. https://doi.org/10.1111/j.1365-2427.2006.01702.x

Lewandowski, J., Schauser, I., Hupfer, M., 2003. Long term effects of phosphorus precipitations with alum in hypereutrophic Lake Süsser See (Germany). Water Res. 37, 3194–3204. https://doi.org/10.1016/S0043-1354(03)00137-4

Lewis, M.A., 1995. Use of freshwater plants for phytotoxicity testing: A review. Environ. Pollut. 87, 319–336. https://doi.org/10.1016/0269-7491(94)P4164-J

Leys, C., Schumann, S., 2010. A nonparametric method to analyze interactions: The adjusted rank transform test. J. Exp. Soc. Psychol. 46, 684–688. https://doi.org/10.1016/j.jesp.2010.02.007

Lin, J., Zhong, Y., Fan, H., Song, C., Yu, C., Gao, Y., Xiong, X., Wu, C., Liu, J., 2017. Chemical treatment of contaminated sediment for phosphorus control and subsequent effects on ammonia-oxidizing and ammonia-denitrifying microorganisms and on submerged macrophyte revegetation. Environ. Sci. Pollut. Res. 24, 1007–1018. https://doi.org/10.1007/s11356-016-7828-1

Liu, J., Danneels, B., Vanormelingen, P., Vyverman, W., 2016. Nutrient removal from horticultural wastewater by benthic filamentous algae Klebsormidium sp., Stigeoclonium spp. and their communities: From laboratory flask to outdoor Algal Turf Scrubber (ATS). Water Res. 92, 61–68. https://doi.org/10.1016/j.watres.2016.01.049

Londo, G., 1975. Rijksinstituut RIN-mededeling gebruikt goed ruimtelijke vegetatieonderzoek gaat wijden spoedig grof zijn. Geringe veranderingen

kingsgraad zijn hij Braun-Blanquet juiste verhouding Doing Segal omrekeningswaarden Braun-Blanquet zijn volgende : zijn eenv 101–106.

Lürling, M., Mackay, E., Reitzel, K., Spears, B.M., 2016. Editorial - a critical perspective on geo-engineering for eutrophication management in lakes.Water Research. 97, 1 - 10. https:// 10.1016/j.watres.2016.03.035.

Lürling, M., Faassen, E.J., 2012a. Controlling toxic cyanobacteria: Effects of dredging and phosphorus-binding clay on cyanobacteria and microcystins. Water Res. 46, 1447–1459. https://doi.org/10.1016/j.watres.2011.11.008

Lürling, M., Faassen, E.J., 2012b. Controlling toxic cyanobacteria: effects of dredging and phosphorus-binding clay on cyanobacteria and microcystins. Water Res. 46, 1447–59. https://doi.org/10.1016/j.watres.2011.11.008

Lürling, M., Mackay, E.B., Reitzel, K., Spears, B.M., 2016. Editorial – A critical perspective on geo-engineering for eutrophication management in lakes. Water Res. 97, 1–10. https://doi.org/10.1016/j.watres.2016.03.035

Lürling, M., Tolman, Y., 2010. Effects of lanthanum and lanthanum-modified clay on growth, survival and reproduction of Daphnia magna. Water Res. 44, 309–319. https://doi.org/10.1016/j.watres.2009.09.034

Lürling, M., van Oosterhout, F., 2013. Controlling eutrophication by combined bloom precipitation and sediment phosphorus inactivation. Water Res. 47, 6527–6537. https://doi.org/10.1016/j.watres.2013.08.019

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. https://doi.org/10.1007/s10750-012-1141-x

Lürling, M., Waajen, G., van Oosterhout, F., 2014. Humic substances interfere with phosphate removal by lanthanum modified clay in controlling eutrophication. Water Res. 54, 78–88. https://doi.org/10.1016/j.watres.2014.01.059

Mackay, E.B., Maberly, S.C., Pan, G., Reitzel, K., Bruere, A., Corker, N., Egemose, S., Hamilton, D., Hatton-ellis, T., Huser, B., Li, W., Meis, S., Moss, B., Lürling, M., Phillips, G., Yasseri, S., Spears, B.M., 2014. Geoengineering in lakes : welcome attraction or fatal distraction ? Inl. Waters 4, 349–356.

https://doi.org/10.5268/IW-4.4.769

Madsen, J.D., Hartleb, C.F., Boylen, C.W., 1991. Photosynthetic characteristics of Myriophyllum spicatum and six submersed aquatic macrophyte species native to Lake George, New York. Freshw. Biol. 26, 233–240. https://doi.org/10.1111/j.1365-2427.1991.tb01732.x

Madsen, T.V., Cedergreen, N., 2002. Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. Freshw. Biol, 47, 283 - 291. https://doi.org/10.1046/j.1365-2427.2002.00802.x

Madsen, T. V., Sand-Jensen, K., 1994. The interactive effects of light and inorganic carbon on aquatic plant growth. Plant. Cell Environ. 17, 955–962. https://doi.org/10.1111/j.1365-3040.1994.tb00324.x

Mäemets, H., Freiberg, L., Haldna, M., Möls, T., 2006. Inter-annual variability of *Potamogeton perfoliatus* stands. Aquat. Bot. 85, 177–183. https://doi.org/10.1016/j.aquabot.2006.03.008

Malecki-Brown, L.M., White, J.R., Brix, H., 2010. Alum application to improve water quality in a municipal wastewater treatment wetland: Effects on macrophyte growth and nutrient uptake. Chemosphere 79, 186–192. https://doi.org/10.1016/j.chemosphere.2010.02.006

Mardsen, M.W., 1989. Lake restoration by reducing external phosphorus loading: the influence of sediment phosphorus release. Freshw. Biol. 21, 139–162.

Márquez-Pacheco, H., Hansen, A.M., Falcón-Rojas, A., 2013. Phosphorous control in a eutrophied reservoir. Environ. Sci. Pollut. Res. 20, 8446–8456. https://doi.org/10.1007/s11356-013-1701-2

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence - A practical guide. J. Exp. Bot. 51, 659–668. https://doi.org/10.1093/jxb/51.345.659

May, L., Carvalho, L., 2010. Maximum growing depth of macrophytes in Loch Leven, Scotland, United Kingdom, in relation to historical changes in estimated phosphorus loading. Hydrobiologia 646, 123–131. https://doi.org/10.1007/s10750-010-0176-0

Mcfarland, D.G., Shafer, D.J., 2011. Protocol considerations for aquatic plant seed bank assessment. J. Aquat. Plant Manag. 49, 9–19.

McMahon, K., and Read, E. 2013. Microbial contributions to phosphorus cycling in eutrophic lakes and wastewater. Annu. Rev. Microbiol.63, 199 - 219.

MEA, 2005. A Report of the Millennium Ecosystem Assessment Contributing Authors, and Sub-global Coordinators Review Editors: José Sarukhán and Anne Whyte (Co-chairs) and MA Board of Review Editors The Cropper Foundation, Trinidad and Tobago.

Meijer, M., de Boois, I., Scheffer, M., Portielje, R., Hosper, H., 1999. Biomanipulation in shallow lakes in The Netherlands : an evaluation of 18 case studies. Hydrobiologia 408–409, 13–30. https://doi.org/64 PDF 12

Meis, S., Spears, B.M., Maberly, S.C., O'Malley, M.B., Perkins, R.G., 2012. Sediment amendment with Phoslock® in Clatto Reservoir (Dundee, UK): Investigating changes in sediment elemental composition and phosphorus fractionation. J. Environ. Manage. 93, 185–93. https://doi.org/10.1016/j.jenvman.2011.09.015

Meis, S., Spears, B.M., Maberly, S.C., Perkins, R.G., 2013. Assessing the mode of action of Phoslock® in the control of phosphorus release from the bed sediments in a shallow lake (Loch Flemington, UK). Water Res. 47, 4460–4473. https://doi.org/10.1016/j.watres.2013.05.017

Middelboe, A.L., Markager, S., 1997. Depth limits and minimum light requirements of freshwater macrophytes. Freshw. Biol. 37, 553–568. https://doi.org/10.1046/j.1365-2427.1997.00183.x

Mohan, B.S., Hosetti, B.B., 1999. Aquatic plants for toxicity assessment. Environ. Res. 81, 259–274. https://doi.org/10.1006/enrs.1999.3960

Mooij, W.M., Hülsmann, S., De Senerpont Domis, L.N., Nolet, B.A., Bodelier, P.L.E., Boers, P.C.M., Dionisio Pires, L.M., Gons, H.J., Ibelings, B.W., Noordhuis, R., Portielje, R., Wolfstein, K., Lammens, E.H.R.R., 2005. The impact of climate change on lakes in the Netherlands: A review. Aquat. Ecol. 39, 381–400. https://doi.org/10.1007/s10452-005-9008-0

Mousavi, S.K., 2002. Boreal chironomid communities and their relations to environmental factors - The impact of lake depth, size and acidity. Boreal Environ. Res. 7, 63–75.

Mucci, M., 2019. From green to transparent waters. Wageningen University.pp 200

Mulbry, W., Kangas, P., Kondrad, S., 2010. Toward scrubbing the bay: Nutrient removal using small algal turf scrubbers on Chesapeake Bay tributaries. Ecol. Eng. 36, 536–541. https://doi.org/10.1016/j.ecoleng.2009.11.026

Murphy, F., Schmieder, K., Baastrup-Spohr, L., Pedersen, O., Sand-Jensen, K., 2018. Five decades of dramatic changes in submerged vegetation in Lake Constance. Aquat. Bot. 144, 31–37. https://doi.org/10.1016/j.aquabot.2017.10.006

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Murphy, K.J., Rørslett, B., Springuel, I., 1990. Strategy analysis of submerged lake macrophyte communities: an international example. Aquat. Bot. 36, 303–323. https://doi.org/10.1016/0304-3770(90)90048-P

Natural England, 2010. Lost life: England's lost and threatened species.

Natural England, n.d. Designated Sites View. Natural England. [WWW Document]. URL https://designatedsites.naturalengland.org.uk/ (accessed 11.13.18).

Nature Conservancy Council, 1989. No TitleGuidelines for selection of Biological SSSIs; Rationale, Operationsl approach and criteria. Peterborough.

Nichols, S.A., Shaw, B.H., 1986. Ecological life histories of three aquatic nuisance plants, Myriophyllum spicatum, Potamogeton crispus and Elodea canadensis. Hydrobiologia 131, 3–21. https://doi.org/10.3391/ai.2009.4.2.2

NICNAS, 2001. National industrial chemicals notification and assessment scheme full public report lanthanum modified clay NA/899.

Nishihiro, J., Araki, S., Fujiwara, N., Washitani, I., 2004. Germination characteristics of lakeshore plants under an artificially stabilized water regime. Aquat. Bot. 79, 333–343. https://doi.org/10.1016/j.aquabot.2004.05.005

Novak, P.A., Chambers, J.M., 2014. Investigation of nutrient thresholds to guide restoration and management of two impounded rivers in south-western Australia. Ecol. Eng. 68, 116–123. https://doi.org/10.1016/j.ecoleng.2014.03.091

Nürnberg, n.d. Internal load release rates.Unpublished studies. 1 - 11.

Nürnberg, G.K., 1984. The prediction of internal phosphorus load in lakes with anoxic hypolimnia. Limnol. Oceanogr. 29, 111–124. https://doi.org/10.4319/lo.1984.29.1.0111

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., R., P., Minchin, R., O'Hara, B., Simpson, G.L., Peter Solymos, M., Stevens, H.H., Szoecs, E., Wagner, H., 2019. Vegan: Community Ecology Package, R Package Version, 2.5 - 5.

Oreska, M.P.J., Aldridge, D.C., 2011. Estimating the financial costs of freshwater invasive species in Great Britain: A standardized approach to invasive species costing. Biol. Invasions 13, 305–319. https://doi.org/10.1007/s10530-010-9807-7

Ozimek, T., 2006. The possibility of submerged macrophyte recovery from a propagule bank in the eutrophic Lake Mikolajskiw (North Poland). Hydrobiologia 570, 127–131.

Ozimek, T., Gulati, R.D., van Donk, E., 1990. Can macrophytes be useful in biomanipulation of lakes? The Lake Zwemlust example. Hydrobiologia 200–201, 399–407. https://doi.org/10.1007/BF02530357

Ozimek, T., van Donk, E., Gulati, R.D., 1993. Growth and nutrient uptake by two species of Elodea in experimental conditions and their role in nutrient accumulation in a macrophyte-dominated lake. Hydrobiologia 251, 13–18. https://doi.org/10.1007/BF00007159

Pacioglu, O., Cornut, J., Gessner, M.O., Kasprzak, P., 2016. Prevalence of indirect toxicity effects of aluminium flakes on a shredder-fungal-leaf decomposition system. Freshw. Biol. 61, 2013–2025. https://doi.org/10.1111/fwb.12529

Paerl, H.W., Scott, J.T., McCarthy, M.J., Newell, S.E., Gardner, W.S., Havens, K.E., Hoffman, D.K., Wilhelm, S.W., Wurtsbaugh, W.A., 2016. It Takes Two to Tango: When and Where Dual Nutrient (N & P) Reductions Are Needed to Protect Lakes and Downstream Ecosystems. Environ. Sci. Technol. 50, 10805–10813. https://doi.org/10.1021/acs.est.6b02575

Pandey, J., Yaduvanshi, M.S., 2005. Sediment Dredging as a Restoration Tool in

Shallow Tropical Lakes: Cross Analysis of Three Freshwater Lakes of Udaipur, India. Environ. Control Biol. 43, 275–281. https://doi.org/10.2525/ecb.43.275

Pelton, D.K., Levine, S.N., Braner, M., 1998. Measurements of phosphorus uptake by macrophytes and epiphytes from the LaPlatte river (VT) using 32P in stream microcosms. Freshw. Biol. 39, 285–299. https://doi.org/10.1046/j.1365-2427.1998.00281.x

Penning, W.E., Dudley, B., Mjelde, M., Hellsten, S., Hanganu, J., Kolada, A., van den Berg, M., Poikane, S., Phillips, G., Willby, N., Ecke, F., 2008. Using aquatic macrophyte community indices to define the ecological status of European lakes. Aquat. Ecol. 42, 253–264. https://doi.org/10.1007/s10452-008-9183-x

Perkins, R.G., Underwood, G.J.C., 2002. Partial recovery of a eutrophic reservoir through managed phosphorus limitation and unmanaged macrophyte growth. Hydrobiologia 481, 75–87. https://doi.org/10.1023/A:1021281019865

Perrow, M., Meijer, M., Dawidowicz, P., Coops, H., 1997. Biomanipulation in shallow lakes: state of the art. Hydrobiologia 33, 355–365. https://doi.org/PDF 13

Perrow, M.R., Jowitt, A.J.D., Stansfield, J.H., Phillips, G.L., 1999. The practical importance of the interactions between fish, zooplankton and macrophytes in shallow lake restoration. Hydrobiologia 395396, 199–210. https://doi.org/10.1023/A:1017005803941

Perrow, M.R., Moss, B., Stansfield, J., 1994. Trophic interactions in a shallow lake following a reduction in nutrient loading: a long-term study. Hydrobiologia 275–276, 43–52. https://doi.org/10.1007/BF00026698

Pettricrew, E.L., and Arocena, J.M. 2001. Evaluation of iron-phosphate as a source of internal lake phosphorus loadings. Sci. of Tot. Env. 266, 87 - 93.

Phillips, G., Bennion, H., Perrow, M.R., Sayer, C.D., Spears, B.M., Willby, N., 2015. A review of lake restoration practices and their performance in the Broads National Park 1980-2013, Report for the Broads Authority, Nowich and Natural England.

Phillips, G., Kelly, A., Pitt, J.A., Sanderson, R., Taylor, E., 2005. The recovery of a very shallow eutrophic lake, 20 years after the control of effluent derived phosphorus. Freshw. Biol. 50, 1628–1638. https://doi.org/10.1111/j.1365-

2427.2005.01434.x

Phillips, G., Willby, N., Moss, B., 2016. Submerged macrophyte decline in shallow lakes: What have we learnt in the last forty years? Aquat. Bot. 135, 37–45. https://doi.org/10.1016/j.aquabot.2016.04.004

Phillips, G.L., Eminson, D., Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4, 103–126. https://doi.org/10.1016/0304-3770(78)90012-8

Pickett, S.T.A., White, P.S., 1985. The Ecology of Natural Disturbance and Patch Dynamics. Academic Press, London.

Poikane, S., Phillips, G., Birk, S., Free, G., Kelly, M.G., Willby, N.J., 2019. Deriving nutrient criteria to support 'good' ecological status in European lakes: An empirically based approach to linking ecology and management. Sci. Total Environ. 650, 2074–2084. https://doi.org/10.1016/j.scitotenv.2018.09.350

Poikane, S., Portielje, R., Denys, L., Elferts, D., Kelly, M., Kolada, A., Mäemets, H., Phillips, G., Søndergaard, M., Willby, N., van den Berg, M.S., 2018. Macrophyte assessment in European lakes: Diverse approaches but convergent views of 'good' ecological status. Ecol. Indic. 94, 185–197. https://doi.org/10.1016/j.ecolind.2018.06.056

Pollux, B.J.A., 2011. The experimental study of seed dispersal by fish (ichthyochory). Freshw. Biol. 56, 197–212. https://doi.org/10.1111/j.1365-2427.2010.02493.x

Prepas, E., Babin, J., Murphy, T.P., Chambers, P.A., Sandland, G.J., Ghadouani, A., Serediak, M., 2001a. Long-term effects of successive CA(OH)2 and CACO3 treatments on the water quality of two eutrophic hardwater lakes. Freshw. Biol. 46, 1089–1103. https://doi.org/10.1046/j.1365-2427.2001.00792.x

Prepas, E., Chambers, P.A., Murphy, T.P., Reedyk, S., Sandland, G., Serediak, M., 2001b. Lime treatment and its effects on the chemistry and biota of hardwater eutrophic lakes. Freshw. Biol. 46, 1049–1060.

Preston, C.D., Croft, J.M., 2001. Aquatic Plants in Britain and Ireland. Harley Books, Colchester.

Puritch, G.S., Barker, A. V., 1967. Structure and Function of Tomato Leaf

Chloroplasts During Ammonium Toxicity. Plant Physiol. 42, 1229–1238. https://doi.org/10.1104/pp.42.9.1229

R Development Core Team, 2019. R: a language and environment for statistical computing. [WWW Document]. R Found. Stat. Comput. Vienna.

Reedyk, S., Prepas, E.E., Chambers, P. a., 2001. Effects of single Ca(OH)2 doses on phosphorus concentration and macrophyte biomass of two boreal eutrophic lakes over 2 years. Freshw. Biol. 46, 1075–1087. https://doi.org/10.1046/j.1365-2427.2001.00790.x

Reitzel, B.K., Jensen, H.S., 2018. Test of lanthanum dispersion from Phoslock under a range of different laboratory conditions. University of South Denmark. 1–29.

Reitzel, K., Andersen, F.T., Egemose, S., Jensen, H.S., 2013. Phosphate adsorption by lanthanum modified bentonite clay in fresh and brackish water. Water Res. 47, 2787–2796. https://doi.org/10.1016/j.watres.2013.02.051

Reitzel, K., Hansen, J., Andersen, F., Hansen, K.S., Jensen, H.S., 2005. Lake restoration by dosing aluminum relative to mobile phosphorus in the sediment. Environ. Sci. Technol. 39, 4134–4140. https://doi.org/10.1021/es0485964

Reitzel, K., Hansen, J., Jensen, H.S., Andersen, F.Ø., Hansen, K.S., Sønderby, L., Reitzel, K., Hansen, J., Jensen, H.S., Andersen, F.Ø., Hansen, K.S., 2003. Testing aluminum addition as a tool for lake restoration in shallow ,. Hydrobiologia 5147579, 1–8. https://doi.org/10.1023/B:HYDR.0000008624.54844.2d

Reitzel, K., Lotter, S., Dubke, M., Egemose, S., Jensen, H.S., Andersen, F.Ø., 2012. Effects of Phoslock® treatment and chironomids on the exchange of nutrients between sediment and water. Hydrobiologia 703, 189–202. https://doi.org/10.1007/s10750-012-1358-8

Rip, W.J., Ouboter, M.R.L., Los, H.J., 2007. Impact of climatic fluctuations on Characeae biomass in a shallow, restored lake in the Netherlands. Hydrobiologia 584, 415–424. https://doi.org/10.1007/s10750-007-0608-7

Robach, F., Hanjnsek, I., Tremolieres, M., 1995. Phosphorus sources for aquatic macrophytes in running waters: water or sediment? Acta Bot. Gallica, 142, 719

- 731. https://doi.org/10.1080/12538078.1995.10515296.

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 237–243.

Robe, W.E., Griffiths, H., 1998. Adaptations for an amphibious life: changes in leaf morphology, growth rate, carbon and nitrogen investment, and reproduction during adjustment to emersion by the freshwater macrophyte *Littorella uniflora*. New Phytol. 140, 9–23. https://doi.org/10.1046/j.1469-8137.1998.00257.x

Rooney, N., Kalff, J., 2000. Inter-annual variation in submerged macrophyte community biomass and distribution: The influence of temperature and lake morphometry. Aquat. Bot. 68, 321–335. https://doi.org/10.1016/S0304-3770(00)00126-1

Ross, G., Haghseresht, F., Cloete, T.E., 2008. The effect of pH and anoxia on the performance of Phoslock®, a phosphorus binding clay. Harmful Algae 7, 545–550. https://doi.org/10.1016/j.hal.2007.12.007

Rybak, M., Joniak, T., 2018. Changes in Chara hispida L. morphology in response to phosphate aluminium coagulant application. Limnol. Rev. 18, 31–37. https://doi.org/10.2478/limre-2018-0004

Rybak, M., Kołodziejczyk, A., Joniak, T., Ratajczak, I., Gąbka, M., 2017. Bioaccumulation and toxicity studies of macroalgae (Charophyceae) treated with aluminium: Experimental studies in the context of lake restoration. Ecotoxicol. Environ. Saf. 145, 359–366. https://doi.org/10.1016/j.ecoenv.2017.07.056

Salgado, J., Sayer, C., Carvalho, L., Davidson, T., Gunn, I., 2010. Assessing aquatic macrophyte community change through the integration of palaeolimnological and historical data at Loch Leven, Scotland. J. Paleolimnol. 43, 191–204. https://doi.org/10.1007/s10933-009-9389-5

Sand-Jensen, K., Borum, J., 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat. Bot. 41, 137–175. https://doi.org/10.1016/0304-3770(91)90042-4

Sand-Jensen, K., Bruun, H.H., Baastrup-Spohr, L., 2017. Decade-long time delays

in nutrient and plant species dynamics during eutrophication and re-oligotrophication of Lake Fure 1900–2015. J. Ecol. 105, 690–700. https://doi.org/10.1111/1365-2745.12715

Sand-Jensen, K., Riis, T., Vestergaard, O., Larsen, S.E., 2000. Macrophyte decline in Danish lakes and streams over the past 100 years. J. Ecol. 88, 1030–1040. https://doi.org/10.1046/j.1365-2745.2000.00519.x

Sand-Jensen, K., Søndergaard, M., 1981. Phytoplankton and Epiphyte Development and Their Shading Effect on Submerged Macrophytes in Lakes of Different nutrient Status. Int. Rev. ges. Hydrobiol. 66, 529–552.

