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Eutrophication homogenizes shallow lake macrophyte assemblages over space and time

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Abstract:	Eutrophication is commonly implicated in the reduction of macrophyte species richness in shallow lakes. However, the extent to which other more nuanced measures of macrophyte diversity, such as assemblage heterogeneity, are impacted concurrently by eutrophication over space and time and the joint influences of other factors (e.g. species invasions and connectivity) remains relatively poorly documented. Using a combination of contemporary and paleoecological data, we examine how eutrophication influences macrophyte assemblage heterogeneity, and how nutrient enrichment interacts with watercourse connectivity, lake surface area, and relative zebra mussel abundance over space (within and among lakes) and time (decades to centuries) at the landscape scale. The study system is the Upper Lough Erne, Northern Ireland, UK, which is composed of a large main lake and several smaller satellite lakes that vary in their hydrological connectivity to the main lake. By applying homogeneity analysis of multivariate dispersions and partial redundancy analysis we demonstrate that contemporary lake macrophyte heterogeneity and species richness are reduced in lakes with intensified eutrophication but are increased in lakes with greater zebra mussel abundance and lake surface area. Watercourse connectivity positively influenced assemblage heterogeneity and explained larger proportions of the variation in assemblage heterogeneity than local environmental factors, whereas variation in species richness was better related to local abiotic factors. Macrophyte fossil data revealed within and among-lake assemblage homogenization post-1960, with the main Lough

	and connected sites showing the highest rates of homogenization due to progressive eutrophication. The long-term and contemporary data collectively indicate that eutrophication reduces assemblage heterogeneity over time by overriding the importance of regional processes (e.g. connectivity) and exerts stronger pressure on isolated lakes. Our results suggest further that in connected lake systems, assemblage heterogeneity may be impacted more rapidly by eutrophication than species richness. This means that early effects of eutrophication in many systems may be underestimated by monitoring that focuses solely on species richness and is not performed at adequate landscape scales.

Running Head: Homogenization of lake assemblages (35 letters including space/40)

Eutrophication homogenizes shallow lake macrophyte assemblages over space and time

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Abstract

Eutrophication is commonly implicated in the reduction of macrophyte species richness in shallow lakes. However, the extent to which other more nuanced measures of macrophyte diversity, such as assemblage heterogeneity, are impacted concurrently by eutrophication over space and time and the joint influences of other factors (e.g. species invasions and connectivity) remains relatively poorly documented. Using a combination of contemporary and paleoecological data, we examine how eutrophication influences macrophyte assemblage heterogeneity, and how nutrient enrichment interacts with watercourse connectivity, lake surface area, and relative zebra mussel abundance over space (within and among lakes) and time (decades to centuries) at the landscape scale. The study system is the Upper Lough Erne, Northern Ireland, UK, which is composed of a large main lake and several smaller satellite lakes that vary in their hydrological connectivity to the main lake. By applying homogeneity analysis of multivariate dispersions and partial redundancy analysis we demonstrate that contemporary lake macrophyte heterogeneity and species richness are reduced in lakes with intensified eutrophication but are increased in lakes with greater zebra mussel abundance and lake surface area. Watercourse connectivity positively influenced assemblage heterogeneity and explained larger proportions of the variation in assemblage heterogeneity than local environmental factors, whereas variation in species richness was better related to local abiotic factors. Macrophyte fossil data revealed within and among-lake assemblage homogenization post-1960, with the main Lake and connected sites showing the highest rates of homogenization due to progressive eutrophication. The long-term and contemporary data collectively indicate that eutrophication reduces assemblage heterogeneity over time by overriding

the importance of regional processes (e.g. connectivity) and exerts stronger pressure on isolated lakes. Our results suggest further that in connected lake systems, assemblage heterogeneity may be impacted more rapidly by eutrophication than species richness. This means that early effects of eutrophication in many systems may be underestimated by monitoring that focuses solely on species richness and is not performed at adequate landscape scales.

Key words: assemblage heterogeneity; hydrological connectivity; lake isolation; landscape ecology; metacommunity; Paleolimnology; spatial variation; temporal variation; zebra mussel

INTRODUCTION

Aquatic macrophyte stands are a key component of shallow lake ecosystems, providing structurally complex habitats for many co-occurring organisms (Jeppesen et al. 1998) and contributing to biogeochemical cycling in shallow lakes (Davidson et al. 2015). However, intensification of anthropogenically-driven processes (e.g. eutrophication, introduction of exotic species and habitat fragmentation) over the last two centuries has commonly resulted in the loss of macrophyte stands and the development of turbid waters and algal blooms (Jeppesen et al. 2000, Scheffer et. al 2001). Many studies have now demonstrated how eutrophication influences species diversity and turnover (e.g. Jeppesen et al. 2000; Scheffer 1998, Scheffer et. al 2001, Sand-Jensen et al. 2008), but the extent to which eutrophication influences assemblage heterogeneity within and among lakes is poorly understood (Donohue et al. 2008). Even less known is how factors such as connectivity amongst sites and invasive species may interact concurrently with eutrophication to jointly influence macrophyte assemblage heterogeneity, although, as outlined below, research suggests that these are likely to be important.

Populations of the invasive zebra mussel (*Dreissena polymorpha* Pallas) can favor plant development and biomass because their suspension-feeding activities increase water clarity (Griffiths 1992, Ibelings et al. 2007, Zhu et al. 2006). These processes are likely to explain how zebra mussels can promote shifts from pelagic- to benthic-dominated food webs (Higgins and Vander Zanden 2010) and may thus potentially counter eutrophication effects.

77 Dispersal and connectivity may also compensate for eutrophication impacts.
78 For example source-sink dynamics may counter or delay extinction. In this scenario
79 dispersal from high ecological quality lakes (sources) may promote colonization and
80 the maintenance of viable populations of sensitive species in low quality lakes
81 (Mouquet and Loreau 2002). Dispersal may additionally facilitate the ability of
82 species to track variation in local environmental conditions according to preferred
83 nutrient enrichment conditions (species-sorting) (Leibold and Norberg 2004).
84 Dispersal and connectivity could therefore be major drivers of macrophyte diversity
85 both within lakes and amongst highly connected lakes, while environmental processes
86 such as eutrophication may exert greater influences on macrophyte diversity in more
87 isolated sites because of diminished dispersal-mediated rescue effects (Brown and
88 Swan 2010). A strong eutrophication pulse may also have more impact in small,
89 disconnected lakes if there is no dilution from elsewhere in the catchment (Strecker
90 and Britatin 2017). Connectivity, however, may also be detrimental. For example,
91 recurrent flooding may act as a homogenizing force, decreasing variation in species
92 composition and environmental conditions between lakes and increasing the spread of
93 both native and non-native species (Levine 2000).
94 This study aims to identify factors driving macrophyte assemblage
95 heterogeneity in a large central lake and in a set of associated well-connected, smaller
96 satellite lakes (loughs) in the Upper Lough Erne (ULE) system, Northern Ireland by
97 collecting and analyzing present-day and paleoecological data. In particular we used
98 homogeneity analyses of multivariate dispersions and partial redundancy analyses to:
99 (i) examine how watercourse connectivity, relative abundances of invasive zebra
100 mussels and lake surface area interact regionally with eutrophication to influence

macrophyte diversity within and between the water bodies in this system over time (last 150 years) and space; and (ii) how spatial and temporal patterns of macrophyte assemblage heterogeneity may differ from those associated with other common measures of macrophyte diversity, such as species richness. Our study provides important insights into the combined effects of environmental change and connectivity on macrophyte diversity across multiple lakes at a time-scale (decades to centuries) relevant to the widespread, eutrophication-driven deterioration of biodiversity in shallow lakes.

METHODS

Study site

The study system is a complex and dynamic riverine system comprising the River Erne that feeds a main central lake (Upper Lough Erne) in Co. Fermanagh Northern Ireland (54.2154° N, 7.5103° W), UK (Fig. 1). The main Lough (Upper Lough Erne) is a large (34.5 km^2), shallow (mean depth 2.3 m), and eutrophic (total phosphorous (TP) ~70 µg L^{-1}) lake. It is surrounded by a series of interconnected, smaller, shallow (<5 m) satellite lakes that vary in degree of enrichment (TP ~ 30-400 µg L^{-1}). The ULE system is a Special Area of Conservation (SAC) under the EC Habitats Directive and supports several species with restricted distributions in the UK. Nevertheless, the ULE system has been affected by progressive eutrophication since the 1960s (Zhou et al. 2000). Prior to the 1900s the ULE system was characterized by lower productivity and greater variation in hydrological connectivity (Salgado et al. 2017). Water-level regulation schemes implemented in the late 1800s and 1940s acted to reduce water-level fluctuation in the main lake but were unsuccessful in preventing

flood events, which periodically inundate much of the ULE area. The zebra mussel invaded the system in the 1990s (Minchin et al. 2003).

Data collection

Nineteen satellite lakes and three basins in the Belleisle, Trannish and Crom areas within the main Lough were selected for study (Fig. 1). Selection criteria included: replication along a gradient of enrichment (total phosphorous [TP], total nitrogen [TN], and chlorophyll *a* [Chl-*a*]), water transparency (secchi disk) and a gradient in watercourse connectivity between the satellite lakes and the main Lough. Data for Chl-*a*, TN and TP and for lake surface areas were obtained from lake condition assessments of the ULE system made for the Northern Ireland Environmental Agency (NIEA) during 2006-2007 (Goldsmith et al. 2008) (Table 1). The Water Management Unit of NIEA provided additional water chemistry data for the Belleisle, Crom and Trannish areas of the main Lough. Water chemistry sampling and laboratory protocols are presented in Appendix S1.

Submerged and floating-leaved macrophyte (angiosperms, bryophytes, and charophytes) abundances and species data were derived from assessments of lake conditions in the ULE system for the NIEA by Goldsmith et al. (2008). Standard Joint Nature Conservation Committee (JNCC) protocols for Site Monitoring (JNCC 2009) were followed. This methodology allows for the characterization of macrophyte assemblages within lakes based on shoreline and boat surveys. Accordingly, data were collected from different sectors of a lake using a combination of two sampling approaches (shoreline and deeper water transects) in each sector to give good spatial coverage (Gunn et al. 2010). In particular, macrophyte data were collected along a

100 m wader-depth shoreline transect by sampling at four depths (25 cm, 50 cm, 75 cm and > 75 cm) at each 20 m interval (20 points in total per shoreline transect). Macrophytes in deeper water were surveyed using a boat to collect data (at depths > 75 cm) along a transect starting at the midpoint of the shoreline transect and running towards the center of the lake. Macrophytes were sampled at every 5 m along this 100 m deeper-water transect (20 points in total). At each point, we used a combination of bathyscope and grapnel sampling, and all aquatic macrophyte species occurring within a $1m^2$ area were recorded using an abundance scale of 0-3, where 0 = absent and 3 = highly abundant. Between two and three sectors were sampled per satellite lake (see Table 1 for details). Representation of the main macrophytes present in each lake was the basis for selecting sectors for sampling – a selection requiring expertise in macrophyte identification and fieldwork experience. This JNCC method has been demonstrated to adequately characterize macrophyte communities in small lakes (< 50 Ha hectares) by sampling two to three sectors (Gunn et al. 2010). Accordingly we sampled 2-3 sectors in the majority of our sites. Exceptions were made for Sarah and Pound Loughs whose small size (< 2 Ha.) precluded surveying more than 1 sector and for Lough 904, where site accessibility prevented surveying more than 1 sector (Goldsmith et al. 2008). The main Lough was divided into three separate study basins and, due to their large size, eight sectors per basin were surveyed. It should be stressed that such sampling along representative transects in a lake will almost certainly not identify all macrophyte species within lakes, but the approach can provide relative data on variation in distributions and abundances (i.e. heterogeneity) of the most typical species within lakes (Gunn et al. 2010). At the same time as surveying for macrophytes we also collected data on relative zebra mussel abundance.

Thus, at each macrophyte sampling point we noted the presence of zebra mussels through direct observations using the bathyscope and/or through individuals collected along with macrophytes when using the rake. Mussel relative abundances within lakes were then quantified using a semi-quantitative scale (0-3) as follows: 0 = no zebra mussels observed in any sampling point; 1 = zebra mussels observed in <10 sampling points; 2 = zebra mussels observed in 10-20 sampling points; and 3 = zebra mussels observed in > 20 sampling points. Consistent sampling of zebra mussels within and amongst lakes provided comparative data of their relative abundances.

To characterize temporal variation in within-lake macrophyte assemblage heterogeneity we used paleoecological methods spanning the last *c.*200 years. We analyzed plant macrofossils from short sediment cores (~ 1 m long) collected during the summers of 2008 and 2009 from 6 of the 21 sites surveyed for present-day data: Trannish basin of the main Lough (core code ULET2), Castle Lough (NCAS3), Cornabrass Lough (CBRAS1), Gole Lough (GOLE1) Killymackan Lough (KILL2) and Lough Head (HEAD1) (Fig. 1). One short sediment core was collected near a basin in the Trannish area from the main Lough (ULET2) using an adapted Livingstone corer (7.4 cm diameter; Livingstone 1955). For the remaining lakes, single sediment cores were collected using a wide-bore (14 cm diameter) "Big-Ben" piston corer (Patmore et al. 2014). Cores were collected from similar macrophyte rich and shallow basins (water depths 90-180 cm). Lithostratigraphic changes in the cores were noted and cores were then extruded in the field at 1-cm intervals.

