White Rose Research Online
eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Leeds.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/200594/

Version: Accepted Version

Article:

Beadle, JM, Holden, J and Brown, LE (Cover date: July 2023) Landscape-scale peatland rewetting benefits aquatic invertebrate communities. Biological Conservation, 283. 110116. ISSN: 0006-3207

https://doi.org/10.1016/j.biocon.2023.110116

© 2023, Elsevier. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/.

Reuse
This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

Landscape-scale peatland rewetting benefits aquatic invertebrate communities

Authors: Jeannie M. Beadle, Joseph Holden & Lee E. Brown*
Affiliations (all authors): School of Geography & water@leeds, University of Leeds, Leeds, LS2 9JT, UK
* Author for correspondence: l.brown@leeds.ac.uk Tel:+44(0)1133433302

Abstract

Many northern hemisphere peatlands have historically been drained, but restoration has sought to raise water tables to support peat-forming vegetation and enhance carbon sinks. In the UK alone, millions of new peatland ponds have been created but their biodiversity remains poorly studied and knowledge to guide conservation is lacking. This study advances understanding of aquatic invertebrate responses to peatland restoration from analyses of: (1) pond colonisation and development up to 18 months after creation; (2) a pond chronosequence spanning 6 months to 15 years, and; (3) a comparison of restored versus naturally-formed ponds. Invertebrate communities established within 4 months of pond creation, but some initial colonisers were no longer found after 6 months. Diversity and abundance peaked at around 5 years then declined. Older pond environmental conditions and biodiversity were generally similar to natural ponds, highlighting restoration success for aquatic biodiversity gains. Co-ordinated, routine monitoring should be implemented to inform conservation approaches for these habitats and their biodiversity, particularly where other land management activities have the potential to impact aquatic systems.

Keywords: Chironomidae; Coleoptera; land management, moorland; pond, succession

1. Introduction

Aquatic habitat modifications due to land management, pollution and flow regulation have driven freshwater species declines globally (WWF, 2020). Peat-dominated wetlands in northern temperate and boreal regions have historically been drained to support extractions for fuel or horticulture and to create land for agriculture, forestry, and infrastructure, also leading to impacts on freshwater species (Ramchunder et al., 2009). These wetlands cumulatively account for an estimated 80%–90% of the 4.23M km^2 of global peat cover (Xu et al., 2018) and their important carbon storage role has led to large-scale restoration schemes to raise water tables. While responses of hydrological and chemical functioning, terrestrial vegetation and greenhouse gases have been studied in detail after restoration (Holden et al., 2018; Chapman et al., 2022; Zou et al., 2022), few studies have focused on aquatic biodiversity.

Re-wetting degraded peatlands typically involves new freshwater pond creation on the surface and behind dams inserted into drainage ditches (Armstrong et al., 2009; Beadle et al., 2015) (Figure S1). Across the UK, the number of newly-created ponds is now likely to exceed one million. Invertebrate communities have been studied in various types of peatland waterbodies, such as old peat cuts and existing ponds on areas of restored peatland (Verberk et al., 2010; Krieger et al., 2019) yet few have looked specifically at invertebrate communities in ditch-blocked ponds (Boyce, 2010; Brown et al., 2016). Most notably, no detailed research has been carried out into how these peatland invertebrate communities form initially then change over time. For some groups such as beetles, human-modified peatlands can provide suitable habitat prior to restoration (e.g. Williams & Gormally, 2010) but it remains unclear which successional processes occur thereafter, and the extent to which biodiversity changes are sustainable over the long-term.

While studies are lacking for peatland ponds, invertebrate colonisation and succession has been well-studied generally in rivers, ponds and lakes. These non-peat studies allow us to hypothesise about peatlands. In non-peatland systems, community assembly depends on dispersal constraints, environmental conditions and species interactions (Belyea and Lancaster, 1999). In peatland pools, taxa from surrounding freshwater habitats need to reach the pool; these can be either strong or weak fliers who actively disperse, or taxa which vector passively by the wind or other organisms. Upon arrival, establishment will depend on environmental constraints (e.g. water quality, habitat availability) and internal influences such as food availability and competitors/predators (e.g. Heino et al., 2015). A consistent finding for new freshwater outside of peatlands is rapid colonisation by Diptera, especially Chironomidae. Chironomids are typically weak fliers but disperse effectively by wind and their short life cycles means there are often adults flying and able to colonise new habitats with a range of physicochemical conditions (Armitage et al., 1995; Baars et al., 2014). In contrast, some groups such as Trichoptera increase steadily through time and Odonata are generally more abundant in later successional stages (Barnes, 1983; Cañedo-Argüelles and Rieradevall, 2011). While Odonata are strong fliers, larval stages can last a few years; their establishment in new peatland ponds may be delayed by low population sizes in general across these landscapes (Brown et al., 2016), plus restricted food or habitat availability in new ponds. The community composition of Coleoptera has also been shown to differ in non-peatland ponds of different ages, with the development of substrate and vegetation cover a key influence on establishment (Pakulnicka and Zawal, 2019).