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

Sayer, C.D., Bennion, H., Davidson, T.A., Burgess, A., Clarke, G., Hoare, D., Frings, P., Hatton-Ellis, T., 2012. The application of palaeolimnology to evidence-based lake management and conservation: Examples from UK lakes. Aquat. Conserv. Mar. Freshw. Ecosyst. 22, 165–180. https://doi.org/10.1002/aqc.2221

Sayer, C.D., Burgess, A., Kari, K., Davidson, T.A., Peglar, S., Yang, H., Rose, N., 2010a. Long-term dynamics of submerged macrophytes and algae in a small and shallow, eutrophic lake: Implications for the stability of macrophyte-dominance. Freshw. Biol. 55, 565–583. https://doi.org/10.1111/j.1365-2427.2009.02353.x

Sayer, C.D., Davidson, T.A., Jones, J.I., 2010b. Seasonal dynamics of macrophytes and phytoplankton in shallow lakes: A eutrophication-driven pathway from plants to plankton? Freshw. Biol. 55, 500–513. https://doi.org/10.1111/j.1365-2427.2009.02365.x

Schaffelke, B., Luning, K., 1994. A circannual rhythm controls seasonal growth in the kelps *Laminaria hyperborea* and *L. digitata* from Helgoland (North sea). Eur. J. Phycol. 29, 49–56. https://doi.org/10.1080/09670269400650471

Schaumburg, J., Schranz, C., Stelzer, D., Vogel, A. 2015. Verfahrensanleitung für die ökologische Bewertung von Seen zur Umsetzung der EG-Wasserrahmenrichtlinie: Makrophyten und Phytobenthos. Phylib. (Stand Februar 2014, Version Oktober 2015). Bayerisches Landesamt für Umwelt (Hrsg.), München.

Scheffer, M., 2001. Alternative attractors of shallow lakes. ScientificWorldJournal. 1, 254–263. https://doi.org/10.1100/tsw.2001.62

Scheffer, M., 1998. Ecology of Shallow Lakes. Kluwer Academic Publishers.

Scheffer, M., Carpenter, S., Foley, J. a, Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature 413, 591–596. https://doi.org/10.1038/35098000

Scheffer, M., Hosper, S.H., Meijer, M.L., Moss, B., Jeppesen, E., 1993. Alternative equilibria in shallow lakes. Trends Ecol. Evol. 8, 275–279. https://doi.org/10.1016/0169-5347(93)90254-M

Scheffer, M., Jeppesen, E., 1998. Alternative Stable states. In Jeppesen E., Søndergaard M., Christoffersen K. (eds) The Structering Role of Submerged Macrophytes in Lakes. Springer, New York, NY, p. 427.

Schindler, D.W., 2006. Recent advances in the understanding and management of eutrophication. Limnol. Oceanogr. 51, 356–363. https://doi.org/10.4319/lo.2006.51.1_part_2.0356

Schindler, D.W., Carpenter, S.R., Chapra, S.C., Hecky, R.E., Orihel, D.M., 2016. Reducing phosphorus to curb lake eutrophication is a success. Environ. Sci. Technol. 50, 8923–8929. https://doi.org/10.1021/acs.est.6b02204

Schoumans, O.F., Chardon, W.J., Bechmann, M.E., Gascuel-Odoux, C., Hofman, G., Kronvang, B., Rubæk, G.H., Ulén, B., Dorioz, J.M., 2014. Mitigation options to reduce phosphorus losses from the agricultural sector and improve surface water quality: A review. Sci. Total Environ. 468–469, 1255–1266. https://doi.org/10.1016/j.scitotenv.2013.08.061

Sederias, J., Colman, B., 2009. Inhibition of *Chara vulgaris* oospore germination by sulfidic sediments. Aquat. Bot. 91, 273–278. https://doi.org/10.1016/j.aquabot.2009.07.006

Sederias, J., Colman, B., 2007. The interaction of light and low temperature on breaking the dormancy of *Chara vulgaris* oospores. Aquat. Bot. 87, 229–234. https://doi.org/10.1016/j.aquabot.2007.06.008

Sereda, J.M., Hudson, J.J., McLoughlin, P.D., 2008a. General empirical models for predicting the release of nutrients by fish, with a comparison between detritivores and non-detritivores. Freshw. Biol. 53, 2133–2144.

https://doi.org/10.1111/j.1365-2427.2008.02029.x

Sereda, J.M., Hudson, J.J., Taylor, W.D., Demers, E., 2008b. Fish as sources and sinks of nutrients in lakes. Freshw. Biol. 53, 278–289. https://doi.org/10.1111/j.1365-2427.2007.01891.x

Sharpley, A., Foy, B., Withers, P., 2000. Practical and Innovative Measures for the Control of Agricultural Phosphorus Losses to Water: An Overview. J. Environ. Qual. 29, 1. https://doi.org/10.2134/jeq2000.00472425002900010001x

Sharpley, A., Jarvie, H.P., Buda, A., May, L., Spears, B., Kleinman, P., 2013. Phosphorus Legacy: Overcoming the Effects of Past Management Practices to Mitigate Future Water Quality Impairment. J. Environ. Qual. 42, 1308–1326. https://doi.org/10.2134/jeq2013.03.0098

Simberloff, D., Gibbons, L., 2004. Now you see them, now you don't! - Population crashes of established introduced species. Biol. Invasions 6, 161–172. https://doi.org/10.1023/B:BINV.0000022133.49752.46

Smith, P.A., Moss, B., Carvalho, L., E, A., Howard, B.J., 1999. Presented at the Institute of Fisheries Management 30th Annual Study Course held at Sparsholt College 1999. Present. Inst. Fish. Manag. 30th Annu. Study Course held Sparsholt Coll. 1999 1–14.

Smith, V.H., Schindler, D.W., 2009. Eutrophication science: where do we go from here? Trends Ecol. Evol. 24, 201–207. https://doi.org/10.1016/j.tree.2008.11.009

Smits, A.J.M., Van Avesaath, Pi.H., Van Der Velde, G., 1990. Germination requirements and seed banks of some nymphaeid macrophytes: *Nymphaea alba* L., *Nuphar lutea* (L.) Sm. and *Nymphoides peltata* (Gmel.) O. Kuntze. Freshw. Biol. 24, 315–326. https://doi.org/10.1111/j.1365-2427.1990.tb00712.x

Smolders, A.J.P., Lamers, L.P.M., Lucassen, E.C.H.E.T., Van Der Velde, G., Roelofs, J.G.M., 2006. Internal eutrophication: How it works and what to do about it—a review. Chem. Ecol. 22, 93–111. https://doi.org/10.1080/02757540600579730

Smolders, A.J.P., Van Riel, M.C., Roelofs, J.G.M., 2000. Accumulation of free amino acids as an early indication for physiological stress (nitrogen overload) due to

elevated ammonium levels in vital *Stratiotes aloides* L. stands. Arch. fur Hydrobiol. 150, 169–175.

Snowden, R.E.D., Wheeler, B.D., 1995. Chemical changes in selected wetland plant species with increasing Fe supply, with specific reference to root precipitates and Fe tolerance. New Phytol. 131, 503–520. https://doi.org/10.1111/j.1469-8137.1995.tb03087.x

Søndergaard, M., Bjerring, R., Jeppesen, E., 2013. Persistent internal phosphorus loading during summer in shallow eutrophic lakes. Hydrobiologia 710, 95–107. https://doi.org/10.1007/s10750-012-1091-3

Søndergaard, M., Bruun, L., Lauridsen, T., Jeppesen, E., Madsen, T.V., 1996a. The impact of grazing waterfowl on submerged macrophytes: In situ experiments in a shallow eutrophic lake. Aquat. Bot. 53, 73–84. https://doi.org/10.1016/0304-3770(95)01013-0

Søndergaard, M., Jensen, J.P., Jeppesen, E., 2003. Role of sediment and internal loading of phosphorus in shallow lakes. Hydrobiologia 506–509, 135–145. https://doi.org/10.1023/B:HYDR.0000008611.12704.dd

Søndergaard, M., Jensen, P.J., Jeppesen, E., 2001. Retention and Internal Loading of Phosphorus in Shallow, Eutrophic Lakes. Sci. World J. 1, 427–442. https://doi.org/10.1100/tsw.2001.72

Søndergaard, M., Jeppesen, E., Jensen, J.P., Amsinck, S.L., 2005. Water Framework Directive: Ecological classification of Danish lakes. J. Appl. Ecol. 42, 616–629. https://doi.org/10.1111/j.1365-2664.2005.01040.x

Søndergaard, M., Jeppesen, E., Jensen, J.P., Lauridsen, T., 2000. Lake restoration in Denmark. Lakes Reserv. Res. Manag. 5, 151–159. https://doi.org/10.1046/j.1440-1770.2000.00110.x

Søndergaard, M., Jeppesen, E., Lauridsen, T.L., Skov, C., Van Nes, E.H., Roijackers, R., Lammens, E., Portielje, R., 2007. Lake restoration: successes, failures and long-term effects. J. Appl. Ecol. 44, 1095–1105. https://doi.org/10.1111/j.1365-2664.2007.01363.x

Søndergaard, M., Johansson, L.S., Lauridsen, T.L., Jørgensen, T.B., Liboriussen, L., Jeppesen, E., 2010. Submerged macrophytes as indicators of the ecological

quality of lakes. Freshw. Biol. 55, 893–908. https://doi.org/10.1111/j.1365-2427.2009.02331.x

Søndergaard, M., Liboriussen, L., Pedersen, A.R., Jeppesen, E., 2008. Lake restoration by fish removal: Short- and long-term effects in 36 Danish lakes. Ecosystems 11, 1291–1305. https://doi.org/10.1007/s10021-008-9193-5

Søndergaard, M., Windolf, J., Jeppesen, E., 1996b. Phosphorus fractions and profiles in the sediment of shallow Danish lakes as related to phosphorus load, sediment composition and lake chemistry. Water Res. 30, 992–1002. https://doi.org/10.1016/0043-1354(95)00251-0

Soomers, H., Winkel, D.N., Du, Y., Wassen, M.J., 2010. The dispersal and deposition of hydrochorous plant seeds in drainage ditches. Freshw. Biol. 55, 2032–2046. https://doi.org/10.1111/j.1365-2427.2010.02460.x

Sorenson, J.C., 1948. A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. K. Danske Vidensk. Selsk. 5, 1–34.

Spears, B.M., Andrews, C., Banin, L., Carvalho, L., Cole, S., De Ville, M., Gunn, I.D.M., Ives, S., Lawlor, A., Leaf, S., Lofts, S., Maberly, S.C., Madgwick, G., May, L., Moore, A., Pitt, J., Smith, R., Waters, K., Watt, J., Winfield, I.J., Woods, H., 2018. Assessment of sediment phosphorus capping to control nutrient concentrations in english lakes. Environment Agency Report Number SC120064/R9.

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. https://doi.org/10.1007/s10750-011-0921-z

Spears, B.M., Gunn, I.D.M., Carvalho, L., Winfield, I.J., Dudley, B., Murphy, K., May, L., 2009. An evaluation of methods for sampling macrophyte maximum colonisation depth in Loch Leven, Scotland. Aquat. Bot. 91, 75–81. https://doi.org/10.1016/j.aquabot.2009.02.007

Spears, B.M., Jones, I.D., 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. https://doi.org/10.1007/s10750-010-0188-9

Spears, B.M., Lürling, M., Yasseri, S., Castro-Castellon, A.T., Gibbs, M., Meis, S., McDonald, C., McIntosh, J., Sleep, D., van Oosterhout, F., 2013a. Lake responses following lanthanum-modified bentonite clay (Phoslock®) application: An analysis of water column lanthanum data from 16 case study lakes. Water Res. 47, 5930–5942. https://doi.org/10.1016/j.watres.2013.07.016

Spears, B.M., Maberly, S.C., Pan, G., Mackay, E., Bruere, A., Corker, N., Douglas, G., Egemose, S., Hamilton, D., Hatton-Ellis, T., Huser, B., Li, W., Meis, S., Moss, B., Lürling, M., Phillips, G., Yasseri, S., Reitzel, K., 2014. Geo-engineering in lakes: A crisis of confidence? Environ. Sci. Technol. 48, 9977–9979. https://doi.org/10.1021/es5036267

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., 2016. A meta-analysis of water quality and aquatic macrophyte responses in 18 lakes treated with lanthanum modified bentonite (Phoslock®). Water Res. 97, 111–121. https://doi.org/10.1016/j.watres.2015.08.020

Spears, B.M., Meis, S., Anderson, A., Kellou, M., 2013b. 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.

Stobbe, A, Gregor, T., Röpke, A., 2014. Long-lived banks of oospores in lake sediments from the Trans-Urals (Russia) indicated by germination in over 300 tears old radiocarbon dated sediments. Aquatic Botany, 119, 84 - 90. https://doi.org/10.1016/j.aquabot.2014.07.004

Stoler, A., Sudol, K., Mruzek, J., Relyea, R., 2018. Interactive effects of road salt and sediment disturbance on the productivity of seven common aquatic macrophytes. Freshw. Biol. 63, 709–720. https://doi.org/10.1111/fwb.13110

Strand, J.A., Weisner, S.E.B.B., 2001. Dynamics of submerged macrophyte populations in response to biomanipulation. Freshw. Biol. 46, 1397–1408. https://doi.org/10.1046/j.1365-2427.2001.00746.x

Strayer, D.L., Dudgeon, D., 2013. Freshwater biodiversity conservation: recent progress and future challenges. J. North Am. Benthol. Soc. 29, 344–358. https://doi.org/10.1899/08-171.1

Tarvainen, M., Sarvala, J., Helminen, H., 2002. The role of phosphorus release by roach [*Rutilus rutilus* (L.)] in the water quality changes of a biomanipulated lake. Freshw. Biol. 47, 2325–2336. https://doi.org/10.1046/j.1365-2427.2002.00992.x

Tarvainen, M., Ventelä A-M., Helminen, H., Sarvala, J. 2005. Nutrient release and resuspension generated by ruffe (*Gymnocephalus cernuus*) and chironomids. Freshwater Biology. 50, 447 - 458.

Thomas, R., Meybeck, M., Beim, A. 1996. Chapter 7: Lakes, water Quality Assessment - a Guide to Use of biota, Sediments and Water in Envionmental Monitoring- Second Edition. UNESCO/WHO/UNEP

Thompson, K., Grime, J.P., 1979. Seasonal Variation in the Seed Banks of Herbaceous Species in Ten Contrasting Habitats Published by : British Ecological Society Stable URL : http://www.jstor.org/stable/2259220. J. Ecol. 67, 893–921.

Titus, J.E., Grisé, D., Sullivan, G., Stephens, M.D., 2004. Monitoring submersed vegetation in a mesotrophic lake: Correlation of two spatio-temporal scales of change. Aquat. Bot. 79, 33–50. https://doi.org/10.1016/j.aquabot.2003.11.006

Tomlinson, M., Devaney, G., Cook, J., Short, V., Stephenson, S. 2019. The Broads Annual Water Plant Monitoring 2014 – 2018. The Broads Authority, Norwich, UK.

Trémolières, M., 2004. Plant response strategies to stress and disturbance: the case of aquatic plants. J. Biosci. 29, 461–470. https://doi.org/10.1007/BF02712119

Van De Haterd, R.J.W., Ter Heerdt, G.N.J., 2007. Potential for the development of submerged macrophytes in eutrophicated shallow peaty lakes after restoration measures. Hydrobiologia 584, 277–290. https://doi.org/10.1007/s10750-007-0593-x

Van Den Berg, M.S., Coops, H., Simons, J., De Keizer, A., 1998a. Competition between *Chara aspera* and *Potamogeton pectinatus* as a function of temperature and light. Aquat. Bot. 60, 241–250. https://doi.org/10.1016/S0304-3770(97)00099-5

Van Den Berg, M.S., Scheffer, M., Coops, H., Simons, J., 1998b. The role of characean algae in the management of eutrophic shallow lakes. J. Phycol. 34,

750–756. https://doi.org/10.1046/j.1529-8817.1998.340750.x

van Donk, E., Grimm, M.P., Heuts, P.G.M., G., B., Everards, K., van Tongeren, O.F.R., 1994. Mesocosms shallow eutrophic lake restoration measures. Arch. Hydrobiol. Beih.40, 283 - 294

Van Donk, E., Van de Bund, W.J., 2002. Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: Allelopathy versus other mechanisms. Aquat. Bot. 72, 261–274. https://doi.org/10.1016/S0304-3770(01)00205-4

Van Katwijk, M.M., Vergeer, L.H.T., Schmitz, G.H.W., Roelofs, J.G.M., 1997. Ammonium toxicity in eelgrass *Zostera marina*. Mar. Ecol. Prog. Ser. 157, 159–173. https://doi.org/10.3354/meps157159

van Oosterhout, F., Goitom, E., Roessink, I., Lürling, M., 2014. Lanthanum from a modified clay used in eutrophication control is bioavailable to the marbled crayfish (*Procambarus fallax* f. virginalis). PLoS One 9. https://doi.org/10.1371/journal.pone.0102410

van Oosterhout, F., Lürling, M., 2012. The effect of phosphorus binding clay (Phoslock®) in mitigating cyanobacterial nuisance: a laboratory study on the effects on water quality variables and plankton. Hydrobiologia 710, 265–277. https://doi.org/10.1007/s10750-012-1206-x

Van Oosterhout, F., Lürling, M., 2011. Effects of the novel “Flock & Lock” lake restoration technique on Daphnia in Lake Rauwbraken (The Netherlands). J. Plankton Res. 33, 255–263. https://doi.org/10.1093/plankt/fbq092

Van Vierssen, W., 1982. Some notes on the germiantion of seeds of *Najas marina* L. Aquat. Bot. 12, 201–203.

Verdonschot, P., Angeler, D., Boria, A., Brucet, S., Feld, C.K., Gunn, I., Johnson, R., Kail, J., Kernan, M., Marzin, A., May, L., Meis, S., Spears, B., 2011. WISER Deliverable D6.4-2: Report on the differences between cause-effect-recovery chains of different drivers within the water categories.

Verdonschot, P.F.M., Spears, B.M., Feld, C.K., Brucet, S., Keizer-Vlek, H., Borja, A., Elliott, M., Kernan, M., Johnson, R.K., 2013. A comparative review of recovery processes in rivers, lakes, estuarine and coastal waters. Hydrobiologia 704,

453–474. https://doi.org/10.1007/s10750-012-1294-7

Waajen, G., van Oosterhout, F., Douglas, G., Lürling, M., 2016a. Management of eutrophication in Lake De Kuil (The Netherlands) using combined flocculant – Lanthanum modified bentonite treatment. Water Res. 97, 83–95. https://doi.org/10.1016/j.watres.2015.11.034

Waajen, G., van Oosterhout, F., Douglas, G., Lürling, M., 2016b. Geo-engineering experiments in two urban ponds to control eutrophication. Water Res. 97, 69–82. https://doi.org/10.1016/j.watres.2015.11.070

Waajen, G., van Oosterhout, F., Lürling, M., 2017. Bio-accumulation of lanthanum from lanthanum modified bentonite treatments in lake restoration. Environ. Pollut. 230, 911–918. https://doi.org/10.1016/j.envpol.2017.07.046

Wade, P., Edwards, R., 1980. The effect of channel maintenance on the aquatic macrophytes of the drainage channels of the monmouthshire levels, south Wales, 1840-1976. Aquat. Bot. 8, 307–322.

Wang, W., 1991. Ammonia toxicity to macrophytes (common duckweed and rice) using static and renewal methods. Environ. Toxicol. Chem. 10, 1173–1177.

Wang, W., 1988. Site-specific barium toxicity to common duckweed, *Lemna minor*. Aquat. Toxicol. 12, 203–212. https://doi.org/10.1016/0166-445X(88)90023-9

Wang, Y., Ding, S., Wang, D., Sun, Q., Lin, J., Shi, L., Chen, M., Zhang, C., 2017. Static layer: A key to immobilization of phosphorus in sediments amended with lanthanum modified bentonite (Phoslock®). Chem. Eng. J. 325, 49–58. https://doi.org/10.1016/j.cej.2017.05.039

Wauer, G., Teien, H.C., 2010. Risk of acute toxicity for fish during aluminium application to hardwater lakes. Sci. Total Environ. 408, 4020–4025. https://doi.org/10.1016/j.scitotenv.2010.05.033

Welch, E.B., Cooke, G.D., 1999. Effectiveness and longevity of phosphorus inactivation with alum. Lake Reserv. Manag. 15, 5–27. https://doi.org/10.1080/07438149909353948

Welch, E.B., Cooke, G.D., 1995. Internal phosphorus loading in shallow lakes: Importance and control. Lake Reserv. Manag. 11, 273–281. https://doi.org/10.1080/07438149509354208

Welch, E.B., Kelly, T.S., 1990. Internal phosphorus loading and macrophytes: An alternative hypothesis. Lake Reserv. Manag. 6, 43–48. https://doi.org/10.1080/07438149009354694

Weltje, L., Brouwer, A.H., Verburg, T.G., Wolterbeek, H.T., de Goeij, J.J.M., 2002. Accumulation and elimination of lanthanum by duckweed (*Lemna minor* L.) as influenced by organism growth and lanthanum sorption to glass. Environ. Toxicol. Chem. 21, 1483–1489. https://doi.org/10.1002/etc.5620210721

Wetzel, R., 2001. Limnology: lake and river ecosystems. Academic Press.pp 1006

WFD - UKTAG, 2014. UKTAG Lake Assessment Method Macrophytes and Phytobenthos Macrophytes (Lake LEAFPACS2). Stirling.

Wheeler, A.B.D., Cook, R.E.D., 1985. Iron Toxicity to Plants in Base-Rich Wetlands : Comparative Effects on the Distribution and Growth of Epilobium hirsutum L . and Juncus subnodulosus Schrank Reviewed 100, 653–669.

WHO, 2011. Guidelines for Drinking-water Quality. Fourth Edition. https://doi.org/10.1007/springerreference_30502

Wiegleb, G., Brux, H., 1991. Comparison of life history characters of broad-leaved species of the genus Potamogeton L. I. General characterization of morphology and reproductive strategies. Aquat. Bot. 39, 131–146. https://doi.org/10.1016/0304-3770(91)90028-4

Willby, N., Pitt, J.A., Phillips, G., 2012. The ecological classification of UK lakes using aquatic macrophytes. Science Report SC010080/SR2. Bristol, UK.

Willby, N.J., 2007. Managing invasive aquatic plants: problems and prospects. Aquat. Conserv. Mar. Freshw. Ecosyst. 17, 659–665. https://doi.org/10.1002/aqc

Willby, N.J., Pitt, J.A., Phillips, G., 2009. *The ecological classification of UK lakes using aquatic macrophytes*. Report - SC010080/R2.

Wingfield, R., Murphy, K.J., Gaywood, M., 2006. Assessing and predicting the success of *Najas flexilis* (Willd.) Rostk. & Schmidt, a rare European aquatic macrophyte, in relation to lake environmental conditions. Hydrobiologia 570, 79–86. https://doi.org/10.1007/s10750-006-0165-5

Wobbrock, J., Findlater, L., Gergle, D., Higgins, J., 2011. The Aligned Rank

Transformation for Nonparametric Factorial Analysis Using only ANOVA Procedurers. Proc. ACM Conf. Huma Factors Comput. Syst. 143–146.

Wolterbeek, H.T., Van Der Meer, A.J.G.M., 1996. A sensitive and quantitative biosensing method for the determination of γ-ray emitting radionuclides in surface water. J. Environ. Radioact. 33, 237–254. https://doi.org/10.1016/0265-931X(96)00003-3

Xiao, C., Dou, W. feng, Liu, G. hua, 2010. Variation in vegetation and seed banks of freshwater lakes with contrasting intensity of aquaculture along the Yangtze River, China. Aquat. Bot. 92, 195–199. https://doi.org/10.1016/j.aquabot.2009.11.007

Xu, Q., Fu, Y., Min, H., Cai, S., Sha, S., Cheng, G., 2012. Laboratory assessment of uptake and toxicity of lanthanum (La) in the leaves of *Hydrocharis dubia* (Bl.) Backer. Environ. Sci. Pollut. Res. 19, 3950–3958. https://doi.org/10.1007/s11356-012-0982-1

Yasseri, S., Epe, T.S., 2016. Analysis of the La:P ratio in lake sediments – Vertical and spatial distribution assessed by a multiple-core survey. Water Res. 97, 96–100. https://doi.org/10.1016/j.watres.2015.07.037

Zaman, T., Asaeda, T., 2013. Effects of NH4-N concentrations and gradient redox level on growth and allied biochemical parameters of *Elodea nuttallii* (Planch.). Flora Morphol. Distrib. Funct. Ecol. Plants 208, 211–219. https://doi.org/10.1016/j.flora.2013.02.009

Zamparas, M., Zacharias, I., 2014. Restoration of eutrophic freshwater by managing internal nutrient loads. A review. Sci. Total Environ. 496, 551–562. https://doi.org/10.1016/j.scitotenv.2014.07.076

Zhang, S., Zhou, Q., Xu, D., Lin, J., Cheng, S., Wu, Z., 2010. Effects of sediment dredging on water quality and zooplankton community structure in a shallow of eutrophic lake. J. Environ. Sci. (China) 22, 218–224.