The cores were dated using a combination of techniques. For the 5 satellite lakes, we used radionuclide measurements of ^{210}Pb (half-life 22.3 years) and ^{137}Cs and ^{241}Am (Appleby et al. 1986). Dates at specific levels were ascribed using the Constant Rate

of Supply (CRS) model (Appleby and Oldfield 1978) (see Appendix S2: Tables S2.1-8; Fig. S2). Due to high sedimentation rates in the top 20 cm of core HEAD1, the CRS dating model covered only the last 23 years. Thus, we cross-correlated the remaining selected sediment samples with the dated profiles of two cores taken from two similar hypertrophic lakes (GOLE1-Gole Lough, included in this study; and DHOW1-Derryhowlght Lough, unpublished data), which had relatively similar sedimentation rates but greater chronological resolution (Appendix S2: Tables S2.1-8; Fig. S2). As funds were not available for dating the core from the main Lough (ULET2), selected levels were estimated from the core ULET3 (unpublished data; Appendix S2: Tables S2.1-8; Fig. S2), an extra ^{210}Pb dated core obtained from Castle Lough (NCAS1; Salgado et al. in press) and three of the study satellite lakes (NCAS3, CBRAS1 and KILL2) which had relatively similar sedimentation rates and similar ranges of total phosphorous concentrations to those observed in the main Lough (See Table 1).

A selected number of sediment slices of 1-cm were analyzed from all lake cores (NCAS3, n=10; CBRAS1, n=13; ULET2, n= 9; KILL2, n=12; GOLE1, n=8, HEAD1=11). Sampling resolution was dictated by intrinsic sedimentation rates within each core (Appendix S2: Tables S2.1-8). All samples were disaggregated in 10% potassium hydroxide (KOH) before sieving. Macrophyte fossils were retrieved from the residues of sieved core material (using mesh sizes of 355 µm and 125 µm) following standard methods (Birks 2001) and were identified by comparison with reference material and various taxonomic guides (e.g. Birks 2001). Macrophyte fossil abundances were estimated by counting seeds, leaves, and spines and the data were standardized as the numbers of fossils per 100 cm^3.

Statistical methods

To understand how variation in macrophyte assemblage heterogeneity amongst lakes differs from variation in other measures of macrophyte diversity, we conducted analyses on both assemblage heterogeneity and species richness. Species richness was measured as the total number of species recorded per site during the contemporary surveys. We defined lake assemblage heterogeneity as the variation in macrophyte species occurrences and abundances between different sampling points within a lake (Anderson et al. 2006), and it was calculated as the mean distance to group median (DGM) in ordination space using homogeneity analysis of multivariate dispersions (HMD) on Bray-Curtis dissimilarities (Anderson 2006). HMD applies an ANOVA under the null hypothesis of no difference in multivariate dispersion among sets of lakes (Anderson 2006). Lakes with greater multivariate dispersion (higher values of mean distance to group median) were characterized by greater species dissimilarities and more heterogeneous macrophyte assemblages; more homogenous macrophyte assemblages characterized lakes with low multivariate dispersion. To assess differences in assemblage heterogeneity among lakes we conducted an overall HMD analysis (each lake being treated as an independent group) using ANOVA. HMD analysis was performed in R using the "*betadisper*" package (R Core Team 2016). We pooled shoreline and boat data for each lake transect and, with exceptions of Sarah and Pound Loughs, 40 randomly chosen points (*set.seed* and *sample* algorithms in R; R core Team 2016) per lake (20 littoral and 20 open water from all transects) were selected for the analysis. We used this stratified sampling design because the variability within a chosen subset of data is lower compared to variation of the entire population, and hence has a high statistical precision while requiring smaller sample

243 sizes in comparison to other approaches (Legendre and Legendre 2012). Absence of
244 macrophyte species in some areas within a lake was common, especially for lakes
245 with high TP and TN concentrations. Because such absences can be equated to
246 reductions in plant heterogeneity, absence was included in our analyses and coded as
247 pseudo-species with an abundance value of 0.01.

248 To identify the unique contributions of eutrophication, relative zebra mussel
249 abundance, watercourse connectivity and lake surface area in determining
250 contemporary macrophyte assemblage heterogeneity and species richness we
251 conducted partial regression analysis (pRA; Borcard et al. 1992, Legendre and
252 Legendre 2012) using the "*varpart*" package in R (R Core Team 2016). Watercourse
253 connectivity predictors were calculated through Asymmetric Eigenvector Maps
254 (AEM) analysis (Blanchet et al. 2008a) using the "*AEM*" package in R (R Core Team
255 2016). AEM variables were derived from a matrix of hydrological connectivity (Fig.
256 1b), based on the presence/absence of links such as rivers and streams between two
257 given sites (Blanchet et al. 2008a, Blanchet et al. 2011). Due to a lack of detailed
258 hydrological knowledge about each watercourse, we assumed that all connecting links
259 shared the same ease of water movement between sites (Appendix S3: Fig. S3; Table
260 S3).

261 Significant environmental variables (log-transformed TP, TN, and Chl-*a* data,
262 zebra mussel abundance and log-transformed lake surface area) and AEM
263 connectivity predictors were detected through forward selection analysis ("*packfor*" in
264 R; R Core Team 2016) by following Blanchet et al. (2008b). Unfortunately secchi
265 disk measurements strongly correlated with the other variables (such as nutrients and
266 zebra mussels) making it very difficult to disentangle the unique effects of each

parameter. Secchi disk data were therefore excluded from the analysis. The explained variation of each independent and shared fraction in the pRA was corrected following Peres-Neto et al. (2006) and expressed as adjusted R^2 ($_{adj}R^2$) values. The significance of each component was tested through 999 random Monte Carlo permutations under the reduced model. We plotted the data to visually assess the direction of the relationships. To observe spatial patterns in significant predictors we divided the macrophyte assemblage heterogeneity and the species richness data sets into three connectivity groups according to water flow directions as follows (Fig. 1): *Group 1*- comprising lakes directly connected to the main Lough via the River Erne (e.g. Castle Lough); *Group 2*- comprising lakes with a lateral connection to the main Lough via tributaries (e.g. Kilmore Lough); *Group* 3- comprising isolated lakes or those laterally connected to the main Lough via one or more intermediate lakes (e.g. Killymackan Lough).

We calculated temporal variation of macrophyte assemblages in each lake by splitting the paleoecological data into two time-intervals on the basis of the environmental history of the system. These were: (i) *c.* pre-1900; and (ii) post-1960 for cores NCAS3, CBRAS1, ULET2 and KILL2. The macrofossil data for GOLE1 core only spanned the last *c.* 110 years so for this core we characterized temporal variation in the plant community for: (i) 1959-1880; and (ii) post-1960. Each temporal lake group contained 4-6 sediment samples per core. Macrofossil abundance data were transformed to a DAFOR (Dominant, Abundant, Frequent, Occasional, Rare) scale (Salgado et al. in press) to reduce bias associated with differential production and preservation of plant structures (e.g. spines, leaves and seeds). We conducted HMD analysis on assemblage heterogeneity both within and among sites (between

time-periods) using Bray-Curtis dissimilarities. Among-site variation between time periods was calculated by grouping all data at each time-period (each time period treated as an independent group) and tested via ANOVA. To visualize temporal variation in lake assemblages we ran non-metric multi-dimensional scaling analysis (NMDS) on Bray-Curtis dissimilarities. We identified characteristic species in each time period using the IndVal method ("*labdsv*" in R; R Core Team 2016) of Dufrêne and Legendre (1997).

RESULTS

Variation in contemporary macrophyte diversity

Forty-five submerged and floating-leaved macrophyte species were sampled across the study sites (Appendix S4: Table S4). HMD analysis revealed significant variation in contemporary macrophyte assemblage heterogeneity amongst the study sites (F= 5.5245, P<0.001). TN (annual average measurements), relative zebra mussel abundance and lake surface area were identified by forward selection as significant (P<0.05) explanatory variables for both macrophyte assemblage heterogeneity and species richness. Three watercourse explanatory variables (AEM1, AEM7, AEM14) for assemblage heterogeneity and two watercourse explanatory variables (AEM2, AEM6) for species richness were also identified.

pRA showed that watercourse connectivity alone explained a significant (P<0.001) 50% of adjusted variation in macrophyte assemblage heterogeneity amongst sites (Fig. 2a). Spatial structure and shared variation between environmental variables explained the following adjusted variation in macrophyte assemblage heterogeneity: (i) watercourse connectivity and TN (1%); (ii) watercourse

connectivity, TN and zebra mussel abundance (2%); (iii) watercourse connectivity, TN, relative zebra mussel abundance and lake surface area (23%); (iv) TN, relative zebra mussel abundance and lake surface area (2%); and (v) watercourse connectivity, TN, and lake surface area (2%). Unexplained residual variation accounted for 28% of variation in macrophyte assemblage heterogeneity amongst sites.

pRA on macrophyte species richness resulted in TN and watercourse predictors explaining a significant (P<0.01) 3% and 21% of the adjusted variation, respectively (Fig. 2b). Spatial structure and shared variation between environmental variables together explained the following variation in macrophyte species richness amongst sites: (i) watercourse connectivity and TN (4%); (ii) TN, zebra mussel abundance, lake surface area and watercourse connectivity (13%); (iii) zebra mussel abundance, watercourse connectivity and lake surface area (1%); (iv) TN, lake surface area and zebra mussel abundance (14%); and (v) TN and lake surface area (10%). Unexplained residual variation accounted for 43% of adjusted variation in macrophyte species richness.

Regression plots of the contemporary macrophyte data revealed that concentrations of TN increased while macrophyte assemblage heterogeneity and species richness declined with lake isolation (Fig. 3). In turn, greater macrophyte species richness and more heterogeneous plant assemblages were associated with greater zebra mussel abundance and larger lake surface areas (Fig. 3).

Temporal trends in macrophyte assemblage variation

HMD analyses of lake macrophyte fossil data indicated a strong homogenization of macrophyte assemblages within the lakes post-1960 (Fig. 4a). We observed greater rates of post-1960 assemblage homogenization in the main Lough

(68%; ΔDGM =-0.15; $DGM_{pre\text{-}1900}$ = 0.22; $DGM_{post\text{-}1950}$ = 0.07), in Cornabrass Lough (40%; ΔDGM =-0.13; $DGM_{pre\text{-}1900}$ = 0.32; $DGM_{post\text{-}1950}$ = 0.19) and in Castle Lough (35%; ΔDGM =-0.05; $DGM_{pre\text{-}1900}$ = 0.16; $DGM_{post\text{-}1950}$ = 0.10) than in the more isolated lakes Killymackan Lough (22%; ΔDGM =-0.04; $DGM_{pre\text{-}1900}$ = 0.20; $DGM_{post\text{-}1950}$ = 0.15), Gole Lough (14%; ΔDGM =-0.04; $DGM_{pre\text{-}1900}$ = 0.25; $DGM_{post\text{-}1950}$ = 0.21) and Lough Head (22%; ΔDGM =-0.04; $DGM_{pre\text{-}1900}$ = 0.28; $DGM_{post\text{-}1950}$ = 0.24). Amongst-lake analysis revealed significant (F= 6.8939; P=0.01) post-1960 homogenization and convergence towards similar macrophyte assemblages across lakes (Fig. 4b). IndVal analysis (Fig. 5) revealed that this post-1960 homogenization of macrophyte assemblages was generally due to declines in abundances of oligo-mesotrophic taxa (including bryophytes, *Isoetes lacustris* L., *Lobelia dortmanna* L., *Najas flexilis* Willd. Rost & Schmidt, *Potamogeton praelongus* Wulfen., *Potamogeton lucens* L.) (Fig. 5) and increases in the abundances of species associated with more eutrophic environments (such as *Lemna minor* L., *Potamogeton pusillus* L., *Potamogeton berchtoldii* Fieber., *Nuphar lutea* L., and *Nymphaea alba* L.).

DISCUSSION

Impacts of eutrophication on macrophyte assemblages in space and time

Our analyses reveal that gradual and progressive nutrient enrichment strongly erodes lake macrophyte assemblage heterogeneity across spatial and temporal scales. Both contemporary and paleoecological data reveal changes indicative of macrophyte homogenization. These changes were manifested post-1960 in the paleoecological data and at TN values >1.1 µg/L in the contemporary data, and are characterized by

increases in the dominance of fine-leaved *Potamogeton* species *(*e.g. *P. pusillus* and *P. berchtoldii*), *E. canadensis* and floating-leaved taxa (water-lilies, and *L. minor*) and decreases in nutrient-intolerant taxa such as *I. lacustris, N. flexilis,* and several broad-leaved *Potamogeton* taxa (Figs. 5, S4; Kolada et al. 2014).

The decline of macrophyte cover and species richness in shallow lakes caused by eutrophication is well documented (e.g. Scheffer 1998, Jeppesen et al. 2000, Kolada et al. 2014). Eutrophication may stimulate a range of responses including gradual vegetational shifts (e.g. from isoetid to more diverse stands of submerged elodeid macrophytes; Arts 2002; Willby et al. 2012), decreases in the seasonal duration of elodeid macrophyte coverage (Sayer et al. 2010a) and apparently sudden shifts from clear water (with abundant and diverse macrophytes) to turbid water conditions (with low transparency and fewer macrophytes; Scheffer 1998, Scheffer et al. 2001, Scheffer et al. 2003). However, despite this relatively large body of research, eutrophication-driven changes in assemblage heterogeneity have received relatively little attention in comparison to studies focusing on patterns of macrophyte abundance and species richness (e.g. Jeppesen et al. 2001, Scheffer et al. 2001, Scheffer et al. 2003, Sayer et al. 2010a). Our analyses of contemporary and paleoecological data provide novel and nuanced insights on eutrophication impacts across the landscape, revealing that satellite lakes connected to the main Lough experienced higher post-1960s rates of macrophyte assemblage homogenization than the more isolated lakes (Fig. 4a). These patterns suggest that prior to 1900 regional processes (e.g. seasonal flooding and variation in water level) were influential in maintaining assemblage heterogeneity concurrently in the main Lough and in proximal satellite lakes (Castle and Cornabrass), but eventually (post-1960s) these

influences were overridden by progressive nutrient enrichment. A paleoecological study by Salgado et al. (2017) addressing macrophyte assemblage variation across three basins in Castle Lough, revealed similar nutrient effects over a decadal to centennial scale (10-100 years), with former drivers of assemblage heterogeneity (e.g. water depth) gradually being displaced by nutrient enrichment, leading eventually to dominance by a few highly competitive macrophyte species. Potential drivers of homogenization include gradual increases in phytoplankton concentrations that restrict macrophyte distributions within lakes and decreases in seasonal duration with macrophytes developing over shorter periods during summer (Sayer et al. 2010a,b). Other mechanisms are reductions in photosynthetic rates and plant growth due to reduced water transparency (Spence 1967), and selection for taxa (e.g. *E. canadensis*) that can grow at lower light levels (Spence and Chrystal 1970).