This study aimed to investigate how invertebrate communities colonise and establish in restored peatland ponds over time, quantify changes in abundances and diversity (alpha, beta), and identify associations between physico-chemical variables and ecological communities. Based on observations and theory developed from studies of pond habitats more generally, for peatlands we expected that: (H_1) chironomids would be dominant colonisers due to high dispersal abilities (Armitage et al., 1995), and tolerance of dystrophic conditions (low pH, high DOC), with other groups such as Coleoptera increasing in abundance over time as food resources (producers, smaller invertebrates) and habitat (vegetation cover) developed; (H_2) taxonomic richness and abundance would rise sharply after pond creation but then quickly reach asymptote because peatland ponds are small habitats with dystrophic conditions that limit establishment of many species (Barnes, 1983; Cañedo-Argüelles and Rieradevall, 2011). However, (H_3) beta-diversity was predicted to be high in young ponds reflecting stochastic dispersal and establishment, but to then decrease after invertebrate taxa had time to establish in multiple ponds (Urban, 2004; Chase, 2007); (H_4) because increasing vegetation cover restricts some taxa (e.g. surface dwellers) but facilitates others (e.g. Coleoptera), intermediate cover should be associated with more diversity, whilst variable depths should support a wider array of taxa by providing heterogeneity of dissolved oxygen availability and refugia from predation (Dowling & Murray, 1981).

2. Methods

2.1. Study sites

The study was undertaken on blanket peatland across the Pennine hills of northern England between 2012-2014. All peatlands were dominated by *Eriophorum* spp. and *Calluna vulgaris* with varying *Sphagnum* spp. cover.

Newly-created ponds

Initial community assembly in newly-blocked ditch ponds was monitored at Moor House (Table S1), where a ~1km^2 area was restored in December 2012. Over 500 new ponds were created in a landscape where naturally-formed ponds are largely absent due to historical modification (Figure S1). Ponds were initially dry until water tables rose, and froze regularly during winter 2012/13. Regular sampling (two monthly) was therefore initiated from 04/2013 to 06/2014 (ages 4 to 18 months), apart from month 14 when snow hindered access and pond surfaces froze. On each visit, five ditches were selected and one pond from each was sampled randomly using a number generator to determine which pool to sample along each ditch. Due to the small size (≤ 3 m^2), ponds were disturbed in their entirety; therefore, sampled ponds were marked with bamboo canes and independent ponds were sampled on every visit.

Multi-peatland chronosequence ponds

Six peatlands were selected for study based on access agreements and the availability of dates for when ponds were created (Table S1). At each peatland, five independent drainage ditches were selected and one pond selected randomly from each sampled in 06/2013. Sampling was repeated in 06/2014 but in different ponds, giving ten ponds per site.

Naturally-formed ponds

Twenty ponds across four peatlands were studied in 07/2012 (Table S1). Due to historical land-use changes, naturally-formed ponds are uncommon in Pennine peatlands. Therefore, random selections were not possible and sampling was undertaken in locations where access was agreed with landowners. Pond numbers thus ranged from 2 (Harwood Fell) to 7 (Butterburn Flow).

2.2. Sampling methods

Invertebrates

Invertebrates were collected using a 250-µm mesh net and preserved in 70% methylated spirits. Two-minute samples were collected from open water, floating vegetation, littoral vegetation and sediments with one-minute then searching for surface taxa. Invertebrates were later sorted then identified (see Supplementary Information). Vegetation cover (primarily *Sphagnum*) was estimated visually before sampling.

Climate

Directly observed data were not available at all locations. However, gridded data (rainfall, air temperature, frost days) were available from the HadUK-Grid (1km). Biological sample were often collected mid-month, therefore data were collated for the preceding whole month, and annual totals aggregated for the previous year.

Pond location and morphology

Pond altitude (m) and location were recorded (Garmin eTrex GPS). Aspect was calculated from the Ordnance Survey Terrain50 grid, then converted to northness using cos(pi*degrees/180): 1= due north, -1 = due south. Pond long/short axes and perimeter were measured with a tape measure. At least 40 depth measurements were taken per pond along regularly spaced transects, with more in larger ponds. Pond volume was estimated from surface area (long x short axes) x mean depth.