Zhang, Y., Jeppesen, E., Liu, X., Qin, B., Shi, K., Zhou, Y., Thomaz, S.M., Deng, J., 2017. Global loss of aquatic vegetation in lakes. Earth-Science Rev. 173, 259–265. https://doi.org/10.1016/j.earscirev.2017.08.013

Zimba, P. V., Hopson, M.S., 1997. Quantification of epiphyte removal efficiency from

submersed aquatic plants. Aquat. Bot. 58, 173–179. https://doi.org/10.1016/S0304-3770(97)00002-8

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and extensions in ecology with R. Springer Science and Business Media.

## Appendix 1.

**Table 1**. The recovery time, macrophyte species, response and nutrient concentrations post-remediation measures in lakes.

| Lake | Restoration | Monitoring length (years) | macrophyte response (years) | First species establishment | Response | Notes | Lack of recovery reason | TP | Water clarity | Chl-*a* | Author(s) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Multiple lakes (n=35) (EU) | ELR | 35 | Lake-specific | - | Abundance increase, growing depth, coverage/percent, volume inhabited rate of re-establishment of submerged macrophyte, species richness, | | Internal loading, lack of seeds/turions, waterfowl grazing, benthic algae competition, dominance of species | 37 – 513 (µg L-) in shallow lakes, 4 – 132 (µg $L^{-1}$) | +77% for shallow lakes, +82% for deep lakes | -76% decline in shallow lakes, -64% in deep lakes | Jeppesen et al., (2005) |
| Multiple lakes (n=21) (EU) | ELR | | Lake-specific | *P. pectinatus, P. crispus, P.perfoliatus, Z. palustris* | Species returned, coverage of lake area (%) | | | 59 (µg $L^{1}$) (summer) | 1.17m (summer) (secchi depth) | | Hilt et al., (2018) |
| Lake Fure (D) | ELR | 115 | 45 years | Elodid species, small angiosperms, *Chara* spp. | Species richness, relative abundance (%), vegetation P index | Species richness increased from 12 - 28 | Internal loading, water clarity | | 4m | | Sand-Jensen (2017) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Multiple lakes (n=4) (EU) | ELR | 8 | Lake-specific | *Chara* spp., *Potamogeton* spp., *Z. peduncullata* | Percent volume inhabited, percent coverage (%) | | Internal loading | 64 (µg $L^{1}$) | Significantly increased | | Lauridsen et al., (2003a) |
| Multiple lakes (n=5) (EU) | ELR + B | 8 | 2 - 5 | *E. canadensis, Chara* spp. *P. crispus* | Percent volume inhabited, percent coverage (%) | | Internal lading, grazing | 85 (µg $L^{1}$) | Significantly increased | | Lauridsen et al., (2003a) |
| Lake Veluwe (NL) | ELR | 35 | 10 | *Chara* spp. | Visual assessment, percent cover (%) | > 30% | | 40 – 60 (µg $L^{-1}$) | >1 $m^{-1}$ | <10 (mg $m^{-3}$) | Ibelings et al., (2007) |
| Lake Constance (DE) | ELR | 38 | 3 | Chara spp. | Species richness, abundance (%) | | | < 7 (µg $L^{1}$) | | | Murphy et al., (20180 |
| Lake Müggelsee (DE) | ELR | 100 | 3 - 13 | *Najas marina Zannichellia palustris Potamogeton friesii* | Maximum colonisation depth (m) | *Potamogeton pectinatus* remained through turbid phase and expanded first | | ~ < 90 (µg $L^{1}$) | | | Hilt et al., (2013) |
| Multiple lakes (n=28) (EU) | ILM | | Lake-specific | Chara spp., *C. demersum E. canadensis/nuttallii, N. marina* | Species returned, coverage of lake area (%) | | | 170 (µg $L^{-1}$) (summer) | 1.10m (summer) (secchi depth) | | Hilt et al., (2018) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Multiple lakes (n=70) (EU) | B | 1 - 12 | Lake-specific | *P. crispus* *E. canadensis* | Cover of submerged macrophytes | | Internal loading, insufficient fish removal, sediment re-suspension & humic acids | 0.05 – 1.4 (mg $L^{-1}$) | 50% increase in 14/20 lakes | 21 – 300 (µg $L^{-1}$) | Søndergaard et al., (2007) |
| Multiple lakes (n=18) (NL) | B | | 0.17 - 3 | *Chara* spp. | Surface area coverage (%) | | Insufficient fish removal | | 40 – 800% improvement in secchi depths | < 15 (µg $L^{-1}$)[1] | Meijer et al., (1999) |
| Lake Terra Nova (NL) | B | | 1 | *C. demersum* | Visual assessment, percentage of sampling sites with macrophytes | Submerged species found in 86% of sampling points. All macrophytes lost 4 years after start of measure | High chlorophyll-*a* and suspended matter concentrations | | | | Immers et al., (2014) |
| Mautby Decoy Broad (UK) | D | | 1 - 2 | F. algae, *C. globularis* | Species, abundance | | | | | | Phillips et al., (2015) |
| Ormesby Great Beoad (UK) | D | | 1 | Fil. algae, *P. pusillus,* *P. pectinatus,* | Species, abundance | | | | | | Phillips et al., (2015) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Buckenham Broad (UK) | D | 18 - 34 | Fil. algae, *C. demersum* | Species, abundance | | | | | Phillips et al., (2015) |
| Calthorpe Broad (UK) | D | 1 | *C. hispida* | Species, abundance | | | 52 (µg L-) | | Phillips et al., (2015) |
| Hassingham Broad (UK) | D | 1 | Fil. algae | Species, abundance | | | | | Phillips et al., (2015) |
| Norton's Broad (UK) | D | 1 | Fil. algae | Species, abundance | | | | | Phillips et al., (2015) |
| Strumpshaw Broad (UK) | D | 16 | Fil. Algae, *U. vulgaris*, *N. marina* | Species, abundance | | | | | Phillips et al., (2015) |
| Upton Little Broad (UK) | D | 1 | *C. vulgaris*, *C. contraria* | Species, abundance | | | | | Phillips et al., (2015) |
| Wheatfen Broad (UK) | D | 3 | F. algae | Species, abundance | | | | | Phillips et al., (2015) |
| Lake Terra Nova (NL) | Fe | 2 | *E. nuttallii* | Visual assessment, percentage of sampling sites with macrophytes | Submerged species found in 51% of sampling points. *E. nuttallii* found in 63% of sampling sites | Humic acid interference binding with Fe, continued P influx | 20 (µg L-) | 20% reduction of 5 year average pre-treatment | Immers et al., (2015) |
| Alton Water reservoir (UK) | Fe dosing on inflows + M +B | 16 10 | *Elodea.* spp. | | Increase attributed to roach kill | Internal loading | 50 – 60 (µg $L^{-1}$) | < 10 (µg $L^{-1}$) | Perkins & Underwood, (2002) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| North Halfmoon Lake (C) | Ca | | 1 - 2 | | Dry weight (g $m^{-2}$), maximum colonisation depth (m) | Cover declined by 80%, growing depth decreased by 1m | Internal loading | 164 (µg $L^{-1}$) | | Declined | Reedyk et al., (2001) |
| Lofty Lake (C) | Ca | | 1 - 2 | | Dry weight (g $m^{-2}$), maximum colonisation depth (m) | Cover declined by 50%, growing depth decreased by 0.5m | Internal loading | 78 (µg $L^{1}$) | | Declined | Reedyk et al., (2001) |
| Halfmoon Lake (C) | Ca | 9 | 2 - 4 | *Potamogeton pectinatus Myriophyllum exalbescens Potamogeton richardsonii Potamogeton zosteriformis* | Macrophyte biomass (%) | | Declined by 95% at 2m, declined by 88% at 1m 1 year after first application but then increased in later years | | 2m | | Prepas et al., (2001a) |
| Lake De Kuil (NL) | Fe + FLMB | | 2 | *E. nuttallii, C. vulgaris,* F. algae, | Macrophyte coverage ($m^2$), maximum growing depth (m) | Coverage increased by 1,161 $m^2$ 2 months post-treatment. | | 20 (µg $L^{-1}$) | 5 m | | Waajen et al (2016a) |

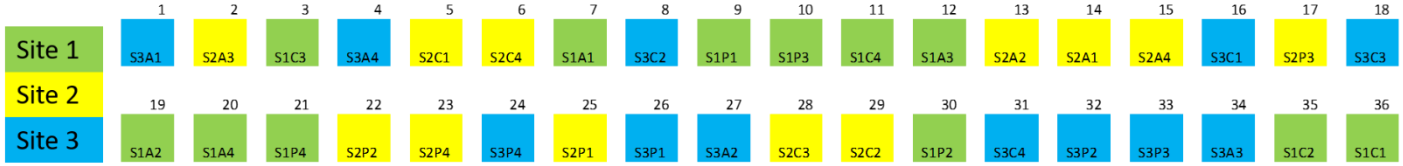
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Macrophyte coverage tripled 1-3 years post-treatment | | | | | |
| Multiple lakes (n=6) (EU) | LMB | | 2 | | Species richness increased, maximum macrophyte growing depth increased | | Humic interference, lack of seeds/turions, waterfowl grazing | 30 (µg $L^{-1}$) | 5.6 m (secchi depth) | 74 (µg $L^{-1}$) | Spears et al., (2016) |
| Loch Flemington (UK) | LMB | 2 | 1 | *C. virgata, A. inundatum* | Species richness, characteristic species, trophic ranking scores, plant ecotype complex, maximum macrophyte growing depth (m) | Remained dominated by *Elodea canadensis* | Non-native invasives present (*Elodea canadensis, Crassula helmsii)* | 27 (µg $L^{-1}$) | 1.4m | 12 (µg $L^{-1}$) | Gunn et al., (2014) |
| Chockyotte irrigation pond (USA) | LMB | | 1 | *Chara* spp. | Surface area growth (%) | < 5% coverage | | 37.3 (µg $L^{-1}$) | 1.8 m | | Bishop & Richardson (2018) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Hickory Meadows irrigation pond (USA) | LMB | 1 | Fil. algae | Percent abundance (%) | ~ 85% | 101 (µg $L^{-1}$) | 1.6 m | | Bishop & Richardson (2018) |

Lake: EU – Europe, UK – United Kingdom, NL – The Netherlands, D – Denmark, DE – Germany, USA – United States of America, C - Canada

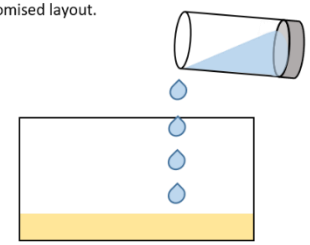
Lake restoration measure: ELR – external phosphorus load reduction, LMB – Lanthanum-modified bentonite, FLM – Flocculant and LMB, B – biomanipulation, D – dredging, ILM – internal lake measures, Fe – iron (III) chloride/ferric dosing, C – $Ca(OH)_2$

## Appendix 2.

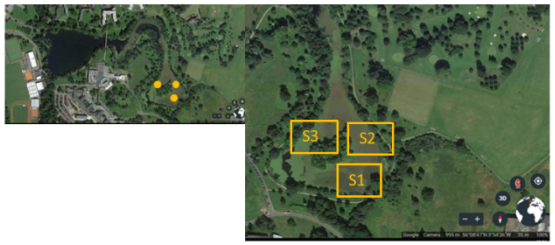



① A randomised germination experimental design with 36 clear containers (17cm(L) x 17cm(W) x 27.5cm(H)) laid out over a single bench in a greenhouse. 4 replicates (4 controls and 4 with LMB addition) with three different sediment collection areas (12 boxes per sediment collection area). An additional 12 boxes were added to the design to account for algal growth impacting macrophyte emergence and establishment in the control treatment after the initial set-up week at week 6 as all control containers contained algal growth. Algal treatment commenced to account for potential biased emergence amongst LMB treated boxes as algal growth was noticeably lower amongst this treatment. Daylight and temperature mimicked outside summer temperature by +/- 2-3°C.

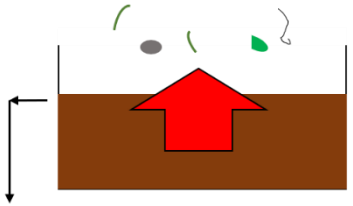
② Aquarium sand was sterilised to give a depth in each container of ~4cm (~1,156cm³). Sand was sterilised by placing enough sand into a container, pouring over 1.7L of boiling water, mixing thoroughly for 10 minutes and then pouring away the water. This was repeated 3 times to ensure sand was washed thoroughly and to ensure that seeds, if present, were no longer viable. Sterilised sand was transferred to a sterilised container with a lid. The containers contained a sand layer of ~4cm to allow for adequate growing depths for submerged aquatic macrophytes. Lids were then placed onto each container to prevent damage in transit and were transported to the greenhouse where they were placed on a bench in a randomised layout.

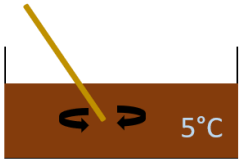


③ Sediment from Airthrey Loch was collected on 21/03/2016 before spring macrophyte emergence. Sediment was randomly sampled from 3 different areas (S1, S2, S3) within the south-eastern arm of the loch. Sediment was collected by hand using a 2m long 4.5 Ø core. Approximately 40 cores were taken to a depth of approx. 5cm depth at each sediment collection location. Sediment from each collection location was placed into a large container and stored at 4°C in the dark a few hours after collection and remained in these conditions until processing of sediment the following day.

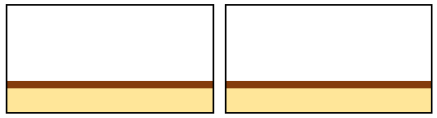



④ Whilst still kept at 4°C as much surface water as possible was removed, if present per container. A 4mm sieve was used to sort the sediment and remove any large stones and any vegetative parts but turions were left in-situ if found. The sieve was used until no more vegetative parts/debris was found.

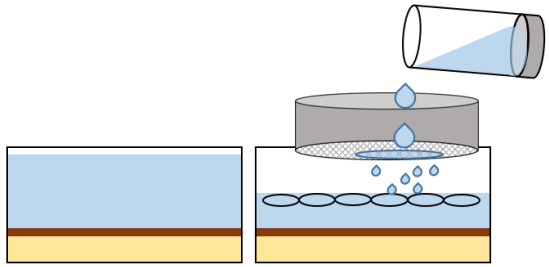





⑤ The sediment from each container was thoroughly mixed and kept at 5°C until ready to begin experimental set-up.

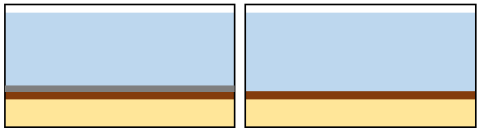


⑥ Container lids were removed from step 2 and ~1 – 1.5cm (~289cm³) of mixed sediment was applied from each sediment collection site into 12 containers on top of the sand (12 boxes per sediment collection site).

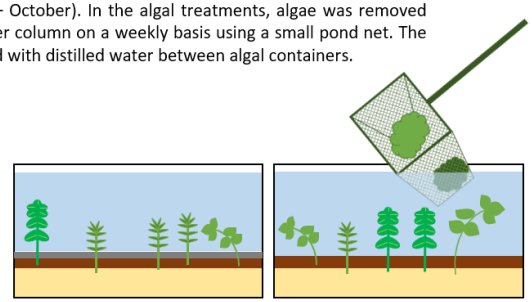


⑦ Before Airthrey Loch water was added to the germination containers, the water was passed through a 2mm sieve to remove any vegetative parts. 8L was then gently added to each container (to a depth of 20cm's) by pouring over bubble wrap to minimise sediment disturbance. Cling film was placed over each container with 5 air holes to prevent excessive evaporation, allow gas exchange and prevent debris falling into containers. The containers were then left to settle for 24 hours.

⑧ After leaving the containers for 24 hours to settle, 12 containers from each sediment collection site were randomly assigned a treatment; control, LMB addition or algal removal. LMB was applied with a dose of 14.7g to the containers that were part of this treatment. The dose was calculated from published doses in Spears et al., (2016). The upper quantile was taken from these published doses and applied to the containers which equated to a dose of 5.1 T/ha. LMB was applied by taking some water out of each container, mixing it with LMB, then returning it to the container by applying over the surface of the water.



⑨ The experiment was left to run for the growing season for 21 weeks (June – October). In the algal treatments, algae was removed from the water column on a weekly basis using a small pond net. The net was rinsed with distilled water between algal containers.



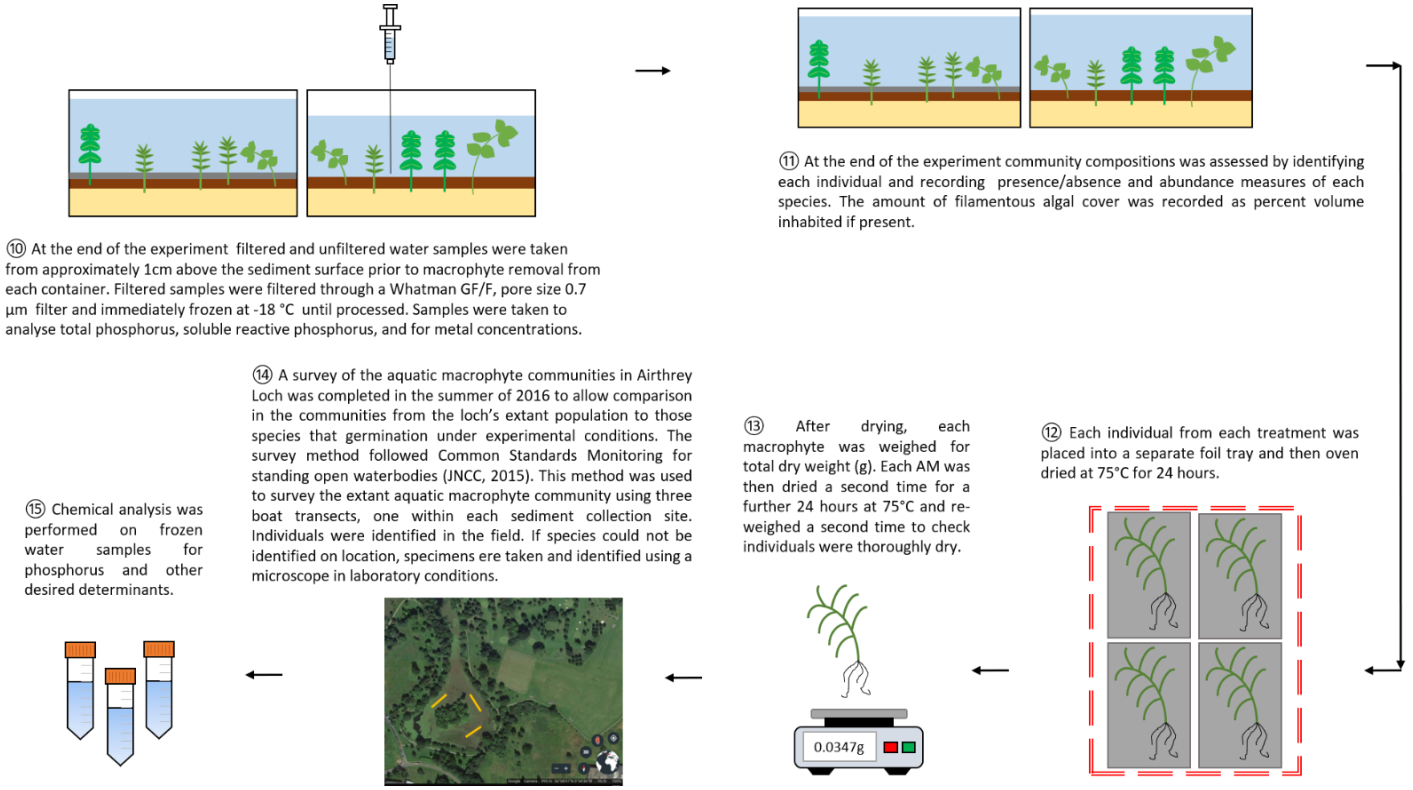



**Figure 1.** Experimental design for the germination experiment used to assess LMB impact on germination success. Airthrey Loch map taken from Google maps (2019) (© Kate Waters-Hart).

## Appendix 2.1. Water quality analysis

### *2.1.1. Phosphorus concentrations in water*

Water samples for the determination of soluble reactive phosphorus (SRP) (the bioavailable portion of dissolved P) and total phosphorus (TP) (all inorganic and organic forms of P) were collected at the beginning and end of the experiment. Samples for SRP were filtered through a 0.7 µm Whatman GF/F filter prior to freezing along with unfiltered samples for TP at -18°C.

Prior to the determination of TP, 5 ml of each sample was digested using potassium persulphate ($K_2S_2O_8$) acid hydrolysis digestion (Eisenreich et al., 1975) to convert all forms of P into ortho-P. 0.1 mg $L^{-1}$ of 30% sulphuric acid ($H_2SO_4$) and 0.5 mg $L^{-1}$ of potassium persulphate were added to each sample. Samples were placed in an autoclave and heated to 120°C for 30 minutes.

Filtered and digested samples were determined using the acid-molybdenum-blue colorimetric method (Murphy and Riley, 1962). For filtered and digested samples, in order to determine colourimetry reactions of ortho-P in water, the reaction of ortho-P with ammonium molybdate ($(NH_4)_6Mo_7O_{24}.H_2O$) and potassium antimonyl tartrate (PAT ($C_8H_4K_2O_{12}Sb_2$) in acid solution ($H_2SO_4$) was required.

During the reaction, a yellow phospho-molybdate complex is formed and was reduced with ascorbic acid ($C_6H_sO_6$) to a stable blue complex: phosphomolybdenum blue. The absorbance of this complex was measured photometricaly at 880 nm using a SEAL AQ2 discrete analyser (SEAL Analytical, US) following the EPA-118-A Rev. 5 method (USEPA 600/R 93/100) for SRP and the EPA-119-C Rev 1A method (EPA 600/ R 93/100) for TP. Both PAT and ascorbic acid were automatically added to digested samples by the auto-analyser. Limits of detection for SRP and TP were 20 µg P $L^{-1}$.

### *2.1.2. Metal concentrations in water*

Water samples for total metal analysis were only collected post-treatment. Water collected for dissolved metal analysis was filtered through 0.7 µm Whatman GF/F filter prior to freezing at -18°C. Samples were defrosted and preserved with 2% Nitric Acid ($HNO_3$). A subset of samples were tested to meet acidic conditions (had a pH ≤ 2). Samples were then digested for 16 hours at 80°C in polypropylene centrifuge tubes and then kept at 4°C prior to analysis.

Concentrations of total metals were determined using Inductively Coupled

Plasma Mass Spectrometry (ICP-MS) using an Agilent 7500ce (with octupole reaction system) employing an RF forward power of 1540 W and reflected power of 1 W, with argon gas flows of 0.81 L $min^{-1}$ and 0.22 L $min^{-1}$ for carrier and makeup flows, respectively. 1.4 mg $L^{-1}$ of sample and standards were spiked with an internal standard (20 µg $L^{-1}$ of 10 mg $L^{-1}$ Rhodium solution) internal standard to account for drift. Sample solutions were taken up into the micro mist nebuliser by a peristaltic pump at a rate of approximately 1.2 mL $min^{-1}$.

The instrument was operated in spectrum acquisition mode and the samples were run in triplicate. The masses analysed for were: $^{44}Ca$, $^{56}Fe$, $^{55}Mn$, $^{137}Ba$, $^{139}La$, $^{141}Pr$ and $^{146}Nd$. Each mass was analysed in fully quant mode (three points per unit mass) and analysed in either standard 'no gas' mode or 'Helium mode' (Appendix 2.1, Table 1) depending on whether correction was required for an interfering polyatomic ion. We used a multi-element standard (ICP multi-element standard solution VI 6% $NHO_3$) for Ca (1000 mg $L^{-1}$- 100000 µg $L^{-1}$), Fe (100 mg $L^{-1}$ – 10000 µg $L^{-1}$), Mn (10 mg $L^{-1}$- 1000 µg $L^{-1}$) and Ba (10 mg $L^{-1}$- 1000 µg $L^{-1}$) and a rare earth element standard (0 - 100 µg $L^{-1}$ prepared from combining rare earth element standards (100 mg $L^{-1}$ 2% $HNO_3$)) for assessing La, Pr and Nd concentrations. Replicate samples were analysed randomly to check the reproducibility of the analysis. Detection limits for Ca, Fe, Mn, Ba, La, Pr and Nd were 67.80, 0.53, 1.10, 1.28, 0.12, 0.04, 0.04 µg $L^{-1}$, respectively.