Homogenization of macrophyte assemblages across sites

The theory of island biogeography (MacArthur and Wilson 1967) and the metacommunity concept (Leibold and Norberg 2004) predict that biodiversity patterns in well-connected landscapes are driven by patch size, habitat quality, environmental heterogeneity and connectivity. Our results support these predictions, revealing that current macrophyte assemblage homogenization and species loss by eutrophication involve interactions of lake surface area, relative zebra mussel abundance, and watercourse connectivity (Fig. 2). We found positive effects of lake surface area and relative zebra mussel abundance on both macrophyte assemblage variation and species richness in the main Lough and in directly connected satellite lakes (connectivity Group 1). The positive effect of habitat size on plant diversity is one of the most supported patterns in ecology (MacArthur and Wilson 1967), and may

409 be explained by greater diversity of niches and a larger area for colonization. Our
410 analyses also revealed reductions of both macrophyte diversity measures associated
411 with increases in nutrient inputs in more isolated sites (Fig. 3).

412 Zebra mussel abundance was higher in the main Lough than in most satellite
413 lakes and this may have improved conditions for macrophyte communities by
414 enhancing water transparency (Griffiths 1992, Ibelings et al. 2007). The capacity of
415 zebra mussel populations to filter substantial volumes of water year-round (Strayer
416 2009) can lead to significant loss of phytoplankton (as suggested by our
417 measurements of Chl-*a*) (Higgins and Vander Zanden 2010). The higher
418 concentrations of TN, lower mussel abundances, and elevated levels of Chl-*a* in more
419 isolated sites (Fig. 3), may promote domination by macrophytes species that tolerate
420 nutrient enrichment (e.g. fine-leaved *Potamogeton* species and *E. canadensis*) and the
421 reduction/displacement of intolerant species (e.g. broad-leaved *Potamogeton* species),
422 resulting in more homogenous assemblages. The rarity of zebra mussels in most
423 isolated lakes could be the result of dispersal limitation (Heino and Moutka 2006) or
424 less favorable conditions for zebra mussel establishment in the organic-rich and silty
425 sediments that characterize most satellite lakes (Strayer 2009).

426 In freshwater systems, connectivity has been characterized as a double-edged
427 sword, promoting diversity but also homogenizing regional communities and abiotic
428 factors and accelerating the spread of invasive species (Strecker and Brittain 2017). In
429 keeping with previous studies (Grant et al. 2012; Strecker and Brittain 2017) we
430 found that increasing connectivity was associated with the occurrence of common
431 taxa, thus increasing local species richness. Highly connected lakes harbored the
432 highest number of species, and were characterized by an average of 5-6 more species

than more isolated lakes (Fig. 3). Disentangling the unique effects of dispersal on species richness is challenging and requires further investigation given the interacting effects of connectivity, relative zebra mussel abundance and lake surface area.

Responses of diversity measures: assemblage heterogeneity vs. *species richness*

Our results revealed declines in both macrophyte assemblage heterogeneity and species richness with increasing eutrophication (Fig. 3). However, the importance of local *vs.* regional factors in explaining variation associated with these diversity measures differed (Fig. 2). Watercourse connectivity was positively associated with macrophyte assemblage heterogeneity and explained a larger proportion of the variation in assemblage heterogeneity than local abiotic factors. Macrophyte species richness, however, was positively associated with zebra mussel abundance and lake surface area and was negatively associated with eutrophication. In addition, local abiotic factors explained a greater proportion of variation in species richness than connectivity. These contrasting patterns indicate that eutrophication effects are variable but sufficiently large to influence species composition in the ULE system while dispersal amongst hydrologically connected sites may ultimately maintain macrophyte species abundances that are sensitive to nutrient enrichment within the system (Amarasekare and Nisbet 2001, Mouquet and Loreau 2002). By analyzing measures of both macrophyte assemblage heterogeneity and species richness our study highlights how regional environmental heterogeneity and spatial gradients in connectivity can influence diversity and dominance and rareness (relative abundance) of plant species in connected landscapes (Amarasekare and Nisbet 2001, Mouquet and Loreau 2002).

CONCLUSION

By combining landscape-scale contemporary and paleoecological perspectives we provide evidence that increasing eutrophication has reduced macrophyte diversity over space and time but that watercourse connectivity moderates eutrophication effects. Isolated lakes were characterized by greater impacts of eutrophication but lower rates of macrophyte assemblage homogenization. In connected lakes rates of macrophyte assemblage homogenization have been higher but heterogeneity in macrophyte assemblages has persisted to the present-day. This heterogeneity enables the main Lough and associated satellite lakes to act collectively as a biodiversity hub, contributing to the integrity and richness of the system through hydrological connections that promote biotic exchange. Our analyses additionally suggest that invasive zebra mussels, large surface areas, source-sink and species-sorting dynamics all contribute to maintaining the relatively high macrophyte assemblage heterogeneity in these connected water bodies. However, our analyses also reveal that eutrophication impacts have been countering some diversity-generating processes, such as connectivity, over time. There is thus a danger of eventual convergence to homogenous macrophyte assemblages across the Upper Lough Erne system. It would be of interest to determine how changes in the relative abundances of component species in this connected landscape have already impacted ecosystem function (Chapin et al. 2000).

ACKNOWLEDGMENTS

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FIGURES

Fig. 1. Map of the Upper Lough Erne (ULE) system. The main Lough is indicated in dark blue with three studied basins Crom, Trannish and Belleisle indicated by a red circle. Contemporary studied satellite lakes are presented in red and lakes having paleoecological data are highlighted with a yellow circle. A number in parenthesis identifies three connectivity groups according to the water flow direction. These are: *Group 1*- lakes directly connected to the main Lough via the River Erne flow; *Group 2*- lakes with a direct lateral connection to the main Lough. *Group* 3- lakes connected laterally to the main Lough via 1 or more intermediate lakes. Flood layers (pale blue) were obtained from SERITT and water layers from Northern Ireland Ordnance Survey (OSi) maps (https://www.osi.ie).

Fig. 2. Venn diagrams of partitioning redundancy analysis performed on the relative contribution of TN, zebra mussel abundance and lake surface area on (a) contemporary lake macrophyte assemblage heterogeneity (measured as distance to group median in the multivariate space); and (b) contemporary macrophyte species-richness. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Fig. 3. Linear relationships of: (a) lake contemporary macrophyte assemblage heterogeneity (measured as distance to group median in the multivariate space) *vs.* TN. zebra mussel abundance and lake surface area; and (b) contemporary lake macrophyte species richness *vs.* TN. zebra mussel abundance and lake surface area. Circles (Group 1), triangles (Group 2), and squares (Group 3) identify the lakes in the three watercourse connectivity groups (Figure 1).

Fig. 4. (a) Variation in lake macrophyte assemblage heterogeneity (measured as distance to group median-DGM in the multivariate space) during two time periods

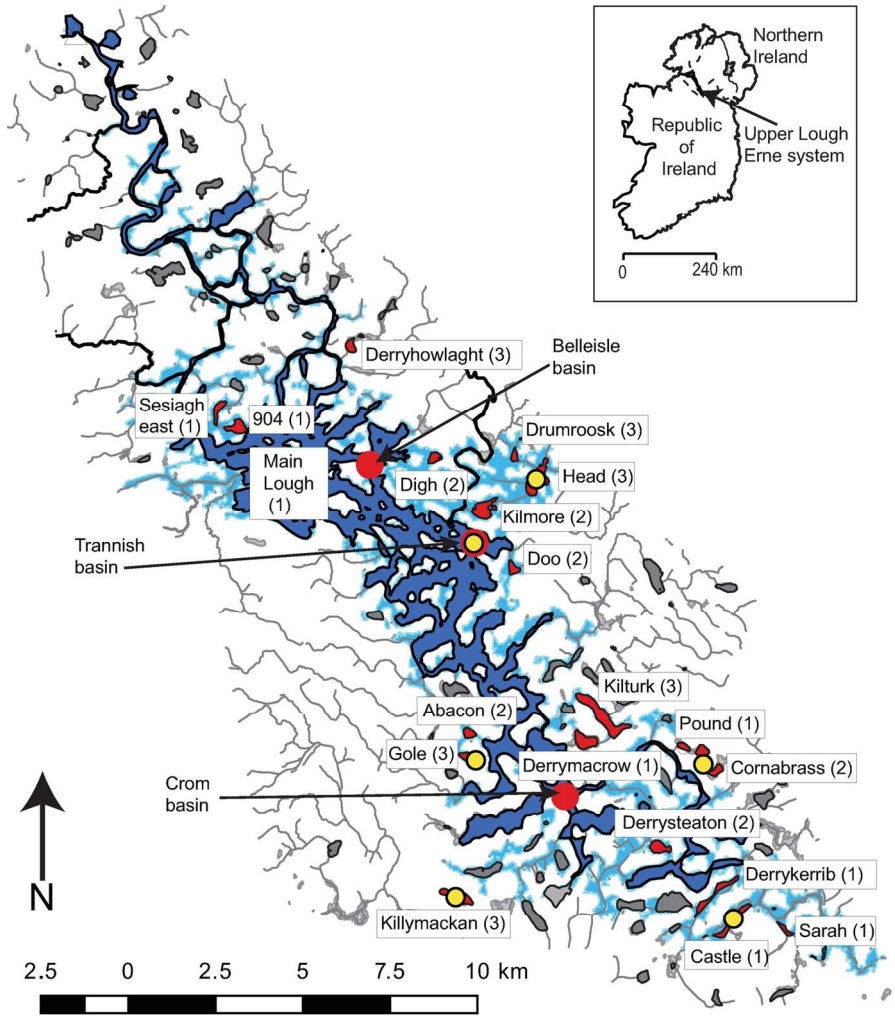
across the lakes. For the main Lough and the satellite lakes Castle, Cornabrass, Killymackan, and Head, the time periods are: *c.* pre-1900 (blue diamonds) and 1960-present (green circles). For Gole Lough the time periods are: 1900-1959 (blue diamonds) and 1960-present (green circles). Rates of variation in macrophyte assemblages between periods (ΔDGM) were calculated as: $DGM_{pre\text{-}1900} - DGM_{post\text{-}1950}$ expressed in percentage. (b) Non-Metric Multi-dimensional Scaling (NMDS) analysis showing each lake distance to group median over the periods *c.* pre-1900 (blue diamonds) and 1960-present (green circles). Dotted lines denote the lake groups at the two time periods showing a significant (F= 6.8939; P=0.01) homogenization among lakes at post-1960.

Fig. 5. Characteristic macrophyte fossil taxa revealed by maximum IndVal abundance values for two time periods in the lakes. For the main Lough and the satellite lakes Castle, Cornabrass, Killymackan, and Head, the time periods are: *c.* pre-1900 (blue diamonds) and 1960-present (green circles). For Gole Lough the time periods are: 1900-1959 (blue diamonds) and 1960-present (green circles). † leaves, ‡ leaf-spine, § sclereids, # megaspores, ¶ oospores, || seeds, * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

664 Table 1. Macrophyte diversity measures, relative zebra mussel abundance, summer and annual averages of total phosphorous (TP), total nitrogen
665 (TN), and chlorophyll-a (Chl-*a*), surface area and number of sectors sampled in 22 sites (19 satellite lakes and in three basins in the main Lough–
666 Upper Lough Erne).

Lake	Macrophyte assemblage heterogeneity	Macrophyte species richness	Zebra mussel (abundance)	Average summer TP (µg/L)	Average summer TN (µg/L)	Average summer Chl-*a* (µg/L)	Annual TN (µg/L)	Annual TP (µg/L)	Annual Chl-*a* (µg/L)	surface area (Ha.)	No. of sectors
Sarah Lough	0.55	12	0	84	0.98	11.5	0.98	61	7	1.6	1
Castle Lough	0.45	18	1	22	0.47	5.9	1.03	29	4.2	13	2
Derrykerrib Lough	0.46	15	1	44	0.45	13.1	0.97	33	8.6	10.5	2
Derrysteaton Lough	0.57	7	1	202	0.76	11.1	1.03	123	7.1	12	2
Cornabrass Lough	0.55	15	0	86	0.54	6.1	1.05	96	5.3	18	3
Pound Lough	0.39	13	0	285	2.76	12	2.25	185	9	1.25	1
Main Lough-Crom	0.59	19	3	65	0.28	5.4	0.28	65	5.4	862.5	8
Killymackan Lough	0.48	17	0	159	0.4	30.1	0.8	111	17.4	19.2	3
Derrymacrow Lough	0.54	13	1	78	0.56	12.8	1	83	8.2	21	3
Kilturk Lough	0.54	17	2	92	0.56	14.7	0.92	111	9	43	3
Abacon Lough	0.55	6	1	105	0.84	18	1.64	100	24.2	7	2
Gole Lough	0.43	7	0	172	0.47	22	1.35	128	13.8	8	3
Mail Lough-Trannish	0.60	21	3	70	0.22	7.2	0.22	79	7.2	862.5	8
Lough Doo	0.56	13	0	45	0.6	5.9	1.18	54	5	5	2
Kilmore Lough	0.55	14	0	228	0.45	11.5	1.09	186	6.5	20	2
Lough Head	0.42	10	0	327	0.51	8.7	1.79	383	9	31	3
Drumroosk Lough	0.43	9	0	238	2.22	14.5	1.99	168	12.9	4	2
Lough Digh	0.60	14	0	62	1.2	11.9	1.4	81.5	10.2	9	2
Main Lough-Belleisle	0.59	15	3	66	0.24	3.1	0.24	66	3.1	862.5	8
Lough 904	0.59	12	1	28.5	1.55	6.8	1.2	43	6.4	11	1
Sessiagh East Lough	0.50	11	1	39	0.6	10.8	0.9	45	7.9	8	3
Derryhowlaght Lough	0.54	9	0	161	1	32.3	1.7	158.8	18.3	4	2