**Table 1.** Ion lenses and quadrupole parameters for no gas mode and helium mode.

| | No gas mode | Helium gas mode (Helium: 6.5 ml $Min^{-1}$) |
|---|---|---|
| Extract 1 (V) | 0 | 0 |
| Extract 2 (V) | -131 | -131 |
| Omega Bias-ce (V) | -20 | -20 |
| Omega Lens (V) | 0 | 0 |
| Cell Entrance (V) | -30 | -30 |
| QP focus (V) | 3 | -12 |
| Cell Exit (V) | -34 | -56 |
| OctP Bias (V) | -6 | -20 |
| QP Bias (V) | -3 | -15 |

## Appendix 2.2

**Table 1.** Mean and standard errors of total and soluble nutrients and metals at the end of the germination experiment for each sediment collection site (Site 1 (S1), Site 2 (S2) and Site 3 (S3)) and treatment group for soluble reactive phosphorus (SRP), total phosphorus (TP) and total lanthanum (TLa) concentrations post-treatment (LOD for TP and SRP = < 20 µg $L^{-1}$; LOD for La = < 0.12 µg $L^{-1}$).

| Measured variables | Control | | | LMB | | | Algal removal | | |
|---|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S1 | S2 | S3 | S1 | S2 | S3 |
| TP (µg $L^{-1}$) | 74.5 ± 9.5 | 54.4 ± 5.4 | 95.5 ± 5.6 | 63.6 ± 18.0 | 50.6 ± 11.5 | 68.4 ± 27.5 | 72.4 ± 6.5 | 68.4 ± 15.8 | 88.1 ± 50.3 |
| SRP (µg $L^{-1}$) | <LOD | <LOD | <LOD | 35.5 ± 21.5 | 21.5 ± 8.7 | 26.0 ± 12.5 | <LOD | <LOD | 24.3 ± 10.7 |
| TLa (µg $L^{-1}$) | <LOD | <LOD | <LOD | 36.6 ± 2.15 | 36.6 ± 4.8 | 73.3 ± 83.7 | - | - | - |

**Table 2.** Results of two-way ANOVA to assess the difference between treatments (control (C) and lanthanum-modified bentonite (LMB)) with sediment collection site (1, 2 and 3) for total phosphorus (TP), soluble reactive phosphorus (SRP) and total lanthanum (TLa) concentrations.

| Variable | C and LMB | | | Sediment collection site | | | Treatment: C and LMB * Sediment collection site | | |
|---|---|---|---|---|---|---|---|---|---|
| | F | *P* | Df | F | *P* | Df | F | *P* | Df |
| TP (µg $L^{-1}$) | 0.651 | 0.430 | 1 | 2.344 | 0.125 | 2 | 0.358 | 0.704 | 2 |
| SRP (µg $L^{-1}$) (K) | 8.766 | **<0.01** | 1 | 0.903 | 0.637 | 2 | 2.133 | 0.221 | 2 |
| TLa (µg $L^{-1}$) (K) | 19.734 | **<0.0001** | 1 | 0.074 | 0964 | 2 | 3.223 | 0.095 | 2 |

K: Non-parametric Kruskal Wallis test

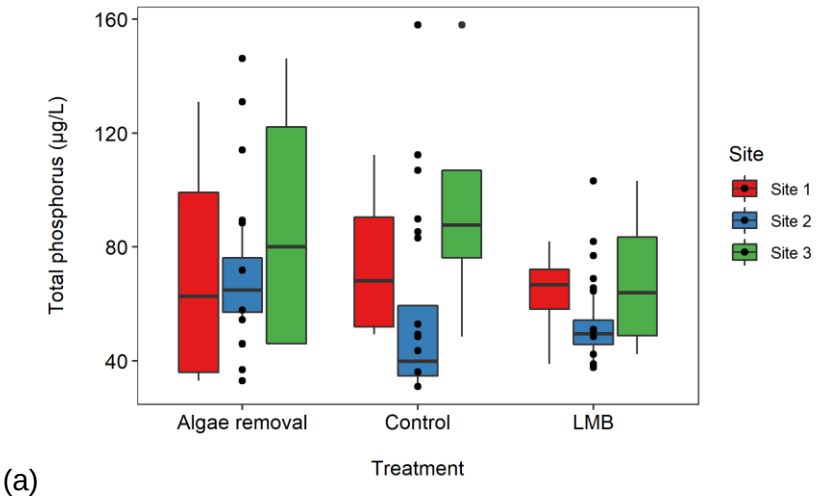
**Table 3.** Results of two-way ANOVA to assess the difference between treatments (control (C) and algae removal (A)) across sediment collection sites (1, 2 and 3) for total phosphorus (TP) concentrations and soluble reactive phosphorus (SRP).

| Variable | C and A | | | Sediment collection site | | | Treatment: C and A * Sediment collection site | | |
|---|---|---|---|---|---|---|---|---|---|
| | **F** | ***P*** | **Df** | **F** | ***P*** | **Df** | **F** | ***P*** | **Df** |
| TP (µg $L^{-1}$) | 0.009 | 0.927 | 1 | 1.228 | 0.316 | 2 | 0.163 | 0.851 | 2 |
| SRP (µg $L^{-1}$) (K) | 1.300 | 0.254 | 1 | 8.131 | **0.05** | 2 | 1.620 | 0.254 | 2 |

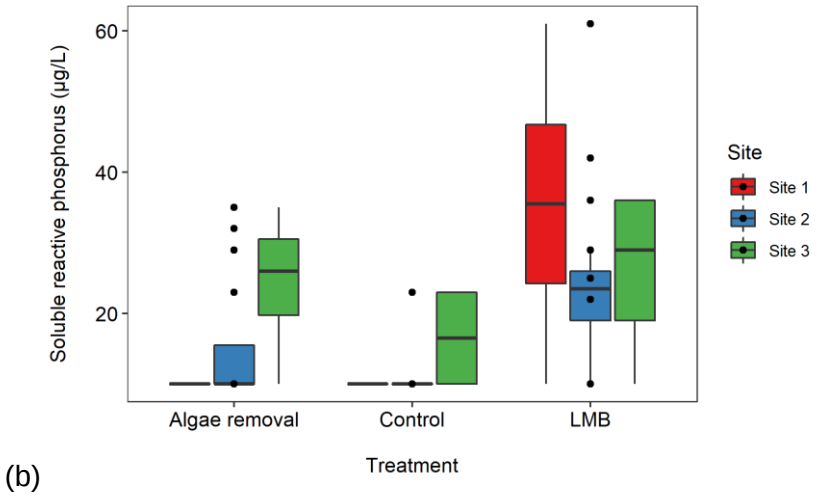
K: Non-parametric Kruskal Wallis test

**Table 4.** Mean and standard errors of measured macrophyte determinants at the end of the germination experiment for each sediment collection site (Site 1 (S1), Site 2 (S2) and Site 3 (S3)) and treatment group for macrophyte species richness, macrophyte dry weight, macrophyte percent volume inhabited (PVI), *Spirogyra* in the water column, *Spirogyra* on the bed sediments, other filamentous algae in the water column, other filamentous algae on the bed sediments and total combined algae as PVI.

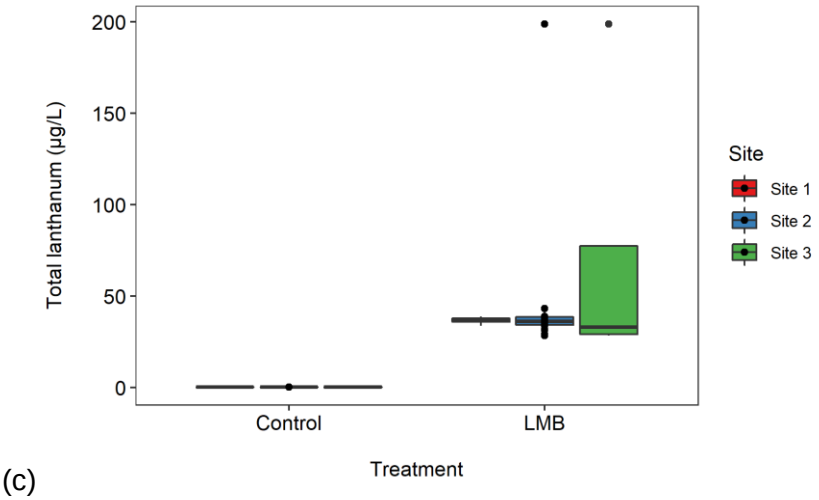
| Measured variables | Control | | | LMB | | | Algal removal | | |
|---|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S1 | S2 | S3 | S1 | S2 | S3 |
| Macrophyte species richness (N) | 1 ± 1.4 | 0.3 ± 0.5 | 1 ± 1.4 | 0.5 ± 1 | 0.3 ± 0.5 | 0.3 ± 0.5 | 1.3 ± 1.89 | 0.5 ± 0.6 | 0.3 ± 0.5 |
| Macrophyte dry weight (g) | 0 ± 0 | 0.11 ± 0.13 | 0 ± 0 | 0.01 ± 0.02 | 0.09 ± 0.17 | 0.21 ± 0.29 | 0.03 ± 0.06 | 0.02 ± 0.05 | 0.01 ± 0.01 |
| Macrophyte biomass (PVI) | 1 ± 0 | 0.3 ± 0.5 | 0.3 ± 0 | 0 ± 0 | 13.5 ± 26.3 | 36.5 ± 41.6 | 8 ± 16 | 0 ± 0 | 0.3 ± 0.5 |
| Spirogyra water column biomass (PVI) | 5 ± 5.8 | 5.8 ± 5.1 | 25 ± 30 | 0 ± 0 | 0 ± 0 | 7.5 ± 2.9 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| Spirogyra bottom sediments biomass (PVI) | 2 ± 2.5 | 24.5 ± 34.8 | 65 ± 41.4 | 0 ± 0 | 0 ± 0 | 8.3 ± 7.9 | 3.8 ± 4.8 | 9.3 ± 7.2 | 15.8 ± 18.4 |
| Filamentous algae water column biomass (PVI) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 2.5 ± 5 | 1.3 ± 1.3 | 0 ± 0 | 0.8 ± 0.8 |
| Filamentous algae bottom sediments biomass (PVI) | 50 ± 57.7 | 12.5 ± 2.5 | 0 ± 0 | 3.8 ± 7.5 | 0 ± 0 | 3.8 ± 7.5 | 56.3 ± 42.7 | 8 ± 5.4 | 22.5 ± 38.6 |
| Total combined algae biomass (PVI) | 57 ± 49.7 | 42.8 ± 33.4 | 90 ± 54.9 | 3.8 ± 7.5 | 0 ± 0 | 22 ± 11.2 | 60 ± 40.2 | 17.3 ± 10.3 | 39 ± 31.5 |




(a)



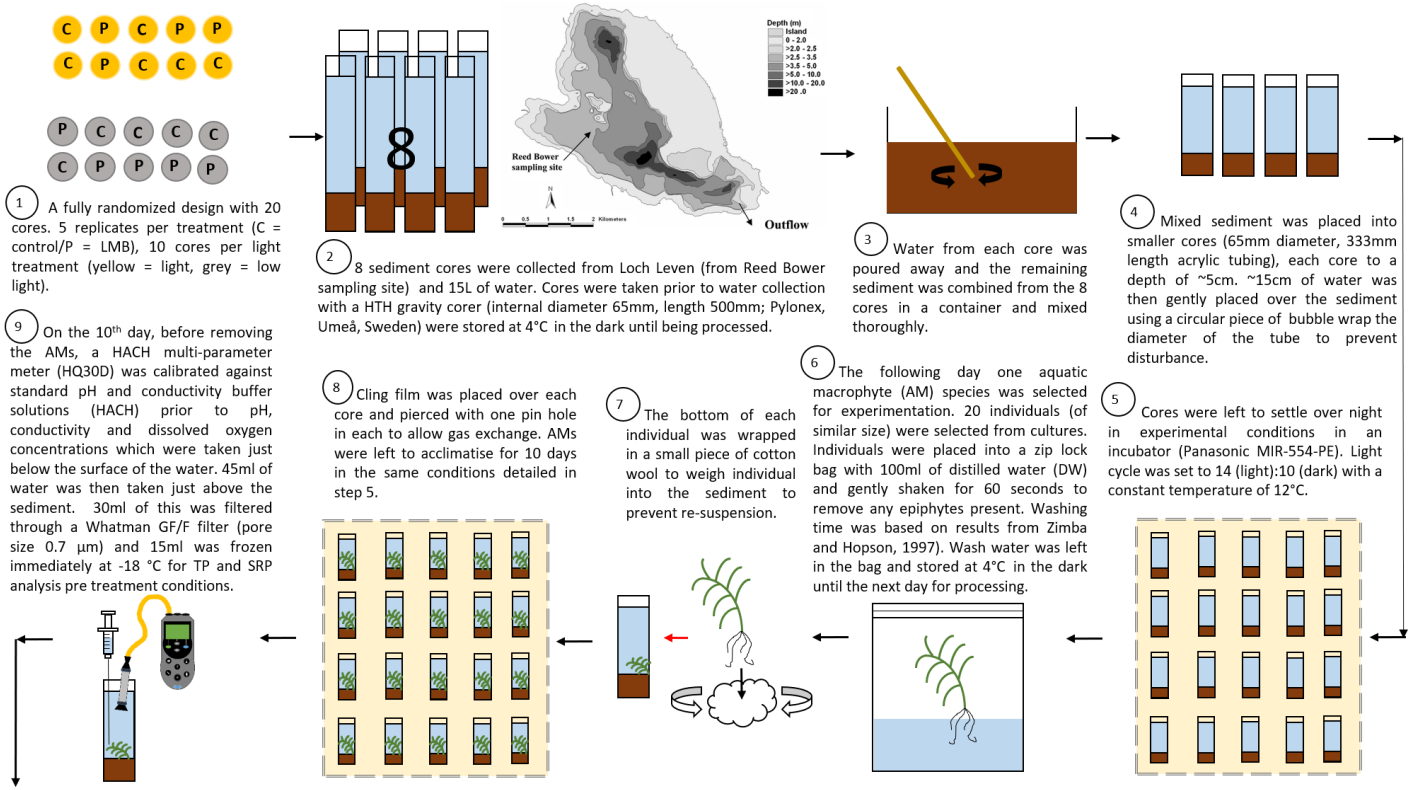

(b)




(c)

**Figure 1.** Total phosphorus (a), soluble reactive phosphorus (b) and total lanthanum (c) concentrations at the end of the germination experiment for sediment collection site and treatment.

# Appendix 3.




1 A fully randomized design with 20 cores. 5 replicates per treatment (C = control/P = LMB), 10 cores per light treatment (yellow = light, grey = low light).

2 8 sediment cores were collected from Loch Leven (from Reed Bower sampling site) and 15L of water. Cores were taken prior to water collection with a HTH gravity corer (internal diameter 65mm, length 500mm; Pylonex, Umeå, Sweden) were stored at 4°C in the dark until being processed.

3 Water from each core was poured away and the remaining sediment was combined from the 8 cores in a container and mixed thoroughly.

4 Mixed sediment was placed into smaller cores (65mm diameter, 333mm length acrylic tubing), each core to a depth of ~5cm. ~15cm of water was then gently placed over the sediment using a circular piece of bubble wrap the diameter of the tube to prevent disturbance.

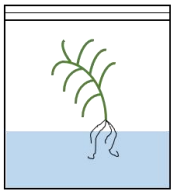
5 Cores were left to settle over night in experimental conditions in an incubator (Panasonic MIR-554-PE). Light cycle was set to 14 (light):10 (dark) with a constant temperature of 12°C.

6 The following day one aquatic macrophyte (AM) species was selected for experimentation. 20 individuals (of similar size) were selected from cultures. Individuals were placed into a zip lock bag with 100ml of distilled water (DW) and gently shaken for 60 seconds to remove any epiphytes present. Washing time was based on results from Zimba and Hopson, 1997). Wash water was left in the bag and stored at 4°C in the dark until the next day for processing.

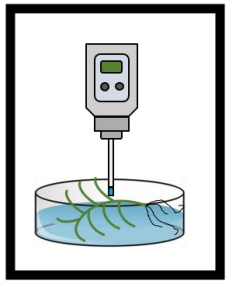
7 The bottom of each individual was wrapped in a small piece of cotton wool to weigh individual into the sediment to prevent re-suspension.

8 Cling film was placed over each core and pierced with one pin hole in each to allow gas exchange. AMs were left to acclimatise for 10 days in the same conditions detailed in step 5.

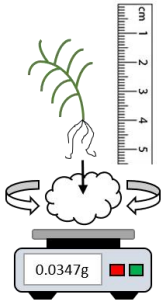
9 On the 10th day, before removing the AMs, a HACH multi-parameter meter (HQ30D) was calibrated against standard pH and conductivity buffer solutions (HACH) prior to pH, conductivity and dissolved oxygen concentrations which were taken just below the surface of the water. 45ml of water was then taken just above the sediment. 30ml of this was filtered through a Whatman GF/F filter (pore size 0.7 µm) and 15ml was frozen immediately at -18 °C for TP and SRP analysis pre treatment conditions.



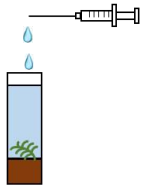
10 Each AM was removed from each core and placed in a zip lock bag with 100ml of DW and gently shake for 60 seconds to remove any epiphytes. Wash water was left in the bag and stored at 4°C in the dark until the next day for processing.



11 Each AM was removed from the zip lock bag and placed in a blacked petri dish with some DW in dark conditions, covered with a black lid and dark adapt for 5 minutes to allow a non-stresses state (Maxwell and Johnson, 2000). A Quantum Yield (QY) of photosystem II (PSII) measurement was then taken using an AquaPen – P AP-P 100 (Photon Systems, Drásov, Czech Republic).

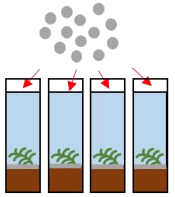



12 Root and shoot lengths (cm) were measured (longest apical shoot/root). AM's were then placed into a 4mm sieve and gently shaken for 2 minutes to remove excess water. Wet weight (total (g)) was then measured. Roots of each AM was again wrapped in a small amount of cotton wool to add weight and then were placed back into each core.

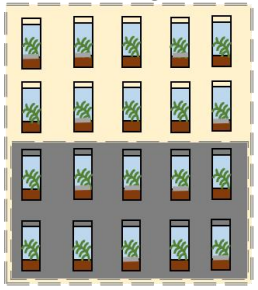


13 45ml of like water was replenished back to core with water stored in the dark at 4°C taken from Loch Leven on the same day original water and sediment cores were collected.

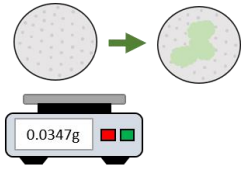
14 Cores were then randomly marked by treatment (see step 1) and half the cores were dosed with 1.6g of LMB (calculated from the 75th percentile of LMB treated lakes data (Spears et al., 2016). LMB was mixed with some of each core's water and then dispensed as a slurry into the core. Cling film was then re-applied to each core.



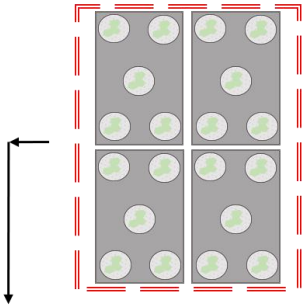
15 All light treatment cores were placed back into the incubator in the same conditions as the acclimatisation period in step 5 and 8. For the dark treatment, cores were placed into a heavy duty black refuge bag to create dark conditions, similar to those at a water depth of 3.5m at Loch Leven. Random pin holes punctured the bag to allow gas exchange. Cores were then placed into the same incubator as the light treatment, in the same conditions. Cores were then left for 20 nights in these conditions.



16 The following day blank filters (Whatman GF/F, pore size 0.7 µm) were weighed, then AM wash water (step 11) was filtered through them.




17 Filters were placed in foil trays and dried for 24 hours at 75°C and then re-weighed to get epiphyte dry weight.



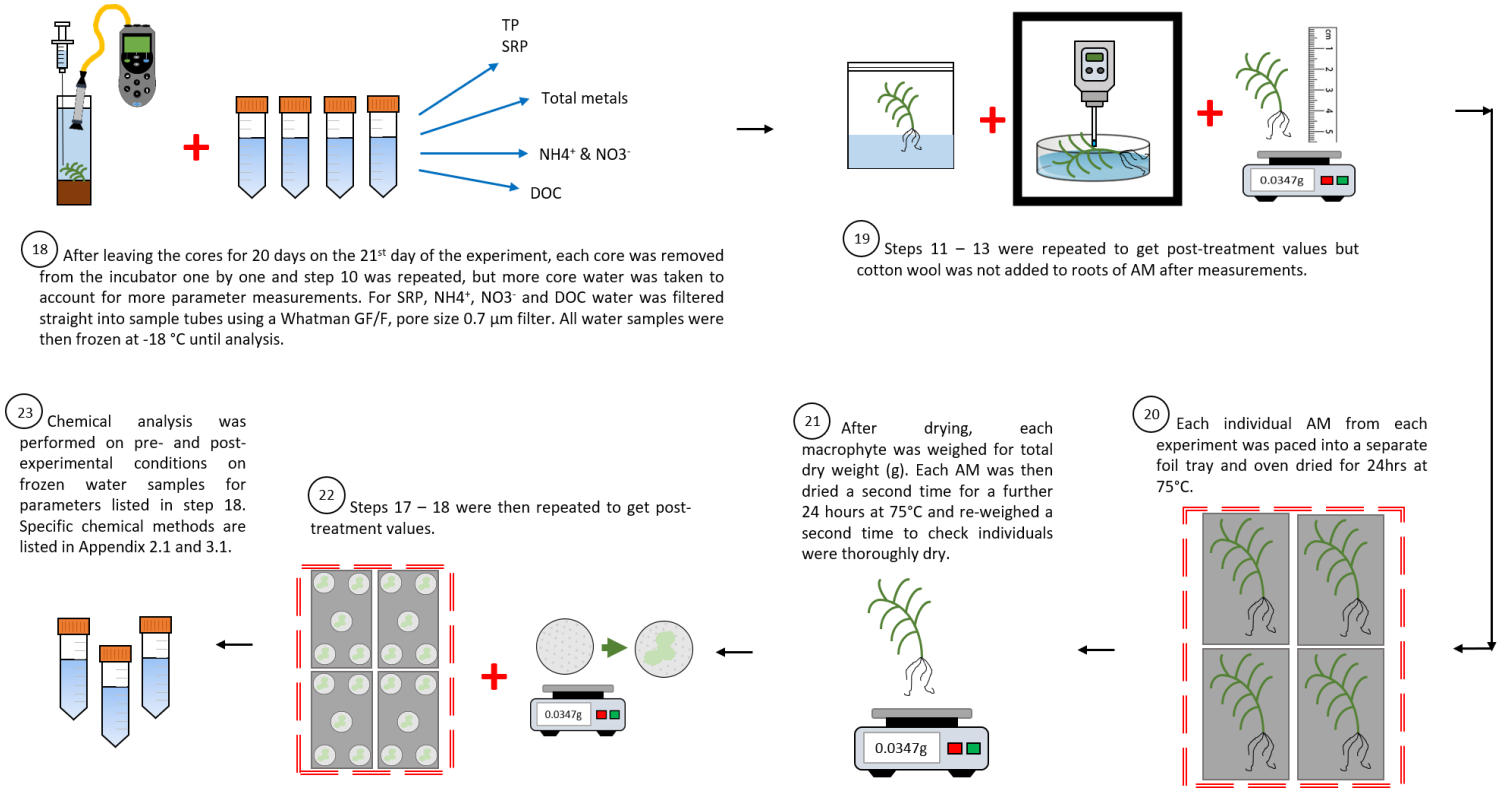



**Figure 1.** Experimental design and method of experimental assays assessing the impact of LMB on different macrophyte species. Loch Leven map taken from Spears et al. (2003) (© Kate Waters-Hart).

### 3.1. Water quality analysis

*3.1.1. Ammonium and nitrate in water*

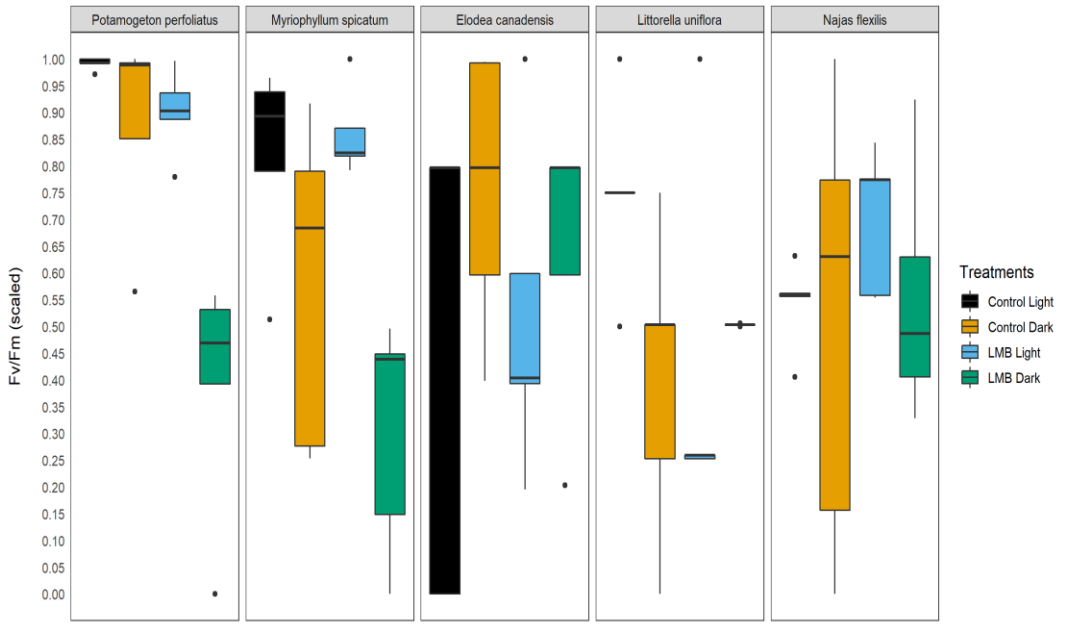
Water samples for ammonium ($NH_4^+$) and nitrate ($NO_3^-$) were only collected post-treatment. Water collected for $NH_4^+$ and $NO_3^-$ analysis was filtered through a 0.7 µm Whatman GF/F filter prior to freezing at -18°C. Concentrations of $NH_4^+$ and $NO_3^-$ were determined using a SEAL AQ2 discrete analyser (SEAL Analytical, US) fitted with cadmium coil following the EPA – 103 – A Rev. 10 for $NH_4^+$ and EPA – 126 – A Rev. 9 (USEPA 600/R 93/100) for $NO_3^-$. Phenol-hypochlorite (for $NH_4^+$) and sulphanilamide ($NO_3^-$ after cadmium coil reduction) where used to deliver the appropriate colorimetry reactions at 660 nm and 520 nm for $NH_4^+$ and $NO_3^-$, respectively. The detection limits for $NH_4^+$ and $NO_3^-$ were 0.004 mg N $L^{-1}$ and 0.01 mg N $L^{-1}$, respectively.

*3.1.2. Dissolved organic carbon in water*

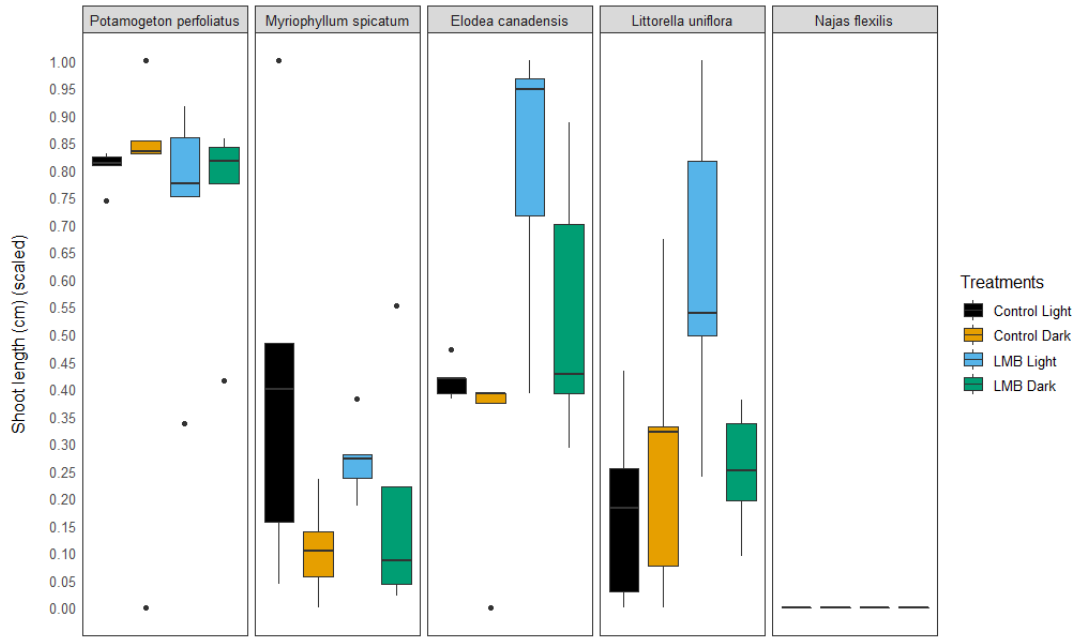
Water samples for dissolved organic carbon (DOC) were only collected post-treatment. Water collected for DOC analysis was filtered through a 0.7 µm Whatman GF/F filter prior to freezing at -18°C. Concentrations of DOC were determined by using a PPM LABTOC analyser (Pollution and Process Monitoring Ltd, UK), with a LOD equivalent to 1% of the calibration standard (50 µg C $L^{-1}$).

**3.2.**

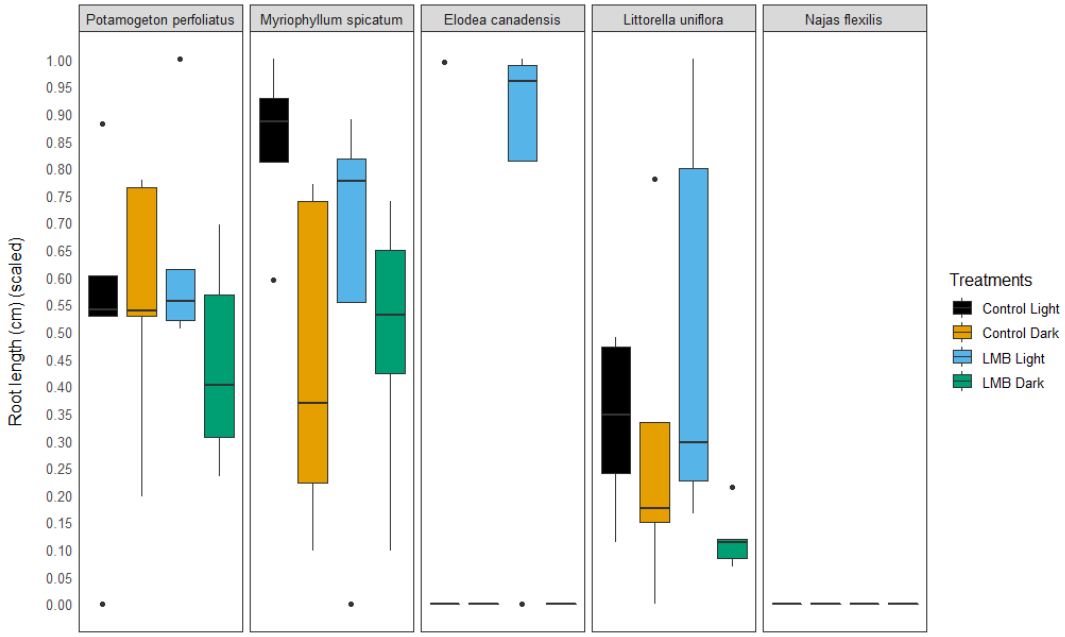
(a)


Potamogeton perfoliatus
Myriophyllum spicatum
Elodea canadensis
Littorella uniflora
Najas flexilis
Fv/Fm (scaled)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Treatments
Control Light
Control Dark
LMB Light
LMB Dark

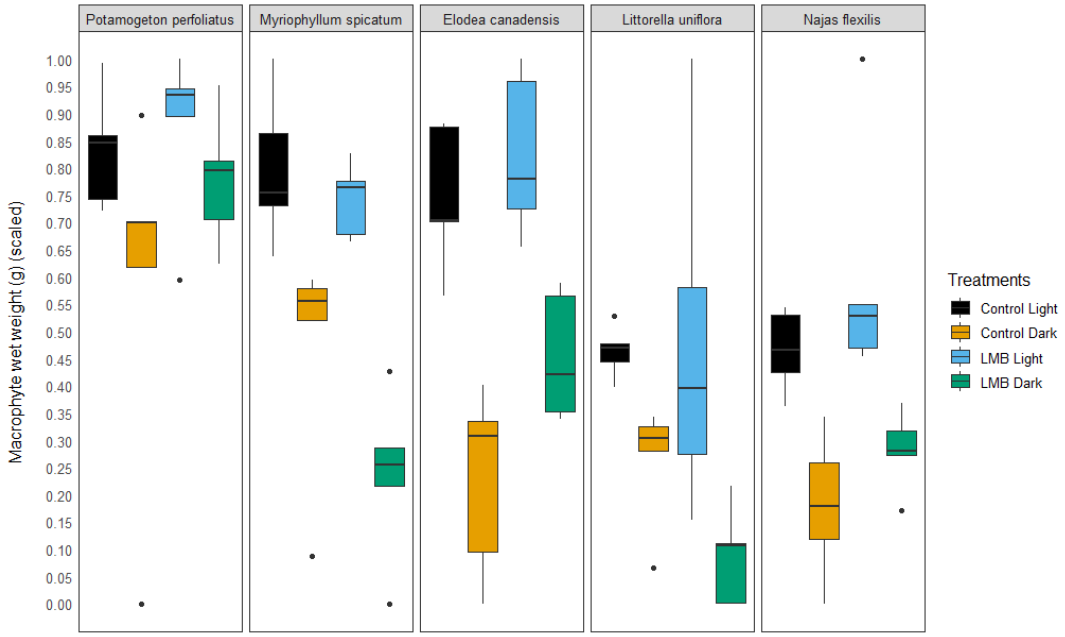
(b)


Potamogeton perfoliatus
Myriophyllum spicatum
Elodea canadensis
Littorella uniflora
Najas flexilis
Shoot length (cm) (scaled)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Treatments
Control Light
Control Dark
LMB Light
LMB Dark

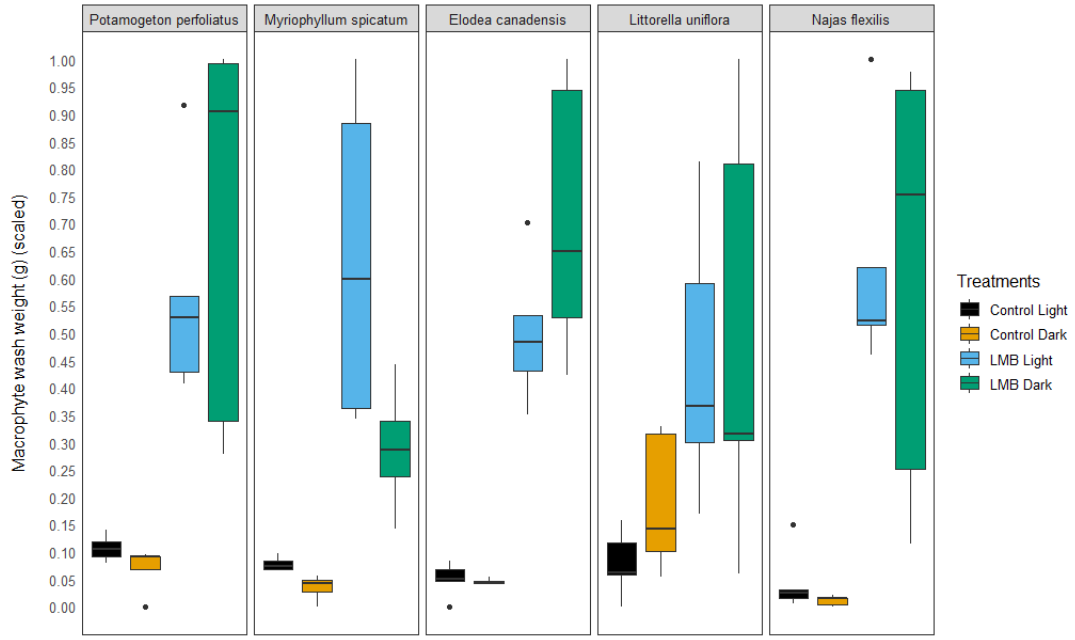
(c)


Potamogeton perfoliatus
Myriophyllum spicatum
Elodea canadensis
Littorella uniflora
Najas flexilis
Root length (cm) (scaled)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Treatments
Control Light
Control Dark
LMB Light
LMB Dark

(d)


Potamogeton perfoliatus
Myriophyllum spicatum
Elodea canadensis
Littorella uniflora
Najas flexilis
Macrophyte wet weight (g) (scaled)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Treatments
Control Light
Control Dark
LMB Light
LMB Dark

(e)




(f)

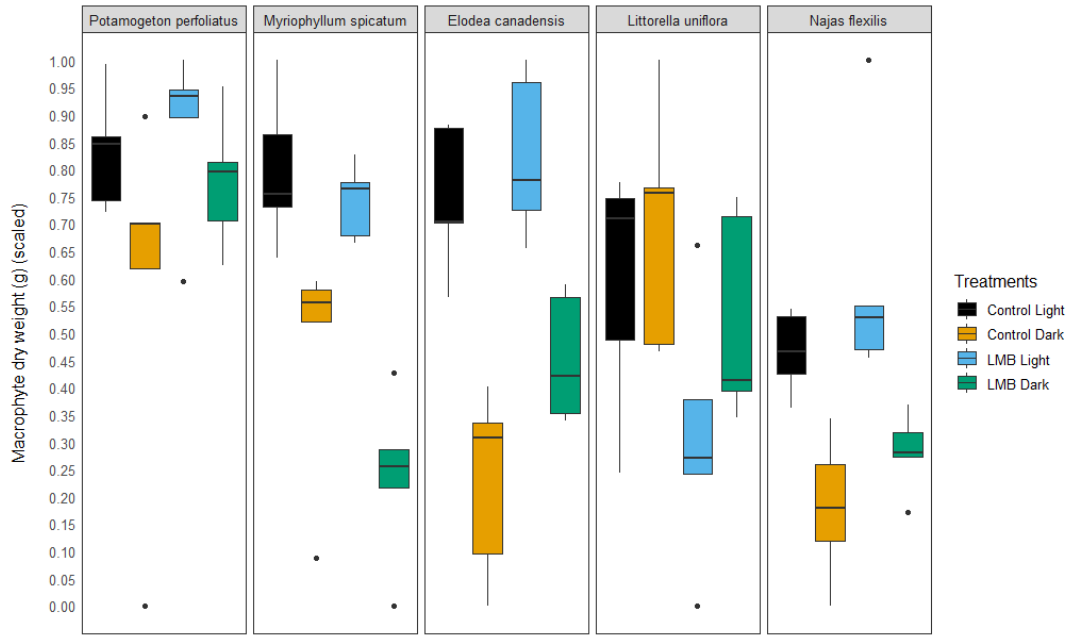



**Figure 1.** Boxplots using delta scaled data to show each macrophyte species response ((a) *Fv/Fm*, (b) shoot length, (c) root length, (d) macrophyte wet weight, (e) macrophyte wash weight and (f) dry weight) following experimental conditions to assess general macrophyte reactions to LMB and reduced light levels.

**Table 1.** Mean and standard deviations for all macrophyte species combined for each measured variable (*Fv/Fm*, shoot length, root length, wet weight, macrophyte wash weight and dry weight).

| **Measured variable** | **Before** | | | | **After** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Light** | | **Dark** | | **Light** | | **Dark** | |
| | **Control** | **LMB** | **Control** | **LMB** | **Control** | **LMB** | **Control** | **LMB** |
| *Fv/Fm* | 0.79 ± 0.02 | 0.79 ± 0.03 | 0.79 ± 0.03 | 0.80 ± 0.03 | 0.69 ± 0.19 | 0.66 ± 0.19 | 0.63 ± 0.25 | 0.52 ± 0.33 |
| Shoot length (cm) | 10.29 ± 4.27 | 11.04 ± 5.14 | 10.48 ± 4.37 | 10.79 ± 4.31 | 10.95 ± 5.02 | 12.60 ± 5.48 | 10.54 ± 4.57 | 11.44 ± 4.68 |
| Root length (cm) | 1.72 ± 2.53 | 0.98 ± 1.53 | 0.92 ± 1.45 | 1.10 ± 2.01 | 4.17 ± 4.15 | 4.16 ± 3.71 | 2.04 ± 3.38 | 1.29 ± 1.59 |
| Wet weight (g) | 0.557 ± 0.338 | 0.602 ± 0.394 | 0.538 ± 0.286 | 0.615 ± 0.321 | 0.729 ± 0.396 | 0.468 ± 0.284 | 0.468 ± 0.284 | 0.592 ± 0.431 |
| Mac wash weight (g) | 0.000 ± 0.001 | 0.000 ± 0.001 | 0.000 ± 0.002 | 0.000 ± 0.001 | 0.001 ± 0.001 | 0.019 ± 0.07 | 0.000 ± 0.0004 | 0.021 ± 0.025 |
| Dry weight (g) | 0.067 ± 0.057 | 0.084 ± 0.079 | 0.056 ± 0.040 | 0.072 ± 0.052 | 0.050 ± 0.032 | 0.058 ± 0.044 | 0.031 ± 0.024 | 0.040 ± 0.024 |

**Table 2.** The mean and standard deviation for individual macrophyte species for each measured growth response (*Fv/Fm*, shoot length, root length, wet weight, macrophyte wash weight and macrophyte dry weight).

| Measured variable | Before | | | | After | | | |
|---|---|---|---|---|---|---|---|---|
| | Light | | Dark | | Light | | Dark | |
| | Control | LMB | Control | LMB | Control | LMB | Control | LMB |
| ***Potamogeton perfoliatus*** | | | | | | | | |
| *Fv/Fm* | 0.80 ± 0.02 | 0.80 ± 0.02 | 0.80 ± 0.02 | 0.81 ± 0.01 | 0.78 ± 0.05 | 0.59 ± 0.15 | 0.61 ± 0.27 | 0.13 ± 0.06 |
| Shoot length (cm) | 11.48 ± 4.58 | 13.14 ± 4.94 | 13.38 ± 3.88 | 13.94 ± 3.40 | 11.36 ± 4.75 | 12.10 ± 4.69 | 13.10 ± 5.99 | 13.40 ± 4.41 |
| Root length (cm) | 4.02 ± 3.07 | 1.94 ± 0.98 | 1.46 ± 1.34 | 3.02 ± 3.18 | 4.70 ± 4.66 | 3.14 ± 1.38 | 2.4 ± 3.38 | 1.86 ± 1.81 |
| Wet weight (g) | 0.76 ± 0.36 | 0.84 ± 0.45 | 0.63 ± 0.18 | 0.87 ± 0.35 | 1.11 ± 0.34 | 1.43 ± 0.81 | 0.66 ± 0.34 | 1.23 ± 0.60 |
| Macrophyte wash weight (g) | 0.0006 ± 0.0012 | 0.0005 ± 0.0005 | 0.0023 ± 0.0036 | 0.0009 ± 0.0009 | 0.0018 ± 0.0015 | 0.0438 ± 0.0192 | 0.0001 ± 0.0002 | 0.0568 ± 0.0340 |
| Macrophyte dried weight (g) | 0.057 ± 0.029 | 0.059 ± 0.041 | 0.037 ± 0.023 | 0.047 ± 0.019 | 0.083 ± 0.035 | 0.093 ± 0.051 | 0.038 ± 0.024 | 0.063 ± 0.024 |
| ***Myriophyllum spicatum*** | | | | | | | | |
| *Fv/Fm* | 0.78 ± 0.03 | 0.77 ± 0.03 | 0.78 ± 0.02 | 0.78 ± 0.02 | 0.33 ± 0.11 | 0.35 ± 0.06 | 0.22 ± 0.13 | 0.11 ± 0.05 |
| Shoot length (cm) | 14.98 ± 1.88 | 15.24 ± 2.04 | 13.72 ± 1.75 | 14.48 ± 2.95 | 17.68 ± 3.74 | 16.82 ± 2.60 | 14.18 ± 1.82 | 15.64 ± 4.36 |
| Root length (cm) | 0.02 ± 0.04 | 0.08 ± 0.13 | 0.14 ± 0.22 | 0 ± 0 | 7.04 ± 2.76 | 4.32 ± 3.07 | 2.68 ± 2.72 | 2.32 ± 1.78 |
| Wet weight (g) | 0.96 ± 0.35 | 1.11 ± 0.16 | 0.93 ± 0.29 | 0.94 ± 0.28 | 1.16 ± 0.27 | 1.29 ± 0.11 | 0.74 ± 0.25 | 0.56 ± 0.22 |
| Macrophyte wash weight (g) | -0.0003 ± 0.0009 | 0.0003 ± 0.0006 | 0.0003 ± 0.0009 | -0.0001 ± 0.0001 | 0.0011 ± 0.0010 | 0.0224 ± 0.0111 | 0.0001 ± 0.0004 | 0.0091 ± 0.0042 |
| Macrophyte dried weight (g) | 0.065 ± 0.022 | 0.094 ± 0.020 | 0.076 ± 0.042 | 0.093 ± 0.028 | 0.079 ± 0.015 | 0.109 ± 0.019 | 0.058 ± 0.028 | 0.055 ± 0.021 |
| ***Elodea canadensis*** | | | | | | | | |
| *Fv/Fm* | 0.80 ± 0.01 | 0.78 ± 0.02 | 0.78 ± 0.01 | 0.80 ± 0.01 | 0.78 ± 0.02 | 0.77 ± 0.01 | 0.78 ± 0.01 | 0.79 ± 0.02 |
| Shoot length (cm) | 9.14 ± 1.44 | 9.40 ± 1.71 | 9.12 ± 1.79 | 8.92 ± 1.56 | 9.38 ± 1.46 | 15.20 ± 5.59 | 8.46 ± 2.11 | 10.74 ± 3.77 |
| Root length (cm) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 2.34 ± 5.23 | 8.20 ± 4.98 | 0 ± 0 | 0 ± 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Wet weight (g) | 0.42 ± 0.06 | 0.45 ± 0.13 | 0.49 ± 0.12 | 0.48 ± 0.17 | 0.58 ± 0.03 | 0.69 ± 0.17 | 0.34 ± 0.12 | 0.44 ± 0.13 |
| Macrophyte wash weight (g) | -0.0002 ± 0.0005 | -0.0006 ± 0.0002 | -0.0005 ± 0.0001 | -0.0004 ± 0.0001 | 0.0001 ± 0.0005 | 0.0142 ± 0.0044 | -0.0004 ± 0.0003 | 0.0213 ± 0.0084 |
| Macrophyte dried weight (g) | 0.034 ± 0.013 | 0.029 ± 0.005 | 0.042 ± 0.020 | 0.046 ± 0.025 | 0.045 ± 0.013 | 0.046 ± 0.009 | 0.032 ± 0.014 | 0.041 ± 0.018 |
| ***Littorella uniflora*** | | | | | | | | |
| *Fv/Fm* | 0.82 ± 0.01 | 0.83 ± 0.01 | 0.83 ± 0 | 0.83 ± 0.01 | 0.82 ± 0.01 | 0.816 ± 0.01 | 0.816 ± 0.01 | 0.82 ± 0.01 |
| Shoot length (cm) | 4.68 ± 1.37 | 3.20 ± 0.90 | 4.50 ± 1.04 | 4.40 ± 0.56 | 5.16 ± 0.99 | 4.64 ± 0.55 | 5.30 ± 0.53 | 5.18 ± 0.66 |
| Root length (cm) | 4.58 ± 1.09 | 2.88 ± 1.98 | 2.98 ± 1.50 | 2.48 ± 1.60 | 6.78 ± 1.23 | 5.14 ± 1.39 | 5.10 ± 5.19 | 2.26 ± 1.32 |
| Wet weight (g) | 0.34 ± 0.07 | 0.26 ± 0.07 | 0.39 ± 0.09 | 0.34 ± 0.07 | 0.42 ± 0.09 | 0.32 ± 0.07 | 0.45 ± 0.11 | 0.36 ± 0.07 |
| Macrophyte wash weight (g) | -0.0005 ± 0.0002 | -0.0007 ± 0.0004 | -0.0008 ± 0.0005 | -0.0006 ± 0.0002 | -0.0005 ± 0.0001 | 0.0007 ± 0.0009 | -0.0003 ± 0.0004 | 0.001 ± 0.002 |
| Macrophyte dried weight (g) | 0.163 ± 0.044 | 0.219 ± 0.049 | 0.105 ± 0.023 | 0.151 ± 0.042 | 0.022 ± 0.004 | 0.017 ± 0.004 | 0.018 ± 0.004 | 0.017 ± 0.002 |
| ***Najas flexilis*** | | | | | | | | |
| *Fv/Fm* | 0.77 ± 0.01 | 0.76 ± 0.01 | 0.75 ± 0.02 | 0.76 ± 0.02 | 0.75 ± 0.02 | 0.76 ± 0.02 | 0.73 ± 0.06 | 0.74 ± 0.02 |
| Shoot length (cm) | 11.18 ± 3.13 | 14.22 ± 2.48 | 11.66 ± 4.46 | 12.22 ± 1.14 | 11.18 ± 3.13 | 14.22 ± 2.48 | 11.66 ± 4.46 | 12.22 ± 1.14 |
| Root length (cm) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| Wet weight (g) | 0.31 ± 0.11 | 0.35 ± 0.15 | 0.25 ± 0.11 | 0.45 ± 0.15 | 0.38 ± 0.17 | 0.49 ± 0.14 | 0.16 ± 0.06 | 0.37 ± 0.13 |
| Macrophyte wash weight (g) | -0.0005 ± 0.0002 | -0.0005 ± 0.0001 | -0.0004 ± 0.0002 | -0.0005 ± 0.0002 | 0.0005 ± 0.0016 | 0.0158 ± 0.0059 | -0.0004 ± 0.0001 | 0.0154 ± 0.0107 |
| Macrophyte dried weight (g) | 0.017 ± 0.005 | 0.019 ± 0.008 | 0.014 ± 0.005 | 0.025 ± 0.009 | 0.020 ± 0.007 | 0.027 ± 0.007 | 0.009 ± 0.002 | 0.021 ± 0.007 |