667



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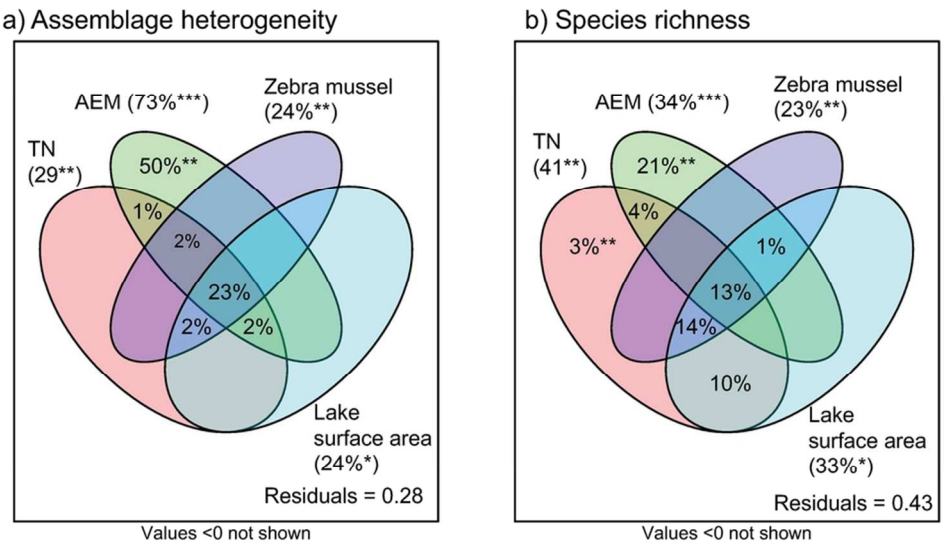

Fig. 2. Venn diagrams of partitioning redundancy analysis performed on the relative contribution of TN, zebra mussel abundance and lake surface area on (a) contemporary lake macrophyte assemblage heterogeneity (measured as distance to group median in the multivariate space); and (b) contemporary macrophyte species-richness. * P ≤ 0.05; **P ≤ 0.01; *** P ≤ 0.001.

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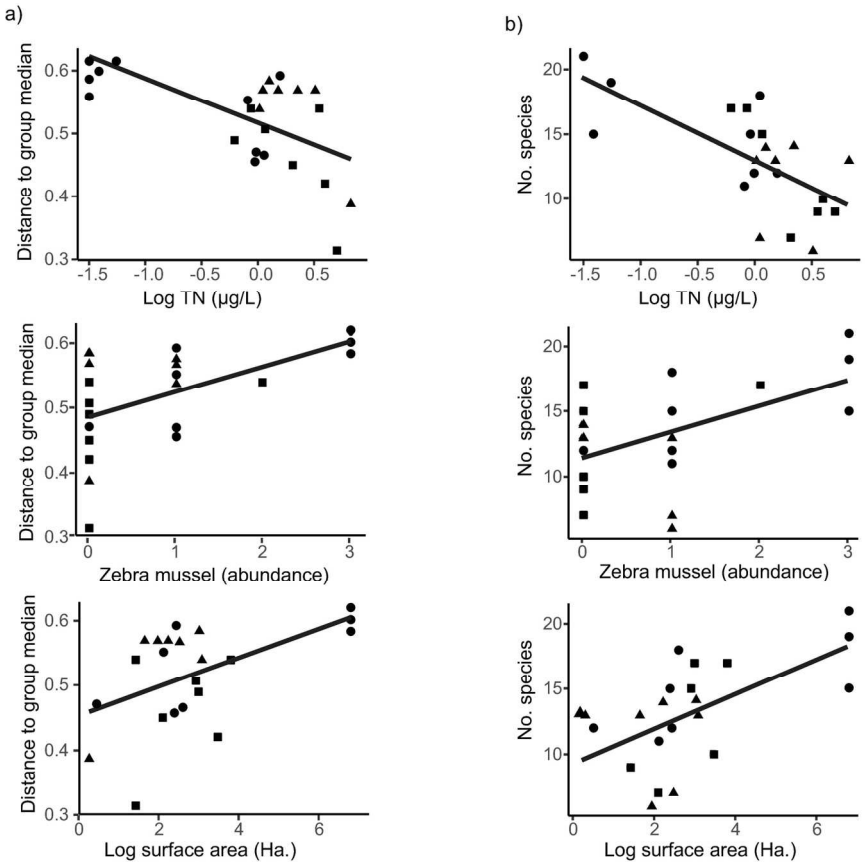

Fig. 3. Linear relationships of: (a) lake contemporary macrophyte assemblage heterogeneity (measured as distance to group median in the multivariate space) vs. TN. zebra mussel abundance and lake surface area; and (b) contemporary lake macrophyte species richness vs. TN. zebra mussel abundance and lake surface area. Circles (Group 1), triangles (Group 2), and squares (Group 3) identify the lakes in the three watercourse connectivity groups (Figure 1).

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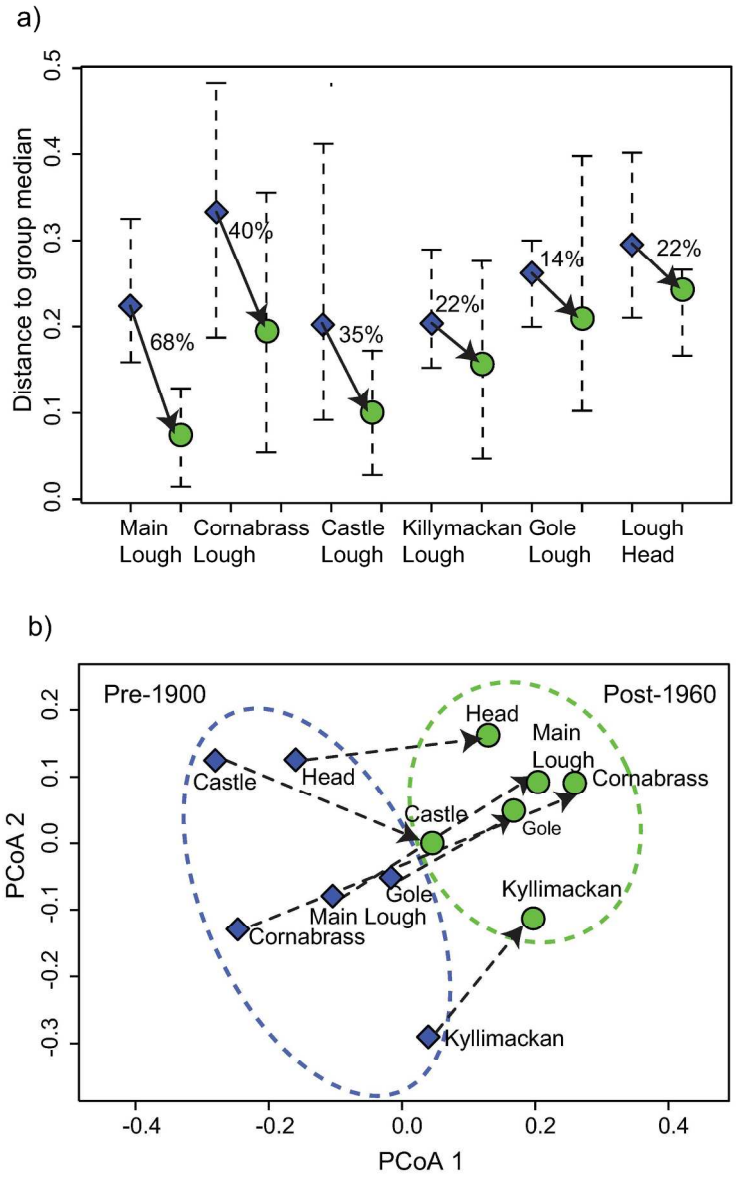

Fig. 4. (a) Variation in lake macrophyte assemblage heterogeneity (measured as distance to group median-DGM in the multivariate space) during two time periods across the lakes. For the main Lough and the satellite lakes Castle, Cornabrass, Killymackan, and Head, the time periods are: c. pre-1900 (blue diamonds) and 1960-present (green circles). For Gole Lough the time periods are: 1900-1959 (blue diamonds) and 1960-present (green circles). Rates of variation in macrophyte assemblages between periods (ΔDGM) were calculated as: DGM pre-1900 – DGM post-1950 expressed in percentage. (b) Non-Metric Multi-dimensional Scaling (NMDS) analysis showing each lake distance to group median over the periods c. pre-1900 (blue diamonds) and 1960-present (green circles). Dotted lines denote the lake groups at the two time periods showing a significant (F= 6.8939; P=0.01) homogenization among lakes at post-1960.

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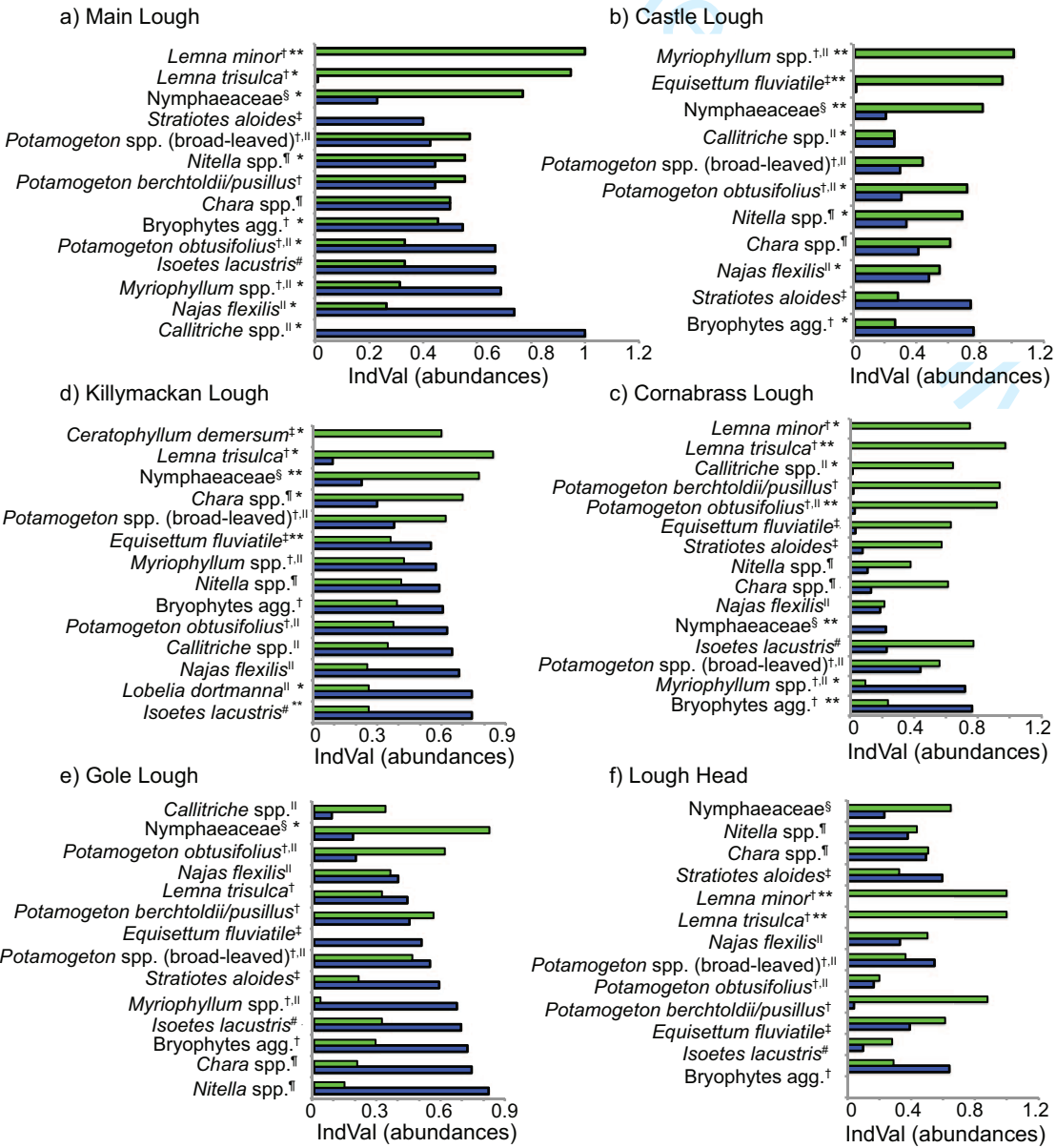
a) Main Lough
Lemna minor† **
Lemna trisulca† *
Nymphaeaceae§ *
Stratiotes aloides‡
Potamogeton spp. (broad-leaved)†,II
Nitella spp.¶ *
Potamogeton berchtoldii/pusillus†
Chara spp.¶
Bryophytes agg.† *
Potamogeton obtusifolius†,II *
Isoetes lacustris#
Myriophyllum spp.†,II *
Najas flexilis II *
Callitriche spp.II *
0 0.2 0.4 0.6 0.8 1 1.2
IndVal (abundances)
b) Castle Lough
Myriophyllum spp.†,II **
Equisettum fluviatile‡**
Nymphaeaceae§ **
Callitriche spp.II *
Potamogeton spp. (broad-leaved)†,II
Potamogeton obtusifolius†,II *
Nitella spp.¶ *
Chara spp.¶
Najas flexilis II *
Stratiotes aloides‡
Bryophytes agg.† *
0 0.4 0.8 1.2
IndVal (abundances)
d) Killymackan Lough
Ceratophyllum demersum‡*
Lemna trisulca† *
Nymphaeaceae§ **
Chara spp.¶ *
Potamogeton spp. (broad-leaved)†,II
Equisettum fluviatile‡**
Myriophyllum spp.†,II
Nitella spp.¶
Bryophytes agg.†
Potamogeton obtusifolius†,II
Callitriche spp.II
Najas flexilis II
Lobelia dortmanna II *
Isoetes lacustris# **
0 0.3 0.6 0.9
IndVal (abundances)
c) Cornabrass Lough
Lemna minor† *
Lemna trisulca† **
Callitriche spp.II *
Potamogeton berchtoldii/pusillus†
Potamogeton obtusifolius†,II **
Equisettum fluviatile‡
Stratiotes aloides‡
Nitella spp.¶
Chara spp.¶
Najas flexilis II
Nymphaeaceae§ **
Isoetes lacustris#
Potamogeton spp. (broad-leaved)†,II
Myriophyllum spp.†,II *
Bryophytes agg.† **
0 0.4 0.8 1.2
IndVal (abundances)
e) Gole Lough
Callitriche spp.II
Nymphaeaceae§ *
Potamogeton obtusifolius†,II
Najas flexilis II
Lemna trisulca†
Potamogeton berchtoldii/pusillus†
Equisettum fluviatile‡
Potamogeton spp. (broad-leaved)†,II
Stratiotes aloides‡
Myriophyllum spp.†,II
Isoetes lacustris#
Bryophytes agg.†
Chara spp.¶
Nitella spp.¶
0 0.3 0.6 0.9
IndVal (abundances)
f) Lough Head
Nymphaeaceae§
Nitella spp.¶
Chara spp.¶
Stratiotes aloides‡
Lemna minor† **
Lemna trisulca† **
Najas flexilis II
Potamogeton spp. (broad-leaved)†,II
Potamogeton obtusifolius†,II
Potamogeton berchtoldii/pusillus†
Equisettum fluviatile‡
Isoetes lacustris#
Bryophytes agg.†
0 0.4 0.8 1.2
IndVal (abundances)