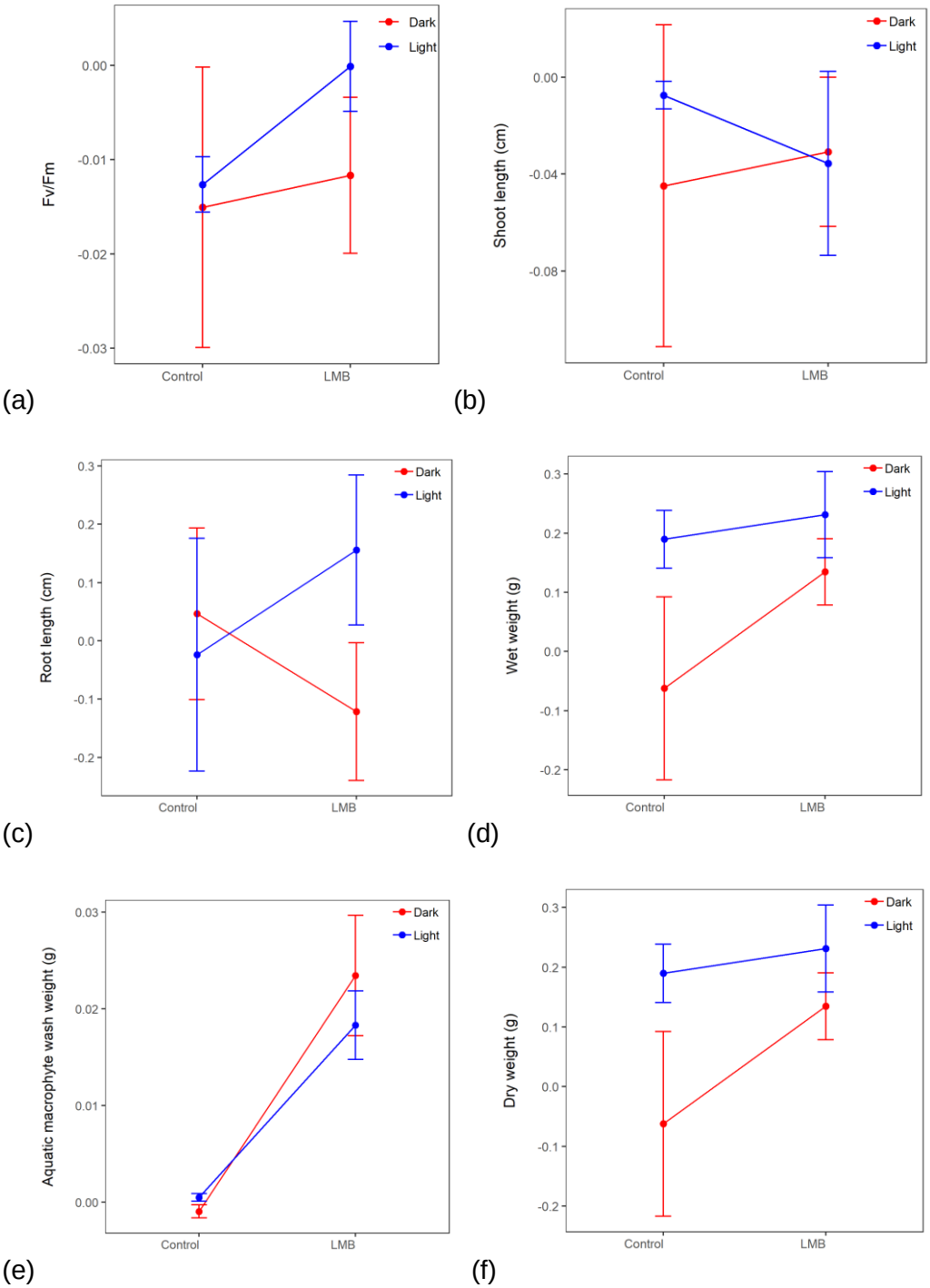



**Figure 2.** *Potamogeton perfoliatus* interaction plots for main effects of treatment (control/LMB) and light (light/dark) with an interaction. Mean and standard errors displayed for delta difference values (post- and pre-treatment conditions) for (a) *Fv/Fm*, (b) shoot length, (c) root length, (d) wet weight, (e) macrophyte wash weight and (f) dry weight.

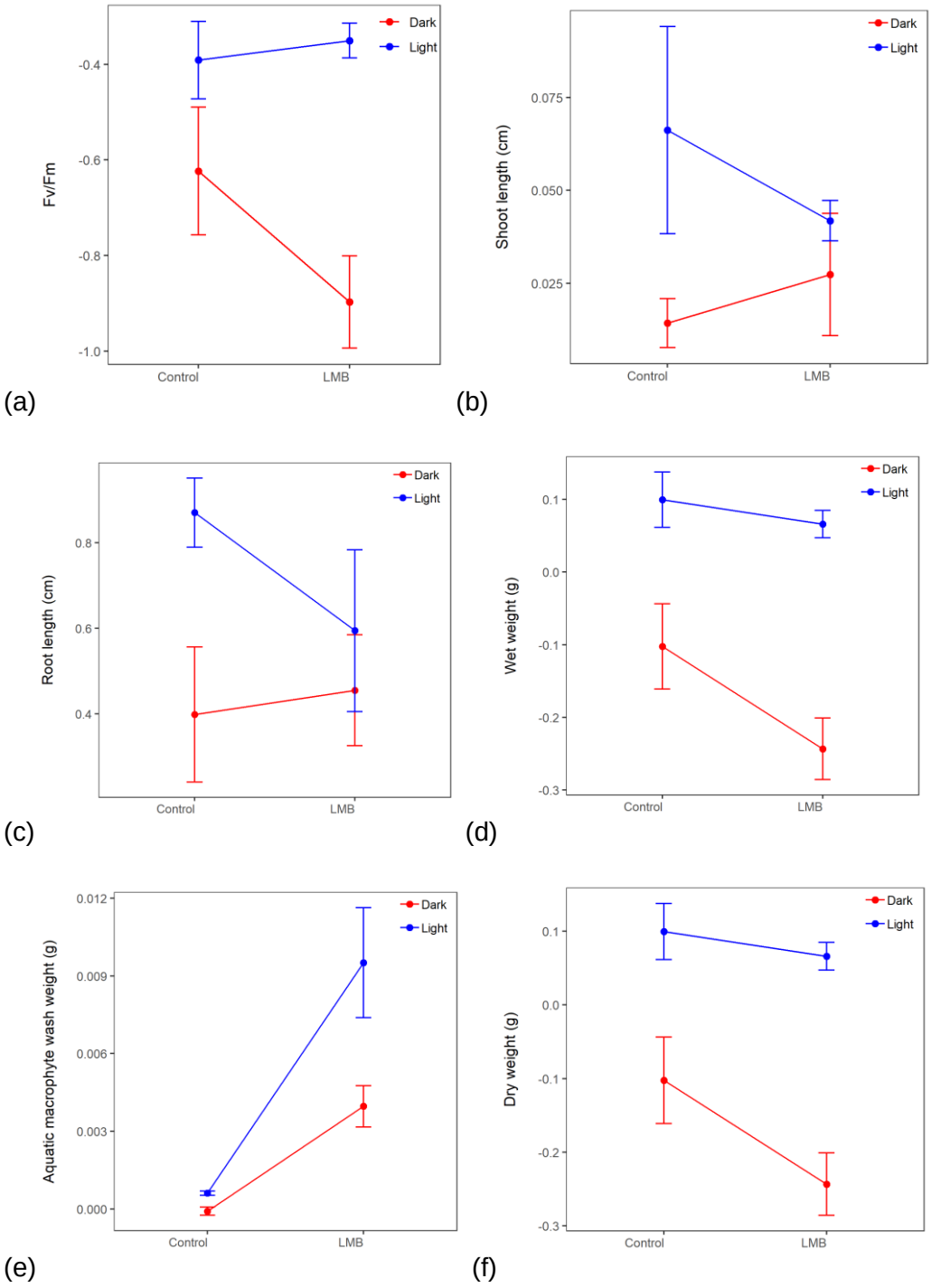



**Figure 3.** *Myriophyllum spicatum* interaction plots for main effects of treatment (control/LMB) and light (light/dark) with an interaction. Mean and standard errors displayed for delta difference values (post- and pre-treatment conditions) for (a) *Fv/Fm*, (b) shoot length, (c) root length, (d) wet weight, (e) macrophyte wash weight and (f) dry weight.

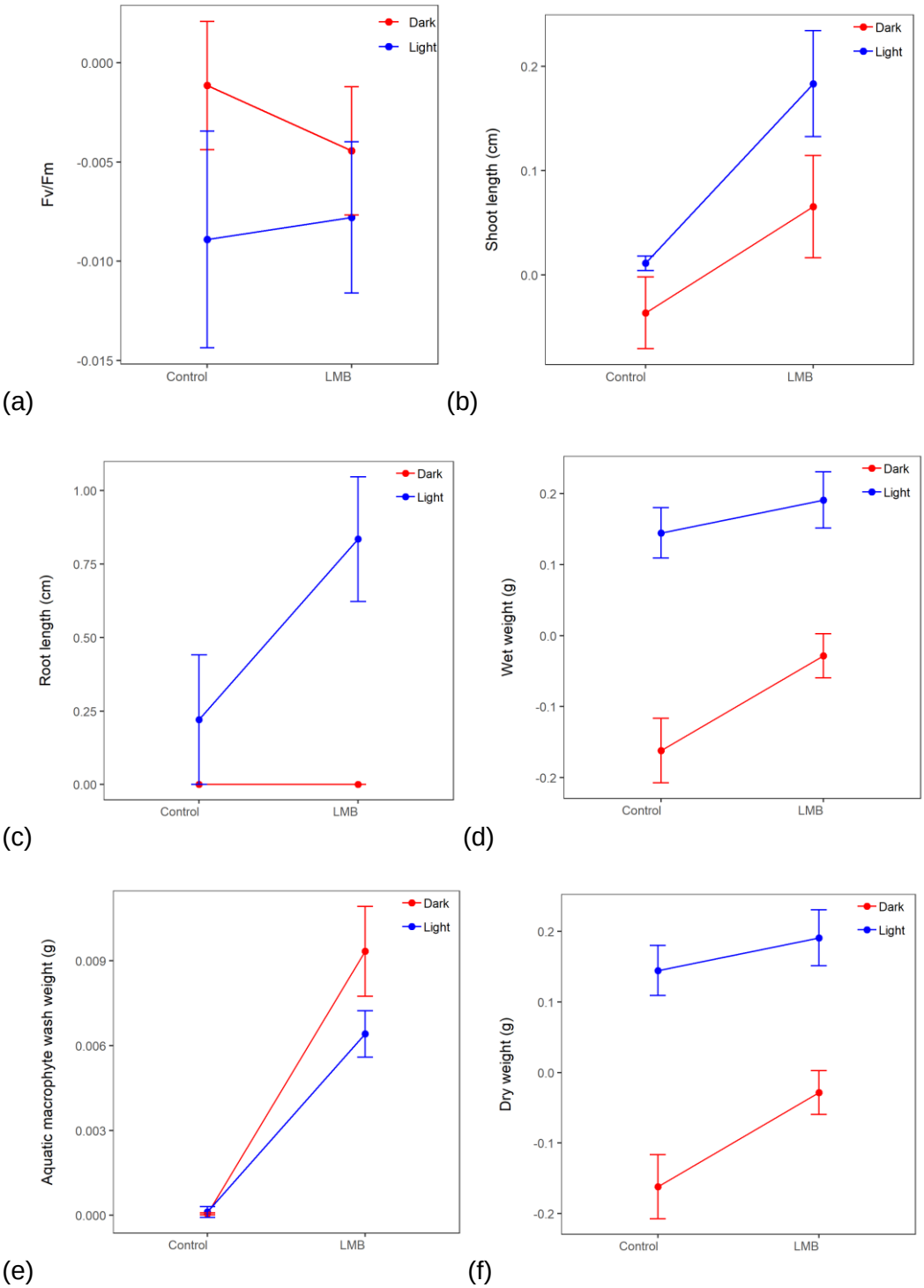



**Figure 4.** *Elodea canadensis* interaction plots for main effects of treatment (control/LMB) and light (light/dark) with an interaction. Mean and standard errors displayed for delta difference values (post- and pre-treatment conditions) for (a) *Fv/Fm*, (b) shoot length, (c) root length, (d) wet weight, (e) macrophyte wash weight and (f) dry weight.

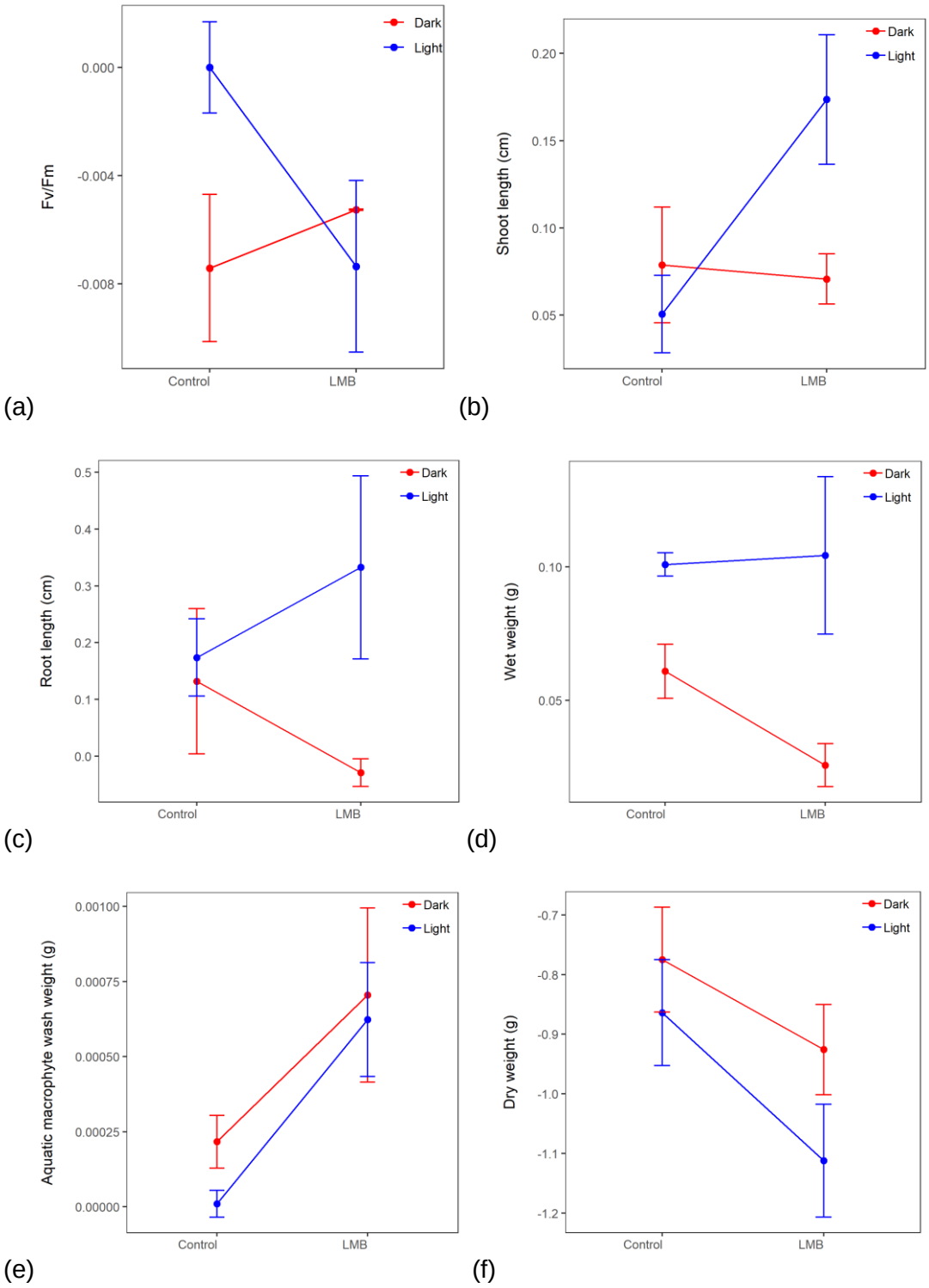



**Figure 5.** *Littorella uniflora* interaction plots for main effects of treatment (control/LMB) and light (light/dark) with an interaction. Mean and standard errors displayed for delta difference values (post- and pre-treatment conditions) for (a) *Fv/Fm*, (b) shoot length, (c) root length, (d) wet weight, (e) macrophyte wash weight and (f) dry weight.

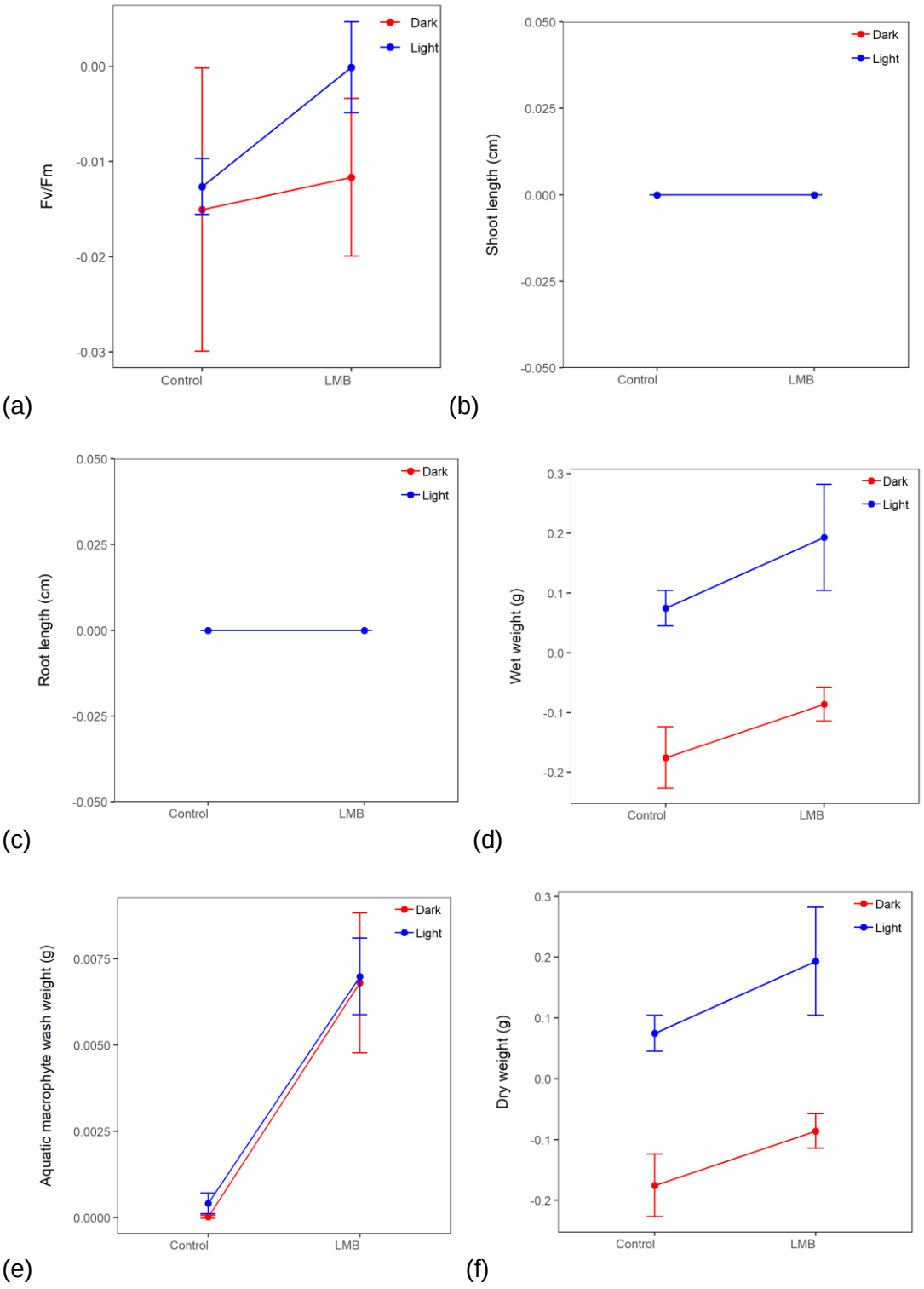



**Figure 6.** *Najas flexilis* interaction plots for main effects of treatment (control/LMB) and light (light/dark) with an interaction. Mean and standard errors displayed for delta difference values (post- and pre-treatment conditions) for (a) *Fv/Fm*, (b) shoot length, (c) root length, (d) wet weight, (e) macrophyte wash weight and (f) dry weight.

**Table 3.** Means and standard deviations for combined macrophyte species for each physico-chemical, nutrient and total chemical variable assessed (Conductivity, pH, Dissolved Oxygen (DO), Dissolved Organic Carbon (DOC), Total Phosphorus (TP), Soluble Reactive Phosphorus (SRP), Ammonium ($NH_4^+$), Nitrate ($NO_3^-$), Total Calcium (Ca), Manganese (Mn), Iron (Fe), Barium (Ba), Lanthanum (La), Neodymium (Nd) and Praseodymium (Pr)).