Supporting Information

Appendix S1

Water chemistry sampling and laboratory protocols

Water chemistry data were obtained from Goldsmith *et al.* (2008). Two water samples were collected from each site using acid-washed polypropylene sample bottles. All samples with the exception of those for chlorophyll-a, TP, and total alkalinity, were filtered on-site and refrigerated prior to analysis. TP was determined by solution spectrometry (phosphomolybdate) after digestion by acid persulphate (Johnes and Heathwaite 1992). TN was determined by solution spectrometry (sulphosalicylic acid) after alkaline persulphate digestion (Wetzel and Likens 1991). Total alkalinity was determined by acidimetric titration in the field. Water samples (250–1000 mL) for the analysis of chlorophyll-a were filtered through Whatman GF/F (0.7 μm) filter papers (Whatman, Clifton, New Jersey, USA) and chlorophyll-a was determined spectrophotometrically (Pye Unicam SP6– 550 UV/VIS, Phillips, Cambridge, UK) by cold extraction in 90% acetone (Talling and Driver 1961). Conductivity and pH were measured in the field. Water colour was determined spectrophotometrically against standard platinum solutions (Wetzel and Likens 1991).

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APPENDIX S2

Table S2.1. Radiometric chronology of core NCAS1 taken from Castle Lough showing the CRS model ^{210}Pb dates and sedimentation rates.

Depth	Dry Mass	Chronology			Sedimentation Rate		
		Date	Age	Std			% Std
cm	g cm^{-2}	AD	yr	±	g cm^{-2}yr^{-1}	cm yr^{-1}	±
0	0	2008	0				
1.5	0.216	2004	4	2	0.0579	0.373	18.7
3.5	0.544	1998	10	2	0.0506	0.3	23.8
6.5	1.058	1984	24	3	0.0265	0.151	28.8
7.5	1.245	1978	30	4	0.0277	0.148	25.2
8.5	1.433	1971	37	5	0.0301	0.153	32.9
9.5	1.638	1963	45	6	0.0294	0.143	33.6
10.5	1.844	1957	51	6	0.0363	0.176	29.5
12.5	2.25	1946	62	9	0.0377	0.18	50.8
13.5	2.4728	1941	67	10	0.0627	0.289	75.3
14.5	2.689	1936	72	11	0.0354	0.163	56.5
15.5	2.906	1929	79	14	0.0266	0.121	54.1
17.5	3.348	1914	94	21	0.0296	0.138	97.5
20.5	3.9811	1887	121	28	0.0181	0.087	117

Table S2.2 Radiometric chronology of core NCAS3 taken from Castle Lough showing the CRS model ^{210}Pb dates and sedimentation rates.

Depth	Dry Mass	Chronology			Sedimentation Rate		
		Date	Age	Std			% Std
cm	g cm^{-2}	AD	yr	±	g cm^{-2}yr^{-1}	cm yr^{-1}	±
0	0	2008	0				

0.5	0.0624	2006	2	2	0.0308	0.247	28.7
1.5	0.1872	2000	8	2	0.0147	0.11	18
2.5	0.3289	1989	19	3	0.0103	0.073	22.2
3.5	0.4707	1972	36	6	0.0088	0.063	35
4.5	0.6125	1957	51	10	0.0135	0.095	45.3
5.5	0.7542	1951	57	11	0.021	0.137	38
6.5	0.9202	1944	64	14	0.0261	0.157	54.7
7.5	1.0862	1938	70	17	0.0288	0.174	55.1
8.5	1.2522	1934	74	18	0.0606	0.344	62.7
9.5	1.4193	1930	78	20	0.0268	0.16	68.8
10.5	1.5864	1923	85	24	0.0197	0.122	92.4
13.5	2.0659	1917	91	31	0.0914	0.68	126.3
15.5	2.3566	1904	104	35	0.043	0.269	149.9

Table S2.3 Radiometric chronology of core CBRAS1 taken from Cornabrass Lough showing the CRS model ^{210}Pb dates and sedimentation rates.

Depth	Dry Mass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2009	0				
0.5	0.0363	2009	0	2	0.1017	0.401	19.2
2.5	0.634	2001	8	2	0.0677	0.214	15.3
4.5	1.2995	1988	21	3	0.0341	0.172	14.6
8.5	1.8224	1973	36	4	0.036	0.262	23
11.5	2.261	1957	52	7	0.0224	0.153	27
13.5	2.5555	1945	64	10	0.0262	0.191	38.6
17.5	3.0829	1920	89	20	0.0161	0.122	69.4

19.5	3.349	1907	102	30	0.0281	0.202	120.8
21.5	3.64	1881	128	36	0.0044	0.027	144.2

Table S2.4 Radiometric chronology of core KILL2 taken from Killymackan Lough showing the CRS model ^{210}Pb dates and sedimentation rates.

Depth	Dry Mass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2009	0				
0.5	0.079	2008	1	2	0.0578	0.417	15
4.5	0.6239	1998	11	2	0.0503	0.382	15
7.5	1.0001	1990	19	2	0.0472	0.361	20.9
11.5	1.5404	1977	32	3	0.0367	0.262	22.5
13.5	1.8406	1966	43	5	0.0336	0.222	23.4
15.5	2.1426	1957	52	8	0.0273	0.182	29.2
17.5	2.4434	1946	63	10	0.029	0.193	29.4
19.5	2.7449	1934	75	12	0.0194	0.127	33.9
21.5	3.0547	1920	89	15	0.0277	0.176	70.1
23.5	3.3759	1911	98	16	0.0373	0.221	86.6
25.5	3.7304	1905	104	18	0.0134	0.072	54.1
27.5	4.1194	1879	130	31	0.0183	0.093	112.2
29.5	4.5161	1835	174	37	0.0090	0.045	123.1

Table S2.5 Radiometric chronology of core ULET3 taken from the main Lough showing the CRS model ^{210}Pb dates and sedimentation rates.

Depth	Dry Mass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2014	0				
0.5	0.0653	2014	0	2	0	0	209.2

1.5	0.2756	2011	3	3	0.0364	0.15	46.1
2.5	0.5506	2002	12	6	0.0254	0.096	52
3.5	0.8034	1991	23	12	0.021	0.085	64.1
4.5	1.045	1983	31	17	0.0387	0.167	110.9
5.5	1.2666	1975	39	23	0.0238	0.105	102
6.5	1.5002	1963	51	29	0.0145	0.06	121.4

50

51 Table S2.6 Radiometric chronology of core GOLE1 taken from Gole Lough showing
52 the CRS model ^{210}Pb dates and sedimentation rates.

Depth	Dry Mass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2009	0				
0.5	0.0311	2009	0	3	0.0926	1.082	19
4.5	0.3853	2004	5	2	0.0614	0.638	16.9
6.5	0.6087	2000	9	2	0.0442	0.351	16.5
9.5	1.0154	1990	19	2	0.0418	0.291	19.8
12.5	1.4702	1981	28	3	0.0661	0.414	32.8
15.5	1.9721	1970	39	4	0.0318	0.194	21.5
17.5	2.2908	1960	49	5	0.0284	0.187	36.4
19.5	2.5802	1947	62	7	0.0184	0.126	31.8
21.5	2.874	1929	80	11	0.0142	0.097	45.9
23.5	3.1688	1909	100	18	0.0148	0.1	74.9
24.5	3.3162	1900	109	21	0.017	0.112	81.5
27.5	3.7736	1876	133	27	0.0103	0.066	114.1

53

54 Table S2.7 Radiometric chronology of core DHOW1 taken from Derryhowlaght
55 Lough showing the CRS model ^{210}Pb dates and sedimentation rates.

Depth	Dry Mass	Chronology		Sedimentation Rate
		Date	Age	

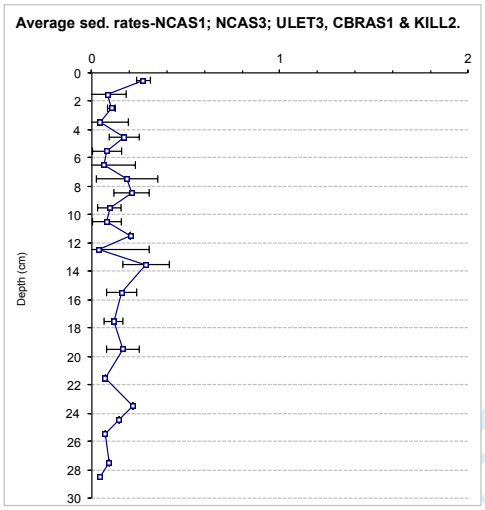
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2009	2009	0			
0.5	0.062	2008	2009	0	0.1282	0.946	29.6
3.5	0.4741	2005	2006	3	0.1733	1.164	50
6.5	0.9556	2001	2002	7	0.0966	0.59	29.7
9.5	1.4554	1995	1997	12	0.1137	0.682	41
12.5	1.9563	1990	1993	16	0.1091	0.625	47.5
15.5	2.5028	1986	1989	20	0.229	1.208	65.6
17.5	2.9042	1984	1988	21	0.3398	1.69	74.5
18.5	3.106	1982	1986	23	0.0413	0.205	28.9
19.5	3.3077	1978	1981	28	0.0861	0.431	57.7
21.5	3.7055	1976	1977	32	0.2172	1.025	47.8
24.5	4.3673	1971	1972	37	0.2427	1.04	81.7
27.5	5.105	1967	1967	42	0.1534	0.633	74.1
29.5	5.5783	1963	1963	46	0.0788	0.339	81.1
30.5	5.802	1961	1961	48	0.1168	0.522	84.3

Table S2.8 Radiometric chronology of core HEAD1 taken from Lough Head showing the CRS model ^{210}Pb dates and sedimentation rates.

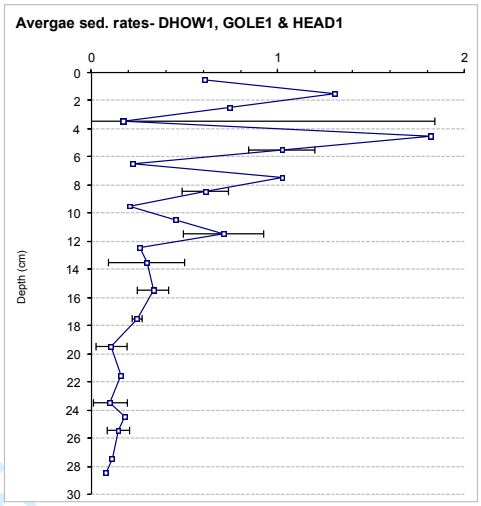
Depth	Dry Mass	Chronology			Sedimentation Rate		
		Date	Age				
cm	g cm^{-2}	AD	yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2008	0				
1.5	0.2335	2007	1	16	0.2205	1.304	71
4.5	0.7613	2006	2	16	0.5278	3	103.2
7.5	1.4254	2002	6	17	0.2361	1.021	98.3
11.5	2.3804	1996	12	17	0.1678	0.708	118.9
15.5	3.3217	1992	16	17	0.1334	0.569	93.3
17.5	3.788	1986	22	15	0.0511	0.209	92.7

Figure S2. Estimated dates of core ULET2 based on cross-correlation with (a) sedimentation rates and (b) the dated profiles of the satellite lakes Castle, Cornabrass, Killymackan and the main Lough (NCAS1, NCAS3, CBRAS1, KILL2, ULET3), and of core HEAD1 based on cross-correlation with (c) sedimentation rates, and (d) the dated profiles of the satellite lakes Gole (GOLE1) and Derryhowlaght (DHOW1).

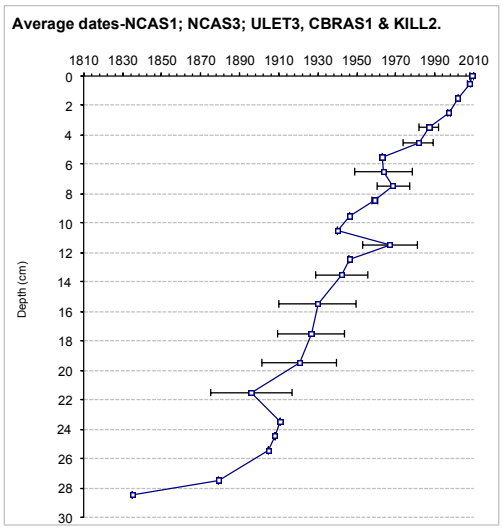
a)



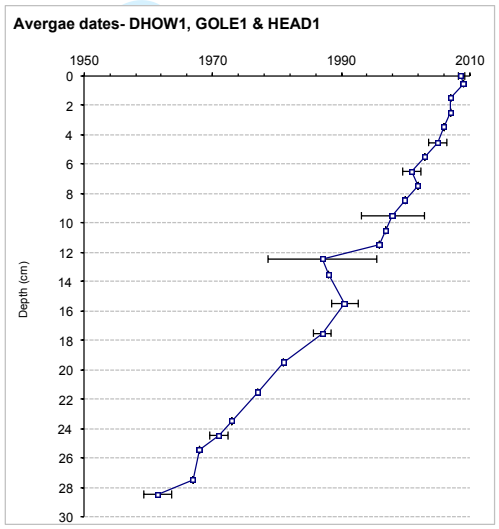
b)



c)



d)



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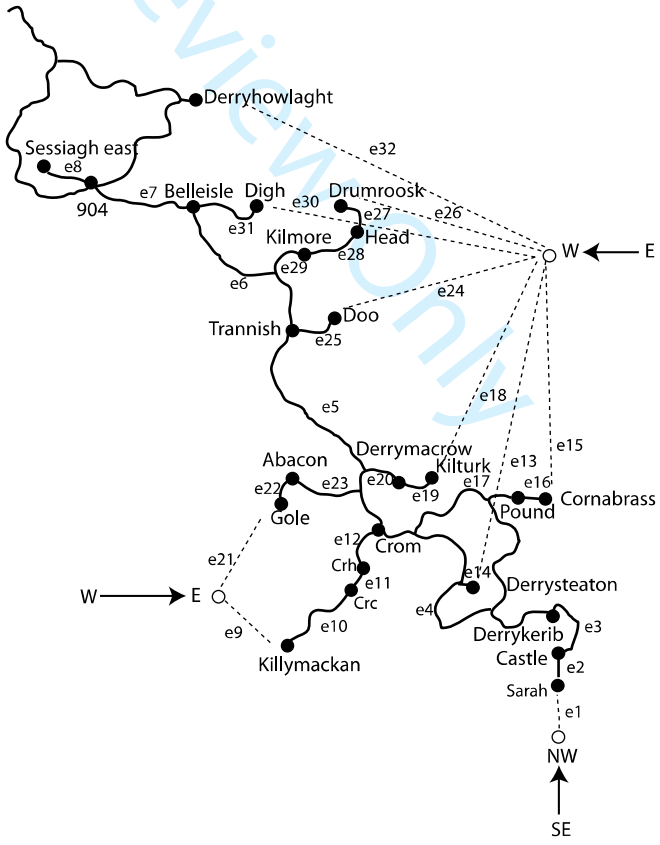
APPENDIX S3

Connectivity predictors

Figure S3. Asymmetric Eigen Model (AEM) construction from a Binary connectivity diagram based on major watercourse features (e.g. river, stream and ditches) of the system, denoting the presence/absence of links (edges) between given sites. The AEM model was constructed by incorporating the longitudinal water flow through the Upper Lough Erne system (i.e. from southeast to northwest) in addition to lateral flows (west-to-east and east-to-west) via streams, rivers or ditches from the associated satellite lakes into the main Lough. Three "imaginary sites" denoted by open circles, were created to represent water flow directions in the binary connectivity matrix.



92 Table S3. Directional sites-by-edges matrix constructed manually following Blanchet et al. (2011).

Lake	e1	e2	e3	e4	e5	e6	e7	e8	e9	e12	e13	e14	e15	e16	e17	e18	e19	e20	e21	e22	e23	e24	e25	e26	e27	e28	e29	e30	e31	e32
Sarah Lough	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Castle Lough	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Derrykerrib Lough	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Derrysteaton Lough	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cornabrass Lough	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pound	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Main Lough-Crom	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Killymackan Lough	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Derrymacrow Lough	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Kilturk Lough	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abacon Lough	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Gole Lough	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Main Lough-Trannish	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
Lough Doo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Kilmore Lough	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
Lough Head	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Drumroosk Lough	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Lough Digh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Main Lough-Belleisle	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Lough 904	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Sessiagh East Lough	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Derryhowlaght Lough	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

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APPENDIX S4

Table S4. Contemporary macrophyte species recorded at 21 sites (19 satellite lakes and three areas of main Lough- the Upper Lough Erne)

Lake	*Callitriche hermaphroditica*	*Ceratophyllum demersum*	*Chara globularis*	*Chara virgata*	*Eleocharis acicularis*	*Elodea canadensis*	*Equisetum fluviatile*	*Fontinalis antipyretica*	*Hydrocharis morsus-ranae*	*Lemna minor*	*Lemna trisulca*	*Litorella uniflora*	*Menyanthes trifoliata*	*Myriophyllum spicatum*	*Myriophyllum verticillatum*	*Nitella flexilis*	*Nitella mucronata* var.	*Nuphar lutea*	*Nymphaea alba*	*Persicaria amphibia*	*Potamogeton alpinus*	*Potamogeton berchtoldii*	*Potamogeton crispus*	*Potamogeton gramineus*	*Potamogeton lucens*	*Potamogeton x angustifolius*	*Potamogeton natans*	*Potamogeton obtusifolius*	*Potamogeton pectinatus*	*Potamogeton perfoliatus*	*Potamogeton polygonifolius*	*Potamogeton praelongus*	*Ranunculus circinatus*	*Sagittaria sagittifolia*	*Sparganium emersum*	*Spirodella polyrhiza*	*Stratiotes aloides*	*Utricularia vulgaris* agg.	*Zannichellia palustris*
Sarah Lough						X			X	X	X		X		X			X	X														X	X	X	X	X	
Castle Lough					X	X	X		X	X	X							X							X		X							X	X		X		
Derrykerrib Lough			X			X		X	X	X	X				X		X	X							X		X							X	X	X	X	X	
Derrysteaton Lough					X	X	X				X							X										X							X				
Cornabrass Lough						X	X		X	X	X	X	X					X			X	X						X		X					X	X			
Pound Lough			X			X			X	X	X		X		X		X	X	X		X							X							X				
Lough Erne-Crom	X				X	X	X				X	X					X	X		X		X		X				X		X					X				
Killymackan Lough	X	X		X		X	X	X		X	X		X					X				X					X		X	X	X	X	X			X			
Derrymacrow Lough						X				X	X							X				X				X	X	X							X	X			
Kilturk Lough						X	X			X	X			X				X				X	X				X	X		X				X		X			
Abacon Lough						X	X				X		X					X									X							X					
Gole Lough						X	X											X		X							X			X				X					
Lough Erne-Trannish						X	X			X	X					X		X				X					X	X	X	X									X
Lough Doo						X	X			X	X		X					X				X				X	X	X	X										
Kilmore Lough			X				X	X		X	X		X	X				X							X		X		X	X			X		X				
Lough Head					X	X	X			X	X			X				X				X						X	X					X					
Drumroosk Lough			X			X	X	X		X	X		X					X										X						X					
Lough Digh						X	X				X							X				X						X						X					
Lough Erne-Belleisle					X	X	X				X			X				X				X					X	X	X	X				X					
Lough 904					X	X		X										X				X					X	X	X						X				
Sessiagh East Lough						X	X			X	X		X					X				X					X	X	X						X				
Derryhowlaght Lough						X	X			X	X			X								X						X							X				

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-General comments:

We have edited various sections of our manuscript, most especially the methods and discussion sections, to address the Reviewers' and the Editor's concerns and also to improve our expression and grammar. Points raised by both Reviewers and the Editor were that it was hard to understand the focus of the study and the methods of data collection and thus that our heterogeneity assessments were unclear. As outlined below, we now include a more detailed description of our methods and analyses and have clarified the main focus of the study.

Reviewer #1:

-This is an interesting and intricate piece of research. It aims to disentangle the effects of eutrophication, the abundance of zebra mussels (an invasive species), and physical features of the landscape, namely lake surface area and connectivity of the lakes in the catchment, on macrophyte species richness and macrophyte vegetation heterogeneity at the present day and before 1900. The authors are to be applauded on a successful outcome. I think they could emphasise more the conservation aspects of their results.

RESPONSE: Further emphasis of the conservation aspects is an excellent suggestion and we have included a conclusion section (Lines 457-476) to better highlight the implications of our results in light of lake management.

-Zebra mussel colonization seems to be a good thing for macrophyte heterogeneity, which is rather surprising. But it is not obvious WHY this should be so.

RESPONSE: We have expanded our discussion on the effects of zebra mussels including consideration of how these effects may be exerted. (Lines 412-425)

-The advantages of connectivity and lake area could also be more specifically commented on.
RESPONSE: We now comment on this more specifically in the discussion (Lines 426-435).

-The paper could benefit from a short 'Conclusions' or Summary section at the end, emphasizing the main results.

RESPONSE: We now include a conclusion (Lines 457-476) to highlight better the implications of our results in the light of lake management.

-Although the science is good, the presentation is rather poor in several respects. The English text is tends to be ungrammatical in places and sometimes it is hard to decipher the meaning. This is very surprising and rather disappointing as at least 5 of the authors are native English speakers! I have made a rather detailed set of suggestions for improvement.

In many instances a statement is made similar to 'Contemporary species richness responded strongly to eutrophication', but it is not explained HOW or WHY this happened – did species richness increase or decrease? This is very irritating throughout the paper. One cannot keep referring to the diagrams to find out the answer. The figures need some attention and clarification.

RESPONSE: We have edited the whole manuscript taking into account concerns about grammar and further explanations of responses.

-Specific comments: We thank the reviewer for such careful and detailed comments that help us to improve our manuscript.

-Title. The title is ungrammatical! Perhaps it could be enlarged to be more informative: A combination of connectivity etc. .. buffers eutrophication-driven ….

RESPONSE: The title has been changed to: "Eutrophication homogenizes shallow lake macrophyte assemblages over space and time"

-L 31. Replace by with of. *AMENDED.*

-L 33. Demonstrated. *AMENDED.*

-L 32-36. This could be better written: Using modern and pre-1900 data-sets of macrophyte occurrence, homogeneity analysis ….. demonstrated that contemporary lake macrophyte community heterogeneity and species richness has decreased with intensified eutrophication but has increased with greater zeb. m. abundance and greater lake surface area.'

AMENDED. Now described as: "By applying homogeneity analysis of multivariate dispersions and partial redundancy analysis we demonstrate that contemporary lake macrophyte heterogeneity and species richness are reduced in lakes with intensified eutrophication but are increased in lakes with greater zebra mussel abundance and lake surface area". LINES 33-37

-L 36. How did lake species-richness respond strongly to eutrophication etc.? … water-course connectivity explained larger portions - than what? You are making a comparison with nothing here.

AMENDED. Now described as: "By applying homogeneity analysis of multivariate dispersions and partial redundancy analysis we demonstrate that contemporary lake macrophyte heterogeneity and species richness are reduced in lakes with intensified eutrophication but are increased in lakes with greater zebra mussel abundance and lake surface area. Watercourse

connectivity positively influenced assemblage heterogeneity and explained larger proportions of the variation in assemblage heterogeneity than local environmental factors, whereas variation in species richness was better related to local abiotic factors." LINES 33-40.

-L 40. After 'communities' insert 'before significant increases in eutrophication' prior to 1900. At that time, water-course connectivity was particularly linked to [insert] 'higher' diversity;

L 42. By 'variation' do you mean heterogeneity or species richness? Insert 'a' before 'few';

L 43. Use 'systems' instead of 'landscapes'.

L 45. End the sentence after richness. Continue: This means that early and telling effects.;

L47. Replace used by recorded).

RESPONSE: We have edited our abstract as follows in view of the above comments: LINES 40-50 "Macrophyte fossil data revealed within and among-lake assemblage homogenization post-1960, with the main Lough and connected sites showing the highest rates of homogenization due to progressive eutrophication. The long-term and contemporary data collectively indicate that eutrophication reduces assemblage heterogeneity over time by overriding the importance of regional processes (e.g. connectivity) and exerts stronger pressure on isolated lakes. Our results suggest further that in connected lake systems, assemblage heterogeneity may be impacted more rapidly by eutrophication than species richness. This means that early effects of eutrophication in many systems may be underestimated by monitoring that focuses solely on species richness and is not performed at adequate landscape scales".

-L 52. …shallow lake [insert] 'ecosystems'.

We have deleted this key word

-L 53. Replace 'species' with 'organisms'. End sentence after the reference. *AMENDED. LINE 57*

-L 54. Replace 'and' with 'They'.

RESPONSE: The wording has been changed as follows: "Aquatic macrophyte stands are a key component of shallow lake ecosystems, providing structurally complex habitats for many co-occurring organisms (Jeppesen et al. 1998) and contributing to biogeochemical cycling in shallow lakes (Davidson et al. 2015). LINES 56-59.

-L 57. Insert 'the' before 'loss'. *AMENDED. LINE 61*

-L 66. Replace mussels with mussel (grammar). *AMENDED. LINE 71*

-L 68. Add comma after freshwaters. *AMENDED.*

-L 75. What are species-sorting processes? Please give a brief explanation of what they are and how they promote community responses to environmental change.

AMENDED. These are described as: Dispersal may additionally facilitate the ability of species to track variation in local environmental conditions according to preferred nutrient enrichment conditions (species-sorting) (Leibold and Norberg 2004). LINES 81-83.

-L 83. Also, a strong eutrophication/pollution pulse will have more impact in a small disconnected lake because there can be no dilution from elsewhere in the catchment.

AMENDED. Now described as: A strong eutrophication pulse may also have more impact in small, disconnected lakes if there is no dilution from elsewhere in the catchment (Strecker and Britatin 2017). LINES 88-90.