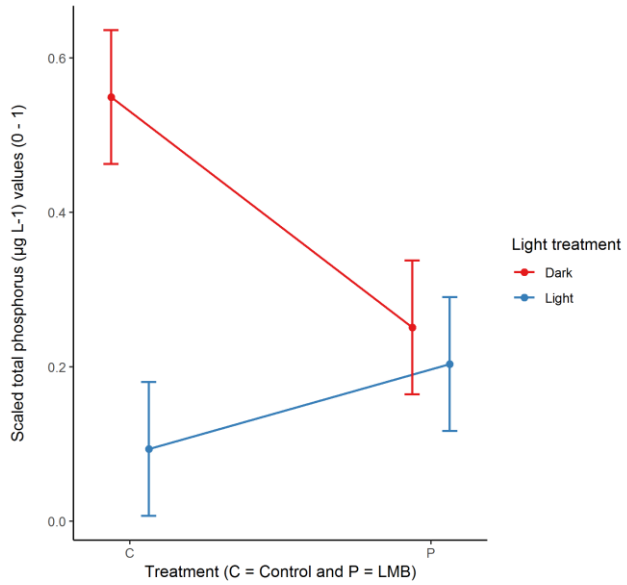
| **Measured variable** | **Before** | | | | **After** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Light** | | **Dark** | | **Light** | | **Dark** | |
| | **Control** | **LMB** | **Control** | **LMB** | **Control** | **LMB** | **Control** | **LMB** |
| Conductivity (µS cm) | 185.59 ± 22.55 | 178.46 ± 32.61 | 182.94 ± 29.48 | 180.93 ± 25.27 | 176.85 ± 27.06 | 230.85 ± 27.06 | 211.62 ± 13.65 | 253.73 ± 18.74 |
| pH | 7.24 ± 1.02 | 7.33 ± 1.02 | 7.32 ± 1.07 | 7.36 ± 1.11 | 7.53 ± 0.92 | 7.10 ± 0.61 | 7.09 ± 0.59 | 6.98 ± 0.57 |
| DO (mg $L^{-1}$) | 8.62 ± 0.25 | 8.47 ± 1.45 | 8.52 ± 1.18 | 8.63 ± 1.19 | 8.98 ± 0.72 | 8.39 ± 1.17 | 7.18 ± 1.28 | 6.83 ± 1.33 |
| DOC (mg $L^{-1}$) | - | - | - | - | 4.52 ± 0.76 | 3.96 ± 0.70 | 4.78 ± 0.85 | 4.23 ± 0.88 |
| TP (µg $L^{-1}$) | 62.1 ± 16.4 | 63.0 ± 18.4 | 63.1 ± 19.2 | 61.6 ± 19.9 | 69.7 ± 26.6 | 93.7 ± 48.4 | 236.7 ± 217.1 | 113.5 ± 126.2 |
| SRP (µg $L^{-1}$) | 36.8 ± 9.2 | 35.8 ± 8.4 | 35.9 ± 8.3 | 35.1 ± 11.4 | 41.6 ± 10.6 | 47.0 ± 12.7 | 84.7 ± 30.6 | 52.4 ± 14.4 |
| $NH_4^+$ (mg $L^{-1}$) | - | - | - | - | 0.03 ± 0.06 | 0.14 ± 0.20 | 0.36 ± 0.22 | 0.49 ± 0.25 |
| $NO_3^-$ (mg $L^{-1}$) | - | - | - | - | 0.01 ± 0.00 | 0.10 ± 0.14 | 0.11 ± 0.08 | 0.11 ± 0.12 |
| TCa (µg $L^{-1}$) | - | - | - | - | 19.31 ± 4.57 | 20.19 ± 3.12 | 22.76 ± 3.80 | 22.64 ± 3.39 |
| TMn (µg $L^{-1}$) | - | - | - | - | 91.87 ± 141.00 | 268.65 ± 319.54 | 652.12 ± 351.16 | 557.24 ± 459.52 |
| TFe (µg $L^{-1}$) | - | - | - | - | 246.39 ± 260.92 | 626.74 ± 929.11 | 1606.95 ± 1630.43 | 1541.98 ±1996.30 |
| TBa (µg $L^{-1}$) | - | - | - | - | 119.93 ± 23.11 | 216.30 ± 30.67 | 160.84 ± 31.36 | 2410.09 ± 46.71 |
| TLa (µg $L^{-1}$) | - | - | - | - | - | 95.12 ± 68.64 | - | 100.26 ± 47.26 |
| TNd (µg $L^{-1}$) | - | - | - | - | - | 0.07 ± 0.07 | - | 0.07 ± 0.07 |
| TPr (µg $L^{-1}$) | - | - | - | - | - | <LOD | - | 0.07 ± 0.20 |

**Table 4.** Model coefficients for all fixed effects with standard error for each dependent physico-chemical, nutrient and chemical variable assessed (Conductivity, pH, Dissolved Oxygen (DO), Dissolved Organic Carbon (DOC), Total Phosphorus (TP), Soluble Reactive Phosphorus (SRP), Ammonium ($NH_4^+$) and Nitrate ($NO_3^-$)).

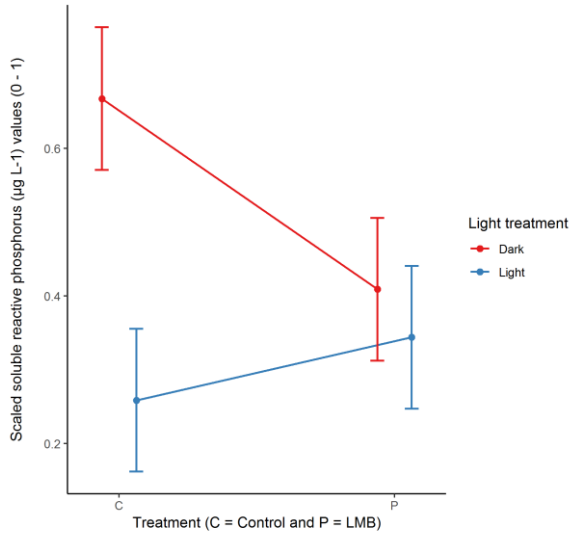
| Response | Fixed effects | Estimate | Standard error | t | *P* |
|---|---|---|---|---|---|
| Conductivity | Intercept | 0.472 | 0.036 | 12.823 | **<0.0001** |
| | Treatment - LMB | 0.429 | 0.052 | 8.245 | **<0.0001** |
| | Light - Light | -0.369 | 0.052 | -70.86 | **<0.0001** |
| | Treatment * Light | 0.205 | 0.074 | 2.785 | **<0.05** |
| pH | Intercept | 0.380 | 0.058 | 6.545 | **<0.0001** |
| | Treatment - LMB | -0.087 | 0.082 | -1.065 | 0.303 |
| | Light - Light | 0.325 | 0.082 | 3.970 | **<0.01** |
| | Treatment * Light | -0.253 | 0.116 | -2.181 | **<0.05** |
| DO | Intercept | 0.356 | 0.062 | 5.704 | **<0.0001** |
| | Treatment - LMB | -0.065 | 0.072 | -0.906 | 0.377 |
| | Light - Light | 0.289 | 0.072 | 4.014 | **<0.001** |
| DOC | Intercept | 0.599 | 0.057 | 10.576 | **<0.0001** |
| | Treatment - LMB | -0.262 | 0.065 | -4.009 | **<0.001** |
| | Light - Light | -0.092 | 0.065 | -1.404 | 0.178 |
| TP | Intercept | 0.549 | 0.044 | 12.580 | **<0.0001** |
| | Treatment - LMB | -0.298 | 0.062 | -4.831 | **<0.001** |
| | Light - Light | -0.455 | 0.062 | -7.380 | **<0.0001** |
| | Treatment*Light | 0.408 | 0.087 | 4.674 | **<0.001** |
| SRP | Intercept | 0.667 | 0.049 | 13.687 | **<0.0001** |
| | Treatment - LMB | -0.258 | 0.070 | -3.745 | **<0.01** |
| | Light - Light | -0.408 | 0.070 | -5.925 | **<0.0001** |
| | Treatment * Light | 0.343 | 0.097 | 3.522 | **<0.01** |
| $NH_4^+$ | Intercept | 0.479 | 0.052 | 8.877 | **<0.0001** |
| | Treatment - LMB | 0.164 | 0.061 | 2.611 | **<0.05** |
| | Light - Light | -0.449 | 0.061 | -7.416 | **<0.0001** |
| $NO_3^-$ | Intercept | 0.431 | 0.084 | 5.127 | **<0.0001** |
| | Treatment - LMB | -0.074 | 0.119 | -0.623 | 0.542 |
| | Light - Light | -0.423 | 0.119 | -3.557 | **<0.01** |
| | Treatment * Light | 0.370 | 0.168 | 2.200 | **<0.05** |

**Table 5.** Random intercept variance, standard deviation and variance components analysis to assess how much of the variation in the model is explained by species-specific macrophyte responses within treatments for each dependent physico-chemical, nutrient and chemical variable assessed (Conductivity, pH, Dissolved Oxygen (DO), Dissolved Organic Carbon (DOC), Total Phosphorus (TP), Soluble Reactive Phosphorus (SRP), Ammonium ($NH_4^+$) and Nitrate ($NO_3^-$).

| Response | Random effects | Variance | Std. Dev. | Variance components analysis (%) |
|---|---|---|---|---|
| Conductivity | Species:Treatment:Light | 0.005 | 0.067 | 28.9 |
| pH | Species:Treatment:Light | 0.008 | 0.088 | 14.5 |
| DO | Species:Treatment:Light | 0.017 | 0.131 | 28 |
| DOC | Species:Treatment:Light | 0.011 | 0.107 | 18.6 |
| TP | Species:Treatment:Light | 0.002 | 0.037 | 3.2 |
| SRP | Species:Treatment:Light | 0.003 | 0.055 | 6.5 |
| $NH_4^+$ | Species:Treatment:Light | 0.010 | 0.098 | 17.8 |
| $NO_3^-$ | Species:Treatment:Light | 0.026 | 0.609 | 35.4 |

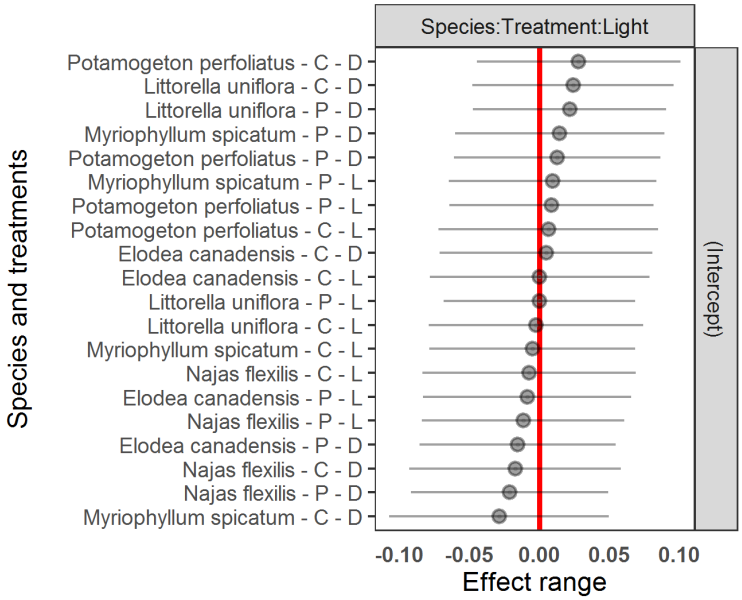



(a)

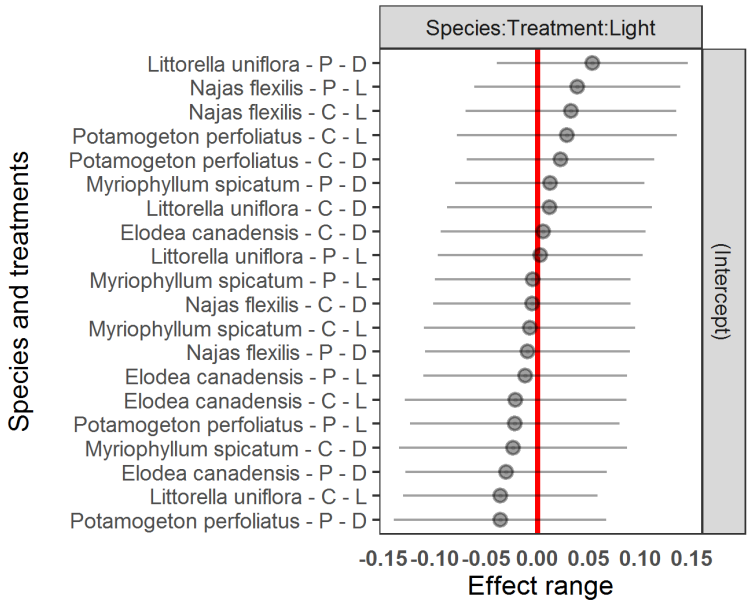



(b)

**Figure 7.** Interaction plots of the main effects with 95% confidence intervals of (a) total phosphorus, (b) soluble reactive phosphorus.




(a)




(b)

**Figure 8.** Median effect estimates of the random effects with 95% confidence intervals for (a) total phosphorus, (b) soluble reactive phosphorus between light within treatment within species. Black values show species with confidence intervals that do not overlap 0 (median) and are either more negative or positive than the global response of each linear mixed effect model.

**Table 6.** Means and standard deviations for each macrophyte species for physico-chemical, and total nutrient and chemical variables (Conductivity, pH, Dissolved Oxygen (DO), Dissolved Organic Carbon (DOC), Total Phosphorus (TP), Soluble Reactive Phosphorus (SRP), Ammonium ($NH_4^+$), Nitrate ($NO_3^-$), Total Calcium (Ca), Manganese (Mn), Iron (Fe), Barium (Ba), Lanthanum (La), Neodymium (Nd) and Praseodymium (Pr)).

| Measured variable | Before | | | | After | | | |
|---|---|---|---|---|---|---|---|---|
| | Light | | Dark | | Light | | Dark | |
| | Control | LMB | Control | LMB | Control | LMB | Control | LMB |
| ***Potamogeton perfoliatus*** | | | | | | | | |
| Conductivity (µS cm) | 174.60 ± 13.91 | 154.84 ± 26.95 | 165.22 ± 27.88 | 164.16 ± 8.19 | 162.74 ± 32.01 | 228.82 ± 31.86 | 216.94 ± 14.04 | 266.20 ± 4.66 |
| pH | 8.49 ± 0.96 | 8.59 ± 0.57 | 8.79 ± 0.84 | 8.93 ± 0.58 | 8.68 ± 0.57 | 7.65 ± 0.25 | 7.60 ± 0.08 | 7.26 ± 0.02 |
| DO (mg $L^{-1}$) | 10.03 ± 1.65 | 10.13 ± 1.28 | 10.03 ± 1.65 | 10.44 ± 0.68 | 8.80 ± 0.96 | 7.64 ± 0.80 | 5.80 ± 0.57 | 4.77 ± 0.90 |
| DOC (mg $L^{-1}$) | - | - | - | - | 4.44 ± 0.96 | 3.29 ± 0.35 | 4.26 ± 0.17 | 4.79 ± 1.58 |
| TP (mg $L^{-1}$) | 82.9 ± 6.2 | 85.0 ± 18.4 | 90.0 ± 15.5 | 86.8 ± 19.8 | 102.8 ± 30.7 | 129.3 ± 38.9 | 339.1 ± 187.9 | 146.2 ± 20.9 |
| SRP (µg $L^{-1}$) | 31.2 ± 12.0 | 36.0 ± 13.1 | 34.0 ± 9.1 | 33.2 ± 5.8 | 38.8 ± 7.5 | 38.4 ± 12.0 | 94.0± 27.4 | 36.6 ± 8.8 |
| $NH_4$+ (mg $L^{-1}$) | - | - | - | - | 0.04 ± 0.02 | 0.13 ± 0.15 | 0.34 ± 0.18 | 0.53 ± 0.29 |
| $NO_3$- (mg $L^{-1}$) | - | - | - | - | 0.01 ± 0.01 | 0.04 ± 0.03 | 0.13 ± 0.06 | 0.03 ± 0.03 |
| TCa (µg $L^{-1}$) | - | - | - | - | 11.91 ± 2.99 | 16.56 ± 2.18 | 17.08 ± 1.36 | 19.07 ± 2.48 |
| TMn (µg $L^{-1}$) | - | - | - | - | 169.97 ± 226.66 | 482.77 ± 434.55 | 738.38 ± 175.88 | 112.70 ± 391.81 |
| TFe (µg $L^{-1}$) | - | - | - | - | 445.16 ± 415.87 | 1435.54 ± 1445.83 | 1858.80 ± 646.82 | 4625.40 ± 1673.02 |
| TBa (µg $L^{-1}$) | - | - | - | - | 92.74 ± 31.52 | 245.50 ± 27.60 | 154.10 ± 18.55 | 298.58 ± 46.53 |
| TLa (µg $L^{-1}$) | - | - | - | - | <LOD | 100.29 ± 114.37 | <LOD | 138.16 ± 12.10 |
| TNd (µg $L^{-1}$) | - | - | - | - | <LOD | 0.13 ± 0.11 | <LOD | 0.09 ± 0.06 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| TPr (µg $L^{-1}$) | - | - | - | - | <LOD | 0.06 ± 0.06 | <LOD | 0.21 ± 0.43 |
| ***Myriophyllum spicatum*** | | | | | | | | |
| Conductivity (µS cm) | 186.18 ± 6.30 | 187.12 ± 0.63 | 187.22 ± 2.33 | 186.62 ± 3.22 | 159.32 ± 9.65 | 194.20 ± 2.12 | 172.24 ± 9.72 | 210.28 ± 2.82 |
| pH | 7.56 ± 0.29 | 7.61 ± 0.32 | 7.51 ± 0.29 | 7.50 ± 0.17 | 8.17 ± 0.28 | 7.99 ± 0.24 | 7.49 ± 0.04 | 7.24 ± 0.06 |
| DO (mg $L^{-1}$) | 8.67 ± 0.65 | 8.26 ± 0.51 | 8.27 ± 0.41 | 8.26 ± 0.44 | 8.31 ± 0.89 | 9.19 ± 0.37 | 5.75 ± 0.43 | 6.53 ± 0.80 |
| DOC (mg $L^{-1}$) | - | - | - | - | 4.08 ± 0.37 | 3.51 ± 0.13 | 4.72 ± 0.59 | 3.55 ± 0.17 |
| TP (µg $L^{-1}$) | 53.5 ± 11.7 | 52.8 ± 10.0 | 55.7 ± 5.0 | 51.6 ± 4.9 | 58.5 ± 17.6 | 118.1 ± 89.3 | 248.4 ± 356.4 | 199.4 ± 279.4 |
| SRP (µg $L^{-1}$) | 35.4 ± 4.6 | 36.8 ± 3.1 | 31.6 ± 7.6 | 30.6 ± 4.9 | 38.8 ± 11.7 | 44.8 ± 12.4 | 77.8 ± 23.7 | 51.6 ± 12.1 |
| $NH_4$+ (mg $L^{-1}$) | - | - | - | - | 0.10 ± 0.11 | 0.34 ± 0.26 | 0.41 ± 0.19 | 0.53 ± 0.19 |
| $NO_3$- (mg $L^{-1}$) | - | - | - | - | <LOD | 0.04 ± 0.03 | 0.07 ± 0.08 | 0.05 ± 0.03 |
| TCa (µg $L^{-1}$) | - | - | - | - | 20.29 ± 3.45 | 23.46 ± 2.01 | 22.34 ± 1.84 | 24.02 ± 3.65 |
| TMn (µg $L^{-1}$) | - | - | - | - | 77.13 ± 103.95 | 516.02 ± 347.19 | 310.34 ± 486.75 | 599.03 ± 654.01 |
| TFe (µg $L^{-1}$) | - | - | - | - | 281.82 ± 133.78 | 1151.58 ± 1041.10 | 1342.40 ± 2520.71 | 1633.60 ± 2092.79 |
| TBa (µg $L^{-1}$) | - | - | - | - | 127.66 ± 23.94 | 229.18 ± 41.56 | 189.64 ± 49.32 | 219.04 ± 68.69 |
| TLa (µg $L^{-1}$) | - | - | - | - | - | 104.84 ± 92.29 | - | 91.57 ± 87.29 |
| TNd (µg $L^{-1}$) | - | - | - | - | - | 0.10 ± 0.05 | - | 0.11 ± 0.12 |
| TPr (µg $L^{-1}$) | - | - | - | - | - | 0.04 ± 0.05 | - | 0.07 ± 0.10 |
| ***Elodea canadensis*** | | | | | | | | |
| Conductivity (µS cm) | 159.22 ± 4.77 | 157.44 ± 3.20 | 159.94 ± 2.85 | 155.46 ± 4.23 | 214.96 ± 27.72 | 223.50 ± 7.10 | 207.34 ± 1.58 | 242.20 ± 5.40 |
| pH | 7.35 ± 0.13 | 7.37 ± 0.27 | 7.41 ± 0.22 | 7.47 ± 0.26 | 7.07 ± 0.28 | 7.01 ± 0.05 | 7.01 ± 0.10 | 6.99 ± 0.12 |
| DO (mg $L^{-1}$) | 8.22 ± 0.68 | 8.19 ± 0.93 | 8.15 ± 0.40 | 8.08 ± 0.83 | 7.54 ± 1.40 | 8.91 ± 0.51 | 6.92 ± 0.61 | 6.84 ± 0.36 |
| DOC (mg $L^{-1}$) | - | - | - | - | 4.66 ± 0.14 | 3.93 ± 0.08 | 5.65 ± 0.40 | 4.17 ± 0.71 |
| TP (µg $L^{-1}$) | 76.7 ± 6.2 | 75.3 ± 9.2 | 76.1 ± 6.9 | 76.5 ± 6.5 | 79.1 ± 14.4 | 85.8 ± 14.6 | 307.5 ± 231.7 | 84.8 ± 8.4 |
| SRP (µg $L^{-1}$) | 41.6 ± 10.3 | 35.2 ± 5.2 | 43.0 ± 6.3 | 48.0 ± 19.9 | 41.8 ± 9.3 | 43.0 ± 6.0 | 111.0 ± 44.0 | 55.0 ± 6.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $NH_4$+ (mg $L^{-1}$) | - | - | - | - | 0.01 ± 0.01 | 0.07 ± 0.09 | 0.56 ± 0.16 | 0.65 ± 0.16 |
| $NO_3$- (mg $L^{-1}$) | - | - | - | - | <LOD | 0.02 ± 0.03 | 0.05 ± 0.03 | 0.08 ± 0.09 |
| TCa (µg $L^{-1}$) | - | - | - | - | 20.47 ± 1.81 | 20.32 ± 1.81 | 25.41 ± 1.95 | 22.02 ± 1.60 |
| TMn (µg $L^{-1}$) | - | - | - | - | 32.53 ± 19.27 | 55.10 ± 53.92 | 782.40 ± 312.57 | 415.82 ± 153.19 |
| TFe (µg $L^{-1}$) | - | - | - | - | 241.24 ± 285.36 | 156.32 ± 121.82 | 2447.38 ± 1920.89 | 665.92 ± 376.31 |
| TBa (µg $L^{-1}$) | - | - | - | - | 89.74 ± 5.81 | 192.24 ± 9.61 | 146.84 ± 22.16 | 224.78 ± 12.05 |
| TLa (µg $L^{-1}$) | - | - | - | - | - | 92.18 ± 16.30 | - | 94.85 ± 10.09 |
| TNd (µg $L^{-1}$) | - | - | - | - | - | 0.04 ± 0.02 | - | 0.04 ± 0.004 |
| TPr (µg $L^{-1}$) | - | - | - | - | - | <LOD | - | <LOD |
| ***Littorella uniflora*** | | | | | | | | |
| Conductivity (µS cm) | 208.34 ± 1.59 | 212.34 ± 5.76 | 209.44 ± 13.5 | 210.34 ± 0.53 | 168.22 ± 1.49 | 211.92 ± 6.05 | 193.50 ± 2.87 | 223.86 ± 4.31 |
| pH | 7.27 ± 0.06 | 7.31 ± 0.02 | 7.24 ± 0.05 | 7.25 ± 0.06 | 6.13 ± 0.05 | 6.07 ± 0.05 | 6.07 ± 0.02 | 6.01 ± 0.01 |
| DO (mg $L^{-1}$) | 7.39 ± 0.64 | 6.70 ± 1.37 | 7.42 ± 0.41 | 7.50 ± 0.33 | 8.97 ± 0.26 | 8.55 ± 0.98 | 7.86 ± 0.32 | 7.80 ± 0.30 |
| DOC (mg $L^{-1}$) | - | - | - | - | 3.99 ± 0.09 | 3.72 ± 0.42 | 4.32 ± 0.39 | 3.57 ± 0.30 |
| TP (µg $L^{-1}$) | 49.5 ± 4.6 | 55.0 ± 11.6 | 47.5 ± 4.5 | 47.9 ± 3.6 | 43.6 ± 2.2 | 60.4 ± 11.0 | 127.1 ± 49.4 | 70.0 ± 15.2 |
| SRP (µg $L^{-1}$) | 42.8 ± 7.2 | 38.8 ± 12.6 | 32.6 ± 10.1 | 28.0 ± 2.7 | 35.0 ± 3.8 | 45.6 ± 9.3 | 71.6 ± 9.6 | 57.4 ± 14.6 |
| $NH_4$+ (mg $L^{-1}$) | - | - | - | - | <LOD | 0.14 ± 0.23 | 0.09 ± 0.04 | 0.22 ± 0.11 |
| $NO_3$- (mg $L^{-1}$) | - | - | - | - | <LOD | 0.35 ± 0.11 | 0.21 ± 0.03 | 0.31 ± 0.09 |
| TCa (µg $L^{-1}$) | - | - | - | - | 20.55 ± 2.08 | 18.30 ± 2.25 | 23.82 ± 3.57 | 23.34 ± 30.9 |
| TMn (µg $L^{-1}$) | - | - | - | - | 4.18 ± 1.66 | 66.34 ± 104.39 | 662.76 ± 146.95 | 205.00 ± 116.79 |
| TFe (µg $L^{-1}$) | - | - | - | - | 43.48 ± 11.94 | 97.50 ± 96.97 | 833.82 ± 410.77 | 202.98 ± 118.29 |
| TBa (µg $L^{-1}$) | - | - | - | - | 115.78 ± 1.67 | 203.16 ± 14.53 | 162.52 ± 19.04 | 230.72 ± 10.31 |
| TLa (µg $L^{-1}$) | - | - | - | - | - | 60.44 ± 32.69 | - | 96.17 ± 52.21 |
| TNd (µg $L^{-1}$) | - | - | - | - | - | 0.03 ± 0.02 | - | 0.03 ± 0.02 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| TPr (µg $L^{-1}$) | - | - | - | - | - | <LOD | - | <LOD |
| ***Najas flexilis*** | | | | | | | | |
| Conductivity (µS cm) | 211.18 ± 5.18 | 212.82 ± 5.18 | 214.88 ± 7.19 | 210.52 ± 1.08 | 213.98 ± 5.02 | 261.40 ± 5.68 | 223.40 ± 5.94 | 270.20 ± 2.68 |
| pH | 5.63 ± 0.11 | 5.64 ± 0.10 | 5.69 ± 0.07 | 5.63 ± 0.03 | 7.15 ± 0.03 | 7.12 ± 0.04 | 7.11 ± 0.02 | 7.12 ± 0.02 |
| DO (mg $L^{-1}$) | 8.88 ± 0.26 | 8.87 ± 0.66 | 8.81 ± 0.47 | 8.86 ± .69 | 9.32 ± 0.26 | 9.39 ± 0.52 | 8.31 ± 0.36 | 7.90 ± 0.57 |
| DOC (mg $L^{-1}$) | - | - | - | - | 5.61 ± 0.16 | 5.07 ± 0.42 | 5.54 ± 0.80 | 4.59 ± 0.44 |
| TP (µg $L^{-1}$) | 48.2 ± 6.7 | 46.7 ± 5.6 | 46.3 ± 5.1 | 45.1 ± 11.1 | 64.5 ± 15.9 | 74.9 ± 3.1 | 142.7 ± 110.4 | 67.0 ± 6.9 |
| SRP (µg $L^{-1}$) | 32.8 ± 7.3 | 36.0 ± 5.2 | 37.2 ± 5.6 | 33.2 ± 2.6 | 53.2 ± 11.8 | 63.2 ± 9.7 | 77.0 ± 24.9 | 53.8 ± 10.0 |
| $NH_4$+ (mg $L^{-1}$) | - | - | - | - | <LOD | <LOD | 0.38 ± 0.20 | 0.52 ± 0.04 |
| $NO_3$- (mg $L^{-1}$) | - | - | - | - | <LOD | 0.06 ± 0.06 | 0.09 ± 0.04 | 0.07 ± 0.04 |
| TCa (µg $L^{-1}$) | - | - | - | - | 23.35 ± 2.21 | 22.33 ± 1.06 | 25.14 ± 2.63 | 24.75 ± 3.54 |
| TMn (µg $L^{-1}$) | - | - | - | - | 175.53 ± 161.46 | 222.99 ± 211.87 | 766.74 ± 399.56 | 440.68 ± 213.17 |
| TFe (µg $L^{-1}$) | - | - | - | - | 220.24 ± 180.95 | 292.74 ± 229.65 | 1552.36 ± 1875.70 | 582.00 ± 574.14 |
| TBa (µg $L^{-1}$) | - | - | - | - | 123.74 ± 6.90 | 211.44 ± 24.67 | 151.12 ± 28.47 | 232.34 ± 25.54 |
| TLa (µg $L^{-1}$) | - | - | - | - | - | 107.83± 56.67 | - | 80.59 ± 19.32 |
| TNd (µg $L^{-1}$) | - | - | - | - | - | 0.08 ± 0.05 | - | 0.05 ± 0.03 |
| TPr (µg $L^{-1}$) | - | - | - | - | - | <LOD | - | <LOD |