-L 87. Datasets of what? Be consistent with data sets and datasets. *AMENDED.*

-L 92. Replace novel with important. *AMENDED.*

-L 101. feeds – grammar. *AMENDED.*

-L 101. Water-level regulation schemes ….. 1940s reduced water-level fluctuation. *AMENDED.*

-L 113. Inundated. *AMENDED.*

-L 114. After polymorpha insert ‘the zebra mussel’ (for clarity). *AMENDED.*

-L 129. Before abundance insert ‘species and’. *AMENDED.*

-L 130. After NIEA insert ‘by’; then Goldsmith et al. (2008). *AMENDED.*

-l. 136. I think transect is a better word than section in this context. One talks of ‘vegetation transects’. *AMENDED.*

-L 138. Replace >1 by ‘more than one’ – transect. *AMENDED.*

-L 140. How did you sample the zebra mussel population? Was it a visual estimate or did you catch them from a known area somehow?

AMENDED. Now described as: “At the same time as surveying for macrophytes we also collected data on relative zebra mussel abundance. Thus, at each macrophyte sampling point we noted the presence of zebra mussels through direct observations using the bathyscope and/or through individuals collected along with macrophytes when using the rake. Mussel relative abundances within lakes were then quantified using a semi-quantitative scale (0-3) as follows: 0 = no zebra mussels observed in any sampling point; 1 = zebra mussels observed in <10 sampling points; 2 = zebra mussels observed in 10-20 sampling points; and 3 = zebra mussels observed in > 20 sampling points. Consistent sampling of zebra mussels within and amongst lakes provided comparative data of their relative abundances. LINES 169-178.

-L 143. Replace ‘for’ by ‘short sediment’. *AMENDED.*

-L 144. What are contemporary surveyed lakes? Does Tranish have one or two ‘n’s. After basin add ‘of’ (grammar).

AMENDED. Now described as: “We analyzed plant macrofossils from short sediment cores (~ 1 m long) collected during the summers of 2008 and 2009 from 6 of the 21 sites surveyed for present-day data: Trannish basin of the main Lough (core code ULET2), Castle Lough (NCAS3), Cornabrass Lough (CBRAS1), Gole Lough (GOLE1) Killymackan Lough (KILL2) and Lough Head (HEAD1) (Fig. 1). LINES 180-185”.

-L 145. Before ULET2 insert ‘core code’. I wondered what these abbreviations are. *AMENDED.*

-L 148. Before sediment insert ‘short’. *AMENDED.*

-L 150. Were cores collected from the middle of the lake, or under certain macrophyte communities?

AMENDED with further description added as: Cores were collected from similar macrophyte rich and shallow basins (water depths 90-180 cm). LINES 189-190.

-L 152. New paragraph for the dating. To say cores were dates by Pb and gamma counting is inadequate, and actually meaningless.

AMENDED. Now reads as: "The cores were dated using a combination of techniques. For the 5 satellite lakes, we used radionuclide measurements of 210Pb (half-life 22.3 years) and 137Cs and 241Am (Appleby et al. 1986). Dates at specific levels were ascribed using the Constant Rate of Supply (CRS) model (Appleby and Oldfield 1978) (see Appendix S2: Tables S2.1-8; Fig. S2). Due to high sedimentation rates in the top 20 cm of core HEAD1, the CRS dating model covered only the last 23 years. Thus, we cross-correlated the remaining selected sediment samples with the dated profiles of two cores taken from two similar hypertrophic lakes (GOLE1-Gole Lough, included in this study; and DHOW1-Derryhowlght Lough, unpublished data), which had relatively similar sedimentation rates but greater chronological resolution (Appendix S2: Tables S2.1-8; Fig. S2). As funds were not available for dating the core from the main Lough (ULET2), selected levels were estimated from the core ULET3 (unpublished data; Appendix S2: Tables S2.1-8; Fig. S2), an extra 210Pb dated core obtained from Castle Lough (NCAS1; Salgado et al. in press) and three of the study satellite lakes (NCAS3, CBRAS1 and KILL2) which had relatively similar sedimentation rates and similar ranges of total phosphorous concentrations to those observed in the main Lough (See Table 1)." LINES192-208.

-L 153. After 'Dates' insert 'at specific levels'.

AMENDED.

-L 154. Why could no precise dates be ascribed to ULET2?

AMENDED. We have now explained as: "As funds were not available for dating the core from the main Lough (ULET2), selected levels were estimated from the core ULET3 (unpublished data; Appendix S2: Tables S2.1-8; Fig. S2), an extra 210Pb dated core obtained from Castle Lough (NCAS1; Salgado et al. in press) and three of the study satellite lakes (NCAS3, CBRAS1 and KILL2) which had relatively similar sedimentation rates and similar ranges of total phosphorous concentrations to those observed in the main Lough (See Table 1)." LINES 202-208.

-L 158. Replace 'for' by 'from'. *AMENDED.*

-L 162. Reorganise sentence: …. Before sieving through mesh sizes of 355µm and 125 µm. Macrophyte fossils were extracted from the residues and various identification guides. *AMENDED. Now reads as: "Macrophyte fossils were retrieved from the residues of sieved core material (using mesh sizes of 355 µm and 125 µm) following standard methods (Birks 2001) and were identified by comparison with reference material and various taxonomic guides (e.g. Birks 2001). Macrophyte fossil abundances were estimated by counting seeds, leaves, and spines and the data were standardized as the numbers of fossils per 100 cm3." LINES 200-207.*

-L 180. Weighting: rare vs abundant species: which is weighted up and which is weighted down? Be precise. *AMENDED.*

-L 186. Move the sentence' HMD applies …' up to line 178, after (Anderson 2006) *AMENDED.*

-L 203. How did you determine the significance of an environmental variable? Or do you mean significant variables were indicated by the forward selection analysis?

AMENDED. We have added the following: "Significant environmental variables (log-transformed TP, TN, and Chl-a data, zebra mussel abundance and log-transformed lake surface area) and AEM connectivity predictors were detected through forward selection analysis ("packfor" in R; R Core Team 2016) by following Blanchet et al. (2008b)." LINES 261-264.

-L 205. Replace 'according to' by 'following' *AMENDED.*

-L 211. When you say each data set do you mean the total (all) data sets? *AMENDED.*

-L 223. Replace 'was comprised' by 'contained'. Move the phrase 'We used HMD analysis on Bray-Curtis dissimilarities' to line 227 before To visualise .*AMENDED.*

-L 228. It is called non-metric multi-dimensionaL scaling. *AMENDED.*

-L 2 34. I do not. *AMENDED.*

-L 241- replace 'predictors' with 'explanatory variables'. *AMENDED.*

-L 242. New paragraph for pRDA. *AMENDED.*

-L 257 and following. Some of these percentages do not agree with Figure 2. See comments on the figure later.

AMENDED. Now reads as: "pRA on macrophyte species richness resulted in TN and watercourse predictors explaining a significant (P<0.01) 3% and 21% of the adjusted variation, respectively (Fig. 2b). Spatial structure and shared variation between environmental variables together explained the following variation in macrophyte species richness amongst sites: (i) watercourse connectivity and TN (4%); (ii) TN, zebra mussel abundance, lake surface area and watercourse connectivity (13%); (iii) zebra mussel abundance, watercourse connectivity and lake surface area (1%); (iv) TN, lake surface area and zebra mussel abundance (14%); and (v) TN and lake surface area (10%). Unexplained residual variation accounted for 43% of adjusted variation in macrophyte species richness." LINES 319-328.

-L 267. Insert 'larger' before lake surface . *AMENDED.*

-L 275. Insert (Fig. 5) after IndVal analysis. *AMENDED.*

-L 276. Replace were with was – grammar! *AMENDED.*

-L 278. Delete 'and'. *AMENDED.*

-l. 279. Delete 'taxa'. *AMENDED.*

-L 291. berchtoldii. *AMENDED.*

-L 292. expense – grammar!.;L 294. Do not need this reference – you are describing your own results. Perhaps it could be moved down to line 298 with Sayer et al.; L 295. Replace 'of' with 'on'; L 298. Better to write: Increases in phytoplankton put stress on macrophytes through shading; L 299. What are 'overwintering species'? Why should they be stressed by phytoplankton increases?

RESPONSE: We have edited this paragraph as follows: "The decline of macrophyte cover and species richness in shallow lakes caused by eutrophication is well documented (e.g. Scheffer

1998, Jeppesen et al. 2000, Kolada et al. 2014). Eutrophication may stimulate a range of responses including gradual vegetational shifts (e.g. from isoetid to more diverse stands of submerged elodeid macrophytes; Arts 2002; Willby et al. 2012), decreases in the seasonal duration of elodeid macrophyte coverage (Sayer et al. 2010a) and apparently sudden shifts from clear water (with abundant and diverse macrophytes) to turbid water conditions (with low transparency and fewer macrophytes; Scheffer 1998, Scheffer et al. 2001, Scheffer et al. 2003). However, despite this relatively large body of research, eutrophication-driven changes in assemblage heterogeneity have received relatively little attention in comparison to studies focusing on patterns of macrophyte abundance and species richness (e.g. Jeppesen et al. 2001, Scheffer et al. 2001, Scheffer et al. 2003, Sayer et al. 2010a). Our analyses of contemporary and paleoecological data provide novel and nuanced insights on eutrophication impacts across the landscape, revealing that satellite lakes connected to the main Lough experienced higher post-1960s rates of macrophyte assemblage homogenization than the more isolated lakes (Fig. 4a). These patterns suggest that prior to 1900 regional processes (e.g. seasonal flooding and variation in water level) were influential in maintaining assemblage heterogeneity concurrently in the main Lough and in proximal satellite lakes (Castle and Cornabrass), but eventually (post-1960s) these influences were overridden by progressive nutrient enrichment. A paleoecological study by Salgado et al. (2017) addressing macrophyte assemblage variation across three basins in Castle Lough, revealed similar nutrient effects over a decadal to centennial scale (10-100 years), with former drivers of assemblage heterogeneity (e.g. water depth) gradually being displaced by nutrient enrichment, leading eventually to dominance by a few highly competitive macrophyte species. Potential drivers of homogenization include gradual increases in phytoplankton concentrations that restrict macrophyte distributions within lakes and decreases in seasonal duration with macrophytes developing over shorter periods during summer (Sayer et al. 2010a,b). Other mechanisms are reductions in photosynthetic rates and plant growth due to reduced water transparency (Spence 1967), and selection for taxa (e.g. E. canadensis) that can grow at lower light levels (Spence and Chrystal 1970).”LINES 365-396.

-L 301. Insert ‘increased’ before ‘phytoplankton’. *AMENDED.*

-L 202. Delete ‘associated’ *AMENDED.*

-L 313. Rephrase: We confirm the prediction in our aquatic ecosystem by showing that macrophyte species loss.

AMENDED. Reads as: “Our results support these predictions, revealing that current macrophyte assemblage homogenization and species loss by eutrophication involve interactions of lake surface area, relative zebra mussel abundance, and watercourse connectivity (Fig. 2).” LINES 401-404.

-L 314. Insert ‘abundance of’ before zebra mussels; L 318. ‘effects’ What stronger effects? Explain; L 319. greater This statement is rather obvious; L 321. Zebra mussels are higher in L Erne – than where? Need the comparison, L 324. Insert ‘thereby’ before ‘reducing. Is Chl-a a proxy for algae? What is the mechanism? Do zebra mussels eat phytoplankton?

RESPONSE: We have edited this paragraph by adding the following: “Zebra mussel abundance was higher in the main Lough than in most satellite lakes and this may have improved conditions for macrophyte communities by enhancing water transparency (Griffiths 1992, Ibelings et al. 2007). The capacity of zebra mussel populations to filter substantial volumes of water year-round (Strayer 2009) can lead to significant loss of phytoplankton (as suggested by our measurements of Chl-a) (Higgins and Vander Zanden 2010). The higher concentrations of TN, lower mussel abundances, and elevated levels of Chl-a in more isolated sites (Fig. 3), may promote domination by macrophytes species that tolerate nutrient enrichment (e.g. fine-leaved Potamogeton species and E. canadensis) and the reduction/displacement of intolerant species (e.g. broad-leaved Potamogeton species), resulting in more homogenous assemblages. The rarity of zebra mussels in most isolated lakes could be the result of dispersal limitation (Heino and Moutka 2006) or less favorable conditions for zebra mussel establishment in the organic-rich and silty sediments that characterize most satellite lakes (Strayer 2009). ” LINES 412-425.

-L 339. Replace ’structural’ with ‘ecosystem’.

RESPONSE: Because of our revision this paragraph is no longer included.

-L 343. We do not know the cause;

RESPONSE: The causes of why species richness related most with local factors while heterogeneity related to connectivity are difficult to discern from our data. Nevertheless, these patterns concur with previous metacommunity studies and in response, we have included a new statement as follow (LINES 446-455): “These contrasting patterns indicate that eutrophication effects are variable but sufficiently large to influence species composition in the ULE system while dispersal amongst hydrologically connected sites may ultimately maintain macrophyte species abundances that are sensitive to nutrient enrichment within the system (Amarasekare and Nisbet 2001, Mouquet and Loreau 2002). By analyzing measures of both macrophyte assemblage heterogeneity and species richness our study highlights how regional environmental heterogeneity and spatial gradients in connectivity can influence diversity and dominance and rareness (relative abundance) of plant species in connected landscapes (Amarasekare and Nisbet 2001, Mouquet and Loreau 2002).”

-L 342. Insert ‘over time’ after ‘responses’

-L 343. Replace ‘responded more’ by was ‘related most’.

-L 346. larger than what? Need a comparison. Larger than species richness? Was the explanation positive or negative? Please write more precisely and informatively

-L 348. What are 'local filtering processes'? I do not understand this sentence

-L 351. Are the effects positive or negative?

-L 352. What is a 'rescue effect'? Is it replacements of lost species?

-L 354. Replace 'structure' with 'distribution'

-L 355. Which species in which loughs?

-L 361. How do anthropogenic disturbances affect lake-macrophyte richness – reduce or enhance it? Ditto for spatial variability (do you mean heterogeneity?).

-l. 362. Move 'over space and time' after 'spatial variability'

-L 363. Replace 'composition' with 'occurrence'. Does 'relative abundance' refer to a species or to total macrophyte cover?

-L 336. Replace 'at' with 'in' – grammar

-L 367. What IS species sorting?