**Table 7.** Individual species responses to measured physico-chemical, total (T) and filtered nutrient and chemical variables (Conductivity, pH, Dissolved Oxygen (DO), Dissolved Organic Carbon (DOC), Total Phosphorus (TP), Soluble Reactive Phosphorus (SRP), Ammonium ($NH_4$+), Nitrate ($NO_3$-), Total Calcium (Ca), Manganese (Mn), Iron (Fe), Barium (Ba), Lanthanum (La), Neodymium (Nd) and Praseodymium (Pr)) for main treatment effects (LMB control) and light treatment (light dark) and an interaction (LMB control*Light dark) using Two-Way ANOVA's and individual Kruskal-Wallis tests with *P* value correction for multiple testing, Aligned rank transformation test for non-parametric interaction testing and Tukey's Post hoc Dunn test (with *P* value adjustment) for significant interaction terms (non-parametric only).

| **Response** | **LMB Control** | | | **Light Dark** | | | **LMB Control*Light Dark** | | | **Post Hoc** |
|---|---|---|---|---|---|---|---|---|---|---|
| | F chi-squared | *P* | Df | F/ chi-squared | *P* | Df | F/ chi-squared | *P* | Df | P |
| ***Potamogeton perfoliatus*** | | | | | | | | | | |
| Conductivity (µS cm) | 63.045 | **<0.0001** | | 28.558 | **<0.0001** | | 4.290 | 0.055 | 16 | |
| pH | 6.659 | **<0.05** | | 11.451 | **<0.001** | | 1.064 | 0.318 | 16 | |
| DO (mg $L^{-1}$) | 3.342 | 0.086 | | 18.111 | **<0.001** | | 0.033 | 0.858 | 16 | |
| DOC (mg $L^{-1}$) | 0.543 | 0.472 | | 2.453 | 0.137 | | 3.990 | 0.063 | 16 | |
| TP (µg $L^{-1}$) ◆ | 0.018 | 0.894 | | 10.877 | **<0.01** | | 5.487 | **<0.05** | 16 | CL – CD** |
| SRP (µg $L^{-1}$) (K) | 4.817 | **<0.05** | 1 | 1.754 | 0.185 | 1 | 8.589 | **<0.05** | 16 | CL – CD *, CD – PD *, PL – CD * |
| $NH_4$+ (mg $L^{-1}$) ◆ | 2.687 | 0.121 | | 20.344 | **<0.001** | | 0.164 | 0.691 | 16 | |
| $NO_3$- (mg $L^{-1}$) ◆ | 4.585 | **<0.05** | | 10.846 | **<0.01** | | 13.033 | **<0.01** | 16 | PD – CD **, CL – CD ***, PL – CD ** |
| TCa (µg $L^{-1}$) | 10.162 | **<0.01** | | 13.636 | **<0.01** | | 1.628 | 0.220 | 16 | |
| TMn (µg $L^{-1}$) | 5.771 | **<0.05** | | 17.277 | **<0.001** | | 0.065 | 0.801 | 16 | |
| TFe (µg $L^{-1}$) | 12.877 | **<0.01** | | 19.333 | **<0.001** | | 2.878 | 0.109 | 16 | |
| TBa (µg $L^{-1}$) | 103.580 | **<0.0001** | | 15.360 | **<0.001** | | 0.080 | 0.780 | 16 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| TLa (µg $L^{-1}$) (K) | 16.309 | **<0.001** | 1 | 0.367 | 0.545 | | 16.00 | **<0.01** | 1 | PD – CD **, CD – PL *, CL – PD **, CL – PL * |
| TNd (µg $L^{-1}$) (K) | 13.865 | **<0.001** | 1 | 0.007 | 0.934 | 1 | 1.871 | 0.285 | 1 | |
| TPr (µg $L^{-1}$) (K) | 3.327 | 0.205 | 1 | 0.237 | 0.654 | 1 | 0.209 | 0.654 | 1 | |
| ***Myriophyllum spicatum*** | | | | | | | | | | |
| Conductivity (µS cm) | 325.417 | **<0.0001** | | 50.035 | **<0.0001** | | 1.365 | 0.260 | 16 | |
| pH | 5.639 | **<0.05** | | 40.251 | **<0.0001** | | 0.002 | 0.961 | 16 | |
| DO (mg $L^{-1}$) | 10.699 | **<0.01** | | 56.357 | **<0.0001** | | 0.586 | 0.455 | 16 | |
| DOC (mg $L^{-1}$) | 29.309 | **<0.0001** | | 4.363 | 0.053 | | 3.392 | 0.084 | 16 | |
| TP (µg $L^{-1}$) (K) | 0.091 | 0.762 | 1 | 5.143 | **<0.05** | 1 | 6.897 | **<0.05** | 1 | CL – CD***, CL – PL* |
| SRP (µg $L^{-1}$) (K) | 0.414 | 0.520 | 1 | 9.871 | **<0.01** | 1 | 5.163 | 0.056 | 16 | |
| $NH_4$+ (mg $L^{-1}$) | 9.294 | **<0.01** | | 46.275 | **<0.0001** | | 0.129 | 0.724 | 16 | |
| $NO_3$- (mg $L^{-1}$) (K) | 0.322 | 0.872 | 1 | 13.205 | **<0.001** | 1 | 0.027 | 0.872 | 16 | |
| ***Elodea canadensis*** | | | | | | | | | | |
| Conductivity (µS cm) | 308.305 | **<0.0001** | | 102.324 | **<0.0001** | | 3.485 | 0.080 | 16 | |
| pH | 8.207 | **<0.05** | | 10.736 | **<0.01** | | 4.616 | **<0.05** | 16 | CL – CD **, CL – PD **, PL – CL * |
| DO (mg $L^{-1}$) | 0.342 | 0.567 | | 30.685 | **<0.0001** | | 0.318 | 0.581 | 16 | |
| DOC (mg $L^{-1}$)◆ | 131.160 | **<0.0001** | | 37.52 | **<0.0001** | | 11.040 | **<0.01** | 16 | PD – CD ****, CL – CD ****, PL – CD ****, CL – PD **, PL – CL *** |
| TP (µg $L^{-1}$) ◆ | 2.592 | 0.127 | | 7.354 | **<0.05** | | 9.952 | **<0.01** | 16 | CD – PD*, CL – CD**, PL – CD* |
| SRP (µg $L^{-1}$) (K) | 1.557 | 0.212 | 1 | 5.147 | **<0.05** | | 13.198 | **<0.01** | 16 | CD – CL **, CD – PD *, CD – PL * |
| $NH_4$+ (mg $L^{-1}$) | 9.294 | **<0.01** | | 46.27 | **<0.0001** | | 0.129 | 0.724 | | |
| $NO_3$- (mg $L^{-1}$) (K) | 0.509 | 0.714 | 1 | 11.08 | **<0.01** | 1 | 0.093 | 0.764 | 16 | |
| ***Littorella uniflora*** | | | | | | | | | | |
| Conductivity (µS cm) | 178.183 | **<0.0001** | | 54.133 | **<0.0001** | | 3.906 | 0.066 | 16 | |
| pH | 6.646 | **<0.05** | | 0.217 | 0.648 | | 0.078 | 0.784 | 16 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DO (mg $L^{-1}$) | 0.308 | 0.586 | | 7.904 | **<0.05** | | 0.002 | 0.964 | 16 | |
| DOC (µg $L^{-1}$) | 12.445 | **<0.01** | | 0.402 | 0.535 | | 2.735 | 0.118 | 16 | |
| TP (µg $L^{-1}$) (K) | 0.013 | 0.910 | 1 | 12.101 | **<0.05** | 1 | 8.592 | **<0.01** | 16 | CL – CD***, CD – PL*, CL – PD* |
| SRP (µg $L^{-1}$)◆ | 0.240 | 0.631 | | 39.274 | **<0.0001** | | 4.796 | **<0.05** | 16 | CL – CD ***, PL – CD **, CL – PD **, PL – PD * |
| $NH_4$+ (mg $L^{-1}$) (K) | 3.369 | 0.09 | 1 | 7.051 | **<0.05** | 1 | 0.412 | 0.530 | 16 | |
| $NO_3$- (mg $L^{-1}$) (K) | 13.972 | **<0.001** | 1 | 0.372 | 0.545 | 1 | 19.338 | **<0.001** | 16 | CD – CL **, CD – PL *, CL – PD ** |
| ***Najas flexilis*** | | | | | | | | | | |
| Conductivity (µS cm) | 786.311 | **<0.0001** | | 23.672 | **<0.001** | | 2.422 | 0.139 | 16 | |
| pH ◆ | 1.289 | 0.273 | | 6.217 | **<0.05** | | 0.659 | 0.429 | 16 | |
| DO (mg $L^{-1}$) | 0.230 | 0.638 | | 9.878 | **<0.01** | | 0.457 | 0.509 | 16 | |
| DOC (mg $L^{-1}$) | 10.757 | **<0.01** | | 1.479 | 0.242 | | 0.826 | 0.377 | 16 | |
| TP (µg $L^{-1}$) | 1.502 | 0.238 | | 4.546 | **<0.05** | | 8.548 | **<0.01** | 16 | CD – PD*, CL – CD* |
| SRP (µg $L^{-1}$)◆ | 0.947 | 0.345 | | 1.000 | 0.332 | | 4.240 | 0.056 | 16 | |
| $NH_4$+ (mg $L^{-1}$) (K) | 0.367 | 0.545 | 1 | 16.309 | **<0.001** | | 16.00 | **<0.01** | 16 | CD – CL *, CD – PL *, CL – PD **, PD – PL ** |
| $NO_3$- (mg $L^{-1}$) ◆ | 1.303 | 0.271 | | 6.483 | **<0.05** | | 4.310 | 0.054 | 16 | 1.303 |

**K** – Non-parametric Kruskal Wallis test

◆ - Logged response variable

Significant Tukey's *Post Hoc* Dunn test - *: <0.05, **: <0.01, ***: <0.001, ****: <0.0001 for listed groups CL (control – light), CD (control – dark), PL – (LMB – light), PD – (LMB – dark)

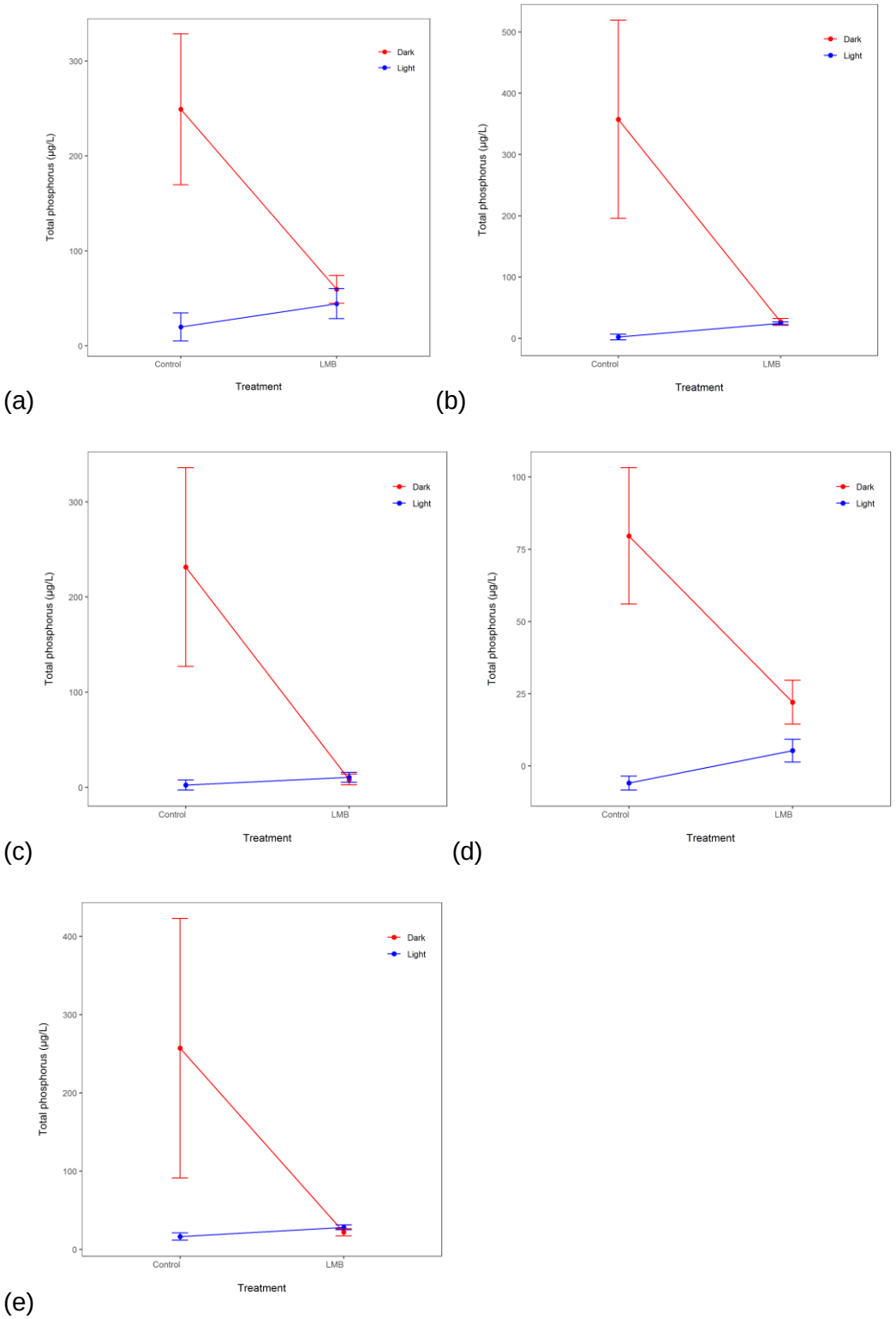
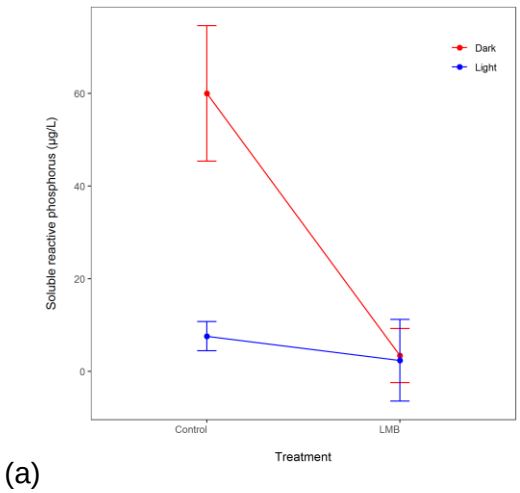
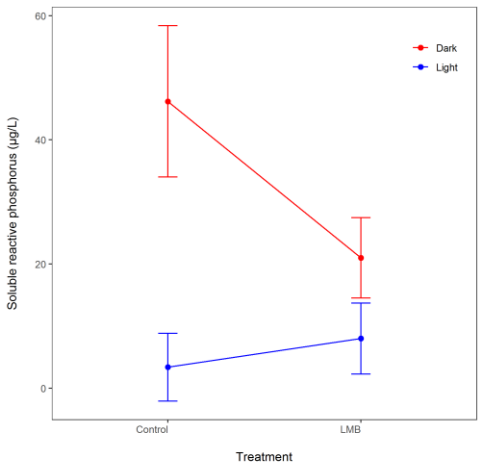


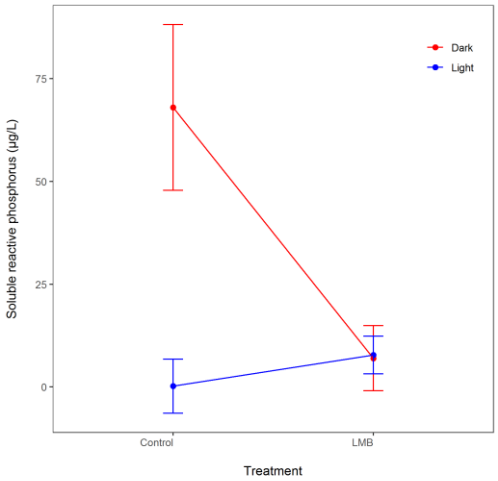

**Figure 9.** Total phosphorus (µg $L^{-1}$) for *Potamogeton perfoliatus* (a), *Myriophyllum spicatum* (b), *Elodea canadensis* (c), *Littorella uniflora* (d) and *Najas flexilis* (e).



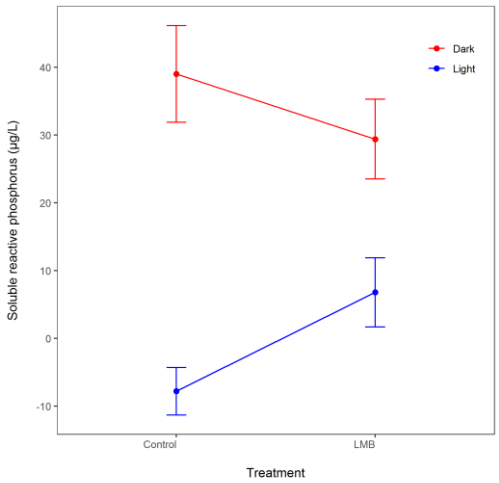

(a)



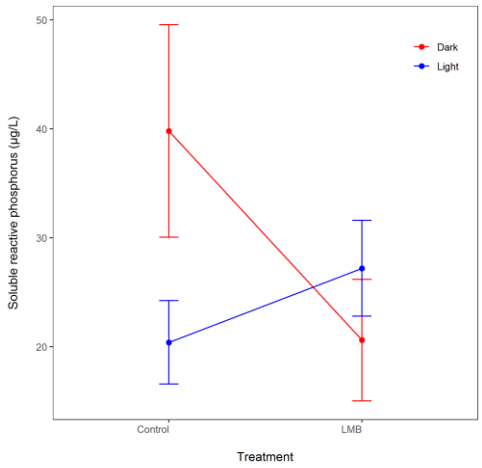

(b)




(c)




(d)




(e)

**Figure 10.** Soluble reactive phosphorus (µg $L^{-1}$) for *Potamogeton perfoliatus* (a), *Myriophyllum spicatum* (b), *Elodea canadensis* (c), *Littorella uniflora* (d) and *Najas flexilis* (e).