-L 368. Replace ',' with 'by'

-L 369. 'influential' – How?; L 369. How are diverse macrophyte populations to be maintained – what is the conservation strategy? Is it to improve connectivity? Or What?

RESPONSE: The various suggestions made above by the reviewer for lines 342-369 were all addressed resulting in major changes in the text including a new conclusion section. See LINES 437- 475.

-Figure 1. This is a very confusing figure. Loch Erne seems to be shown in two shades of grey. Flood layers and water layers are mentioned in the caption. Are these the grey shades? It says the sites are black – but the Lough Erne sites are not black. Nor are their positions indicated. L Erne is such a complicated shape that I think it would be clearer if its shoreline was outlined in heavier black. Is it Tranish or Trannish? *AMENDED.*

-Figure 2b. I do not think the % numbers are correct, according to the text. 1% should be moved to the intersection of AEM and area. 2% should be written both at the overlap of TN and zebra mussels, and at the overlap of AEM and zebra mussels.

AMENDED. Editions have made on the text. LINES 319-328.

-Figure 3a. What is 'centroid'? What are its units? Ii cannot interpret this figure. What are the dotted polygons surrounding the points? Is each point (one for each lake) a mean value of the dissimilarities? Or what?

RESPONSE: We have edited the legend and simplified the figure. LINES 646-657.

Figure 5. It does not affect the diagram much, but why is present sometimes above and sometimes below the pre 1960 bars? *AMENDED.*

-Reviewer 2:

The decline in macrophyte coverage and diversity in shallow lakes due to eutrophication is one of the best documented processes in limnology (e.g. see Scheffer papers listed below). The general pattern of ecosystem changes along the regime shift from the clear water (high water clarity and abundant and divers macrophyte community) to turbid water (low water transparency and few macrophytes) phases is very well known. Nevertheless some aspects of this process maybe poorly documented. The authors used a "…combine ecological and paleoecological approach to examine how eutrophication, watercourse connectivity to a main central lake, lake surface area and zebra mussel abundance interact to influence macrophyte species-richness and community heterogeneity over spatial (within and among lakes) and temporal (decadal to centennial) scales in the Upper Lough Erne shallow lake system…". In general this study represent an interesting approach and potentially can be a useful contribution, however there are several serious problems with the manuscript, including a lack of major literature sources, overstated novelty of the problem, and not adequate methods used to collect field data.

-Decline in macrophyte coverage and diversity in shallow lakes due to eutrophication was described in classical papers by Scheffer and others (e.g. Scheffer, M. Ecology of Shallow Lakes. Chapman and Hall, London, 1998; Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature 413: 591-596; Scheffer, M., Carpenter, S. R. 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. Trends Ecol Evol 18:648-56). RESPONSE: We agree with the reviewer about the importance of the body of literature he/she refers to. Accordingly, we now include further reference to this body of work in our manuscript (LINES: 62-63; LINES: 363-377). However it is important to stress that the body of literature suggested by this Reviewer focuses on patterns of ecosystem change and regime shifts between phytoplankton and macrophyte dominance. In contrast, our manuscript focuses on concurrent changes in macrophyte assemblage structure, assemblage heterogeneity and species richness across relatively broad spatial and temporal scales. We believe our study therefore makes a novel and complementary contribution to the body of research highlighted by the reviewer.

-There are numerous publications on the positive impact of zebra mussels on macrophytes (e.g. Karatayev, A. Y., Burlakova, L. E., Padilla, D. K. 2002. Impacts of zebra mussels on aquatic communities and their role as ecosystem engineers. In: Leppakoski E, Gollach S, Olenin S, editors. Invasive aquatic species of Europe: distribution, impacts and management. Dordreicht,

The Netherlands: Kluwer Academic Publishers; Karatayev, A. Y., Burlakova, L. E., Padilla, D. K. 1997. The effects of Dreissena polymorpha (Pallas) invasion on aquatic communities in eastern Europe. J Shellfish Res 16: 187–203.) as well on macrophyte recovery in turbid lakes after the invasion of Dreissena (e.g. Zhu, B., Fitzgerald, D. G., Mayer, C. M., Rudstam, L. G., Mills, E. L. 2006. Alteration of ecosystem function by zebra mussels in Oneida Lake: impacts on submerged macrophytes. Ecosystems 9:1017–1028; Ibelings, B., Portielje, R., Lammens, E. R. R., et al. 2007. None of these papers are cited in the manuscript, creating a false impression of novelty.

RESPONSE: We thank the reviewer for highlighting these important studies on zebra mussel effects. Initially, we only cited the paper by Higgins and Vander Zanden (2010) because this is the latest and more comprehensive review of zebra mussel effects on various aquatic biota (including macrophytes) and cites several of the suggested references by the reviewer (e.g. Karatayev et al 1997; Karatayev et al. 2002) plus many more. Nevertheless, we have now included the suggested studies by Ibelings et al. (2006) and Zhu et al. 2006 paper (LINE 73; LINE 414). We would point out that the novelty of our study is not to highlight the positive effects of zebra mussels per se but to show how variation in mussel spatial distributions, along with lake area and hydrological connectivity can collectively contribute to the homogenization of lake macrophyte assemblages by eutrophication. To further clarify our results and highlight the novelty of our study, we have made several changes to the discussion section to better explain that the observed positive effects of zebra mussels on macrophyte diversity have been previously reported. See Lines 412-425.

-Lines 78-80. Authors wrote: "Dispersal and connectivity could therefore be major drivers of macrophyte diversity within and amongst highly connected sites via source-sink dynamics,…" Please clarify if you are talking about sites within a lake or among lakes.

RESPONSE: AMENDED, now reads as: "Dispersal and connectivity may also compensate for eutrophication impacts. For example source-sink dynamics may counter or delay extinction. In this scenario dispersal from high ecological quality lakes (sources) may promote colonization and the maintenance of viable populations of sensitive species in low quality lakes (Mouquet and Loreau 2002). Dispersal may additionally facilitate the ability of species to track variation in local environmental conditions according to preferred nutrient enrichment conditions (species-sorting) (Leibold and Norberg 2004). LINES 77-83.

-Lines 118-120. Authors wrote: "Selection criteria included: replication along a gradient of enrichment (total phosphorous [TP], total nitrogen [TN], and chlorophyll a [Chl-a], multiple macrophyte sampling points within lakes…" How many points per lake? How these points were selected? Randomly? Stratified? Stratified-random design?

RESPONSE: We have included a more detailed description of our methods, sampling protocols and analyses as follow (LINES 140-169): We appreciate that it was inappropriate simply to refer

to the standard JNCC method that we employed and have therefore added further information to enable readers to understand our sampling approach as follows: "Standard Joint Nature Conservation Committee (JNCC) protocols for Site Monitoring (JNCC 2009) were followed. This methodology allows for the characterization of macrophyte assemblages within lakes based on shoreline and boat surveys. Accordingly, data were collected from different sectors of a lake using a combination of two sampling approaches (shoreline and deeper water transects) in each sector to give good spatial coverage (Gunn et al. 2010). In particular, macrophyte data were collected along a 100 m wader-depth shoreline transect by sampling at four depths (25 cm, 50 cm, 75 cm and > 75 cm) at each 20 m interval (20 points in total per shoreline transect). Macrophytes in deeper water were surveyed using a boat to collect data (at depths > 75 cm) along a transect starting at the midpoint of the shoreline transect and running towards the center of the lake. Macrophytes were sampled at every 5 m along this 100 m deeper-water transect (20 points in total). At each point, we used a combination of bathyscope and grapnel sampling, and all aquatic macrophyte species occurring within a 1m2 area were recorded using an abundance scale of 0-3, where 0 = absent and 3 = highly abundant. Between two and three sectors were sampled per satellite lake (see Table 1 for details). Representation of the main macrophytes present in each lake was the basis for selecting sectors for sampling – a selection requiring expertise in macrophyte identification and fieldwork experience. This JNCC method has been demonstrated to adequately characterize macrophyte communities in small lakes (< 50 Ha hectares) by sampling two to three sectors (Gunn et al. 2010). Accordingly we sampled 2-3 sectors in the majority of our sites. Exceptions were made for Sarah and Pound Loughs whose small size (< 2 Ha.) precluded surveying more than 1 sector and for Lough 904, where site accessibility prevented surveying more than 1 sector (Goldsmith et al. 2008). The main Lough was divided into three separate study basins and, due to their large size, eight sectors per basin were surveyed. It should be stressed that such sampling along representative transects in a lake will almost certainly not identify all macrophyte species within lakes, but the approach can provide relative data on variation in distributions and abundances (i.e. heterogeneity) of the most typical species within lakes (Gunn et al. 2010)."

-LINES 236-243: "We pooled shoreline and boat data for each lake transect and, with exceptions of Sarah and Pound Loughs, 40 randomly chosen points (set.seed and sample algorithms in R; R core Team 2016) per lake (20 littoral and 20 open water from all transects) were selected for the analysis. We used this stratified sampling design because the variability within a chosen subset of data is lower compared to variation of the entire population, and hence has a high statistical precision while requiring smaller sample sizes in comparison to other approaches (Legendre and Legendre 2012)."

Did you measure water transparency? This is one of the most relevant parameter for macrophyte community assessment and one of the easiest parameter to measure. The littoral depth (as the maximum depth colonized by rooted macrophytes, see Higgins and Vander Zanden, 2010) in the lake is another good parameter to use.

RESPONSE: This is an excellent point and we did measure this (using a secchi disk). Unfortunately this variable strongly correlated with the other variables (such as nutrients and zebra mussels) making it very difficult to disentangled the unique effects of each parameter Thus, we decided to exclude it. This information is, now provided in the manuscript in LINE: 130; LINES: 264-267.

-Lines 129-130. Authors wrote: "…abundance data were derived from assessments of macrophyte site conditions…" How many sites per lake? How these sites were selected? What is the difference between "sampling points" and sites?

RESPONSE: We have dealt with this point above (in response to Reviewer comment about LINES 118-120).

-Lines 133-137. Authors wrote: "Macrophyte surveys involved shoreline and open water (using boat) assessments conducted on 100 m representative sections of each lake using a bathyscope and a double-headed rake. Macrophyte abundances at forty points (20 shoreline + 20 open water) per 100 m section were recorded and at least two 100 m sections per site were surveyed…" So you surveyed only two sections per lake? How their "representativeness" was determined?
RESPONSE: We have included a new column in Table 1 showing the number of sectors per lake and have made our sampling approach more explicit. And we have clarified the sampling in our response above (see response to Reviewer comments about LINES 118-120).

-Note that the forty points per section are pseudo replicates and cannot be used as true replicates in lake-wide assessment and comparisons between lakes. In fact you have only two replicates per lake which is not enough to do within lake variability. With this data you can get only very rough general idea about macrophyte community in a lake. Macrophyte distribution is extremely patchy and depends on many environmental factors, like substrate, depth, wave activity, etc. Therefore, two sections per lake is not nearly enough.

RESPONSE: These points made by the reviewer are an excellent concern and we agree that our *methods section was misleading in the sense of data acquisition and analysis leading us to change our text as described in response to Reviewer's comments on LINES 118-120 above.*

-Lines 139-140. Authors wrote: "The abundance of D. polymorpha at each lake was estimated with the same scale [0>3] used for macrophytes." Again, having only two sites for estimation of Dreissena abundance is not enough. Dreissena distribution is extremely patchy (ranging from none to very high) depending on many environmental factors. With the current sampling design you can only detect the presence of Dreissena, but not estimate even relative abundance.
RESPONSE: We agree with the reviewer about the potential problems with the patchy distribution of zebra mussels. Nevertheless, our macrophyte sampling protocol at different

sectors of a lake including shorelines and boat transects (LINES 140-178) enabled surveying carefully different lake zones and when present, zebra mussels were easy to see directly through the bathyscope or to collect with a rake. Although highly patchy distributions may have been a minor complication, employing consistent sampling across lakes enabled us to obtain comparative data and our subsequent analyses identified zebra mussel effects that are consistent with expectations of variation in relative zebra mussel abundances (e.g. that macrophyte diversity and abundance would be positively impacted by our semi-quantitative measures of zebra mussel abundance due to their filtering activities). We are therefore confident that our semi-quantitative scale of zebra mussel abundance (0-3) reasonably approximates relative variation in their abundances between lakes. We have described our results by referring to relative zebra mussel abundance in key places and have included a more detailed description of our methods and analysis in LINES (169-178).

-Lines 171-172. Authors wrote: "…we conducted analysis on species richness and community heterogeneity at the within lake and among lakes scale…" I don't think you can do this within a lake having only two true replicates per lake.

RESPONSE: We have revised wording, describing that assemblage heterogeneity and richness were measured at the contemporary level among lakes (Lines 220-224), whereas for the paleo-data we measured within (between time periods) and among lake-variation again by comparing between time periods (LINES 280-282).

Result section. I don't have much comments for the result section, with the exception that I don't think authors used adequate methods to collect field data used in their analysis.

RESPONSE: We have clarified our methods and aims as outlined above.

-Lines 286-288. Authors wrote: "Our analyses show that gradual and progressive nutrient enrichment strongly erodes within lake macrophyte richness and community heterogeneity across spatial and temporal scales." In general I agree with this statement, however this is not a new discovery and this is the place (discussion) to compare authors results with the abundant literature data.

RESPONSE: The reviewer made this point earlier and we have revised our manuscript to both highlight this previous work and to stress how our study provides novel and complementary insights (see our response to the Reviewer's General comments). In addition, as far as we are aware, characterizing how macrophyte assemblage heterogeneity is influenced concurrently by multiple factors in shallow lakes over time and at the landscape scale has not been addressed in detail. With these points in mind we have revised the relevant discussion section in LINES (365-393; see response to Reviewer 1 comments about Lines 292-299).

-Lines 300-301. Authors wrote: “In turn, absences of such species over spring and late-summer/autumn result in phytoplankton, thus placing pressure on remaining macrophytes” I don’t understand this sentence. Please rephrase.

RESPONSE: We have revised this whole paragraph (LINES 355-385) and this sentence is no longer included