Pond water quality

Electrical conductivity (EC), dissolved oxygen (DO), water temperature and pH were measured (HACH HQ30d) in the upper water column. The pH sensor failed during month 12, therefore missing values were inferred from the pH~Mg relationship across all other samples (r=0.81). A 50 mL water sample was filtered (0.45-µm) on site and analysed for total nitrogen (TN) and phosphorus (TP), dissolved organic carbon (DOC), aluminium (Al), iron (Fe) and Silica (Si) using an Analytikjena multi N/C® 2100, a San++ Continuous Flow Analyzer and a Thermo Fisher iCAP7600 ICP-OES.

2.3. Data analysis

Invertebrate biodiversity

For the newly-created and chronosequence ponds, abundances were summed across replicates for each pond age then used to identify temporal patterns. Taxa found in newly-created ponds in months 4 and 6 but which were subsequently absent were noted as first colonisers. The three most abundant taxa across newly-created and chronosequence ponds were analysed against pond age using regression (see below). Naturally-formed ponds were not included in temporal analyses as their age could not be determined.

For each pond, taxonomic richness and the community total abundance, plus richness, abundance and relative abundance of Chironomidae and Coleoptera, were calculated. Chironomidae and Coleoptera were selected as the most taxonomically rich and abundant groups which can serve as peatland pond condition indicators (Ozoliņš et al., 2021). For newly-created and chronosequence ponds, beta diversity (turnover, nestedness and Sørensen) was calculated using betapart in R. Beta diversity was analysed (i) within age categories (averaged across five replicates) using regression (below); (ii) between all ponds using multiple regression on distance matrices (MRM) using ecodist in R.

Generalized linear models (GLM) and generalized additive model (GAM) regressions were developed for newly-created ponds to assess relationships between age and biological variables. Analyses were undertaken using the R packages lme4, MASS and mgcv. GAMs were used where initial plots suggested non-linear relationships, with smooth terms for age and k_{max}=5. Model and family combinations appropriate to each analysis were developed (See Table S4). Centred climate (monthly) co-variables (rainfall, frost days (correlated with air temperature; r^2=0.87) were included in models.

Due to spatial autocorrelation in some chronosequence data, generalized linear and additive mixed-effect models were developed (GLMM, GAMM) to assess if there were relationships between age and biological characteristics. Random intercepts (GLMM) or smooths (GAMM) were specified based on samples collected from the same peatlands (site) and for sampling years (2013/14). Whilst Year was represented by only 2 levels, overall sample size was large enough that its inclusion did not influence model results other than minor changes to Site random effect estimates from which we were not making inferences (cf. Gomes, 2022). Analyses were undertaken using the r packages nlme and mgcv (See Table S4 for models). Due to strong correlations among climate variables, and between climate and elevation, model co-variables (centred) were restricted to air temperature (previous month), rainfall (previous month, previous year) and aspect (northness). Chronosequence beta diversity (within) analyses included only age and two random effects due to small sample sizes.

Environment-biodiversity relationships

To aid interpretation of relationships between environmental parameters (climate, pond location/morphology and water quality parameter groups (Table S5)) with biological datasets, principal components analysis (PCA) was used to reduce dimensionality. PCAs were run individually for each group, after initial analyses combining all 24 parameters produced a large numbers of PCs with low % variances. PCAs were conducted separately for newly-created ponds, and for Chronosequence and naturally-formed pond data combined. For newly-created ponds, the climate conditions group included only monthly observations because many were <1 year when sampled. Analyses were undertaken using the princomp function in R with a correlation matrix. PCs with cumulative variance >70% were retained.

Non-metric multidimensional scaling (NMDS) analyses were undertaken on relative abundance data using the vegan package in R, with Bray-Curtis dissimilarity scores, 3d solutions to minimise stress, and 10,000 iterations. One sample from newly-created ponds (month 6) was omitted because it contained only one individual and this heavily affected the solution. Relationships between axes 1/2 and environmental parameters were examined using envfit. Envfit was run using retained PCs and vegetation cover, with age included to understand development over time. Indicator Species Analysis (999 permutations) was utilised to determine which taxa contributed to dissimilarity over time using R package indicspecies. This relies on categorical groupings of samples, so for newly-created ponds two groups were defined to compare 4-6 months with 16-18 months. For chronosequence ponds, groups were defined corresponding to youngest, intermediate and established (0.5-2.5y, 3-8y, 10-15y, respectively) ponds so that age groups were not confounded by location. Age was unavailable for naturally-formed ponds so these formed a combined group. All indicator taxa ($p<0.05$) in one of the three age groups/natural ponds were examined; if they were unique to only one peatland these were omitted from further analysis and interpretation. All analyses were undertaken using RStudio 2022.02.3.

3. Results

3.1. Newly-created ponds: Invertebrate biodiversity

4560 invertebrates were collected from 35 ponds (Table S2). Chironomidae were the most abundant group (79.5%), followed by Hemiptera (8.5%), Coleoptera (6%) and Plecoptera (6%), although relative abundance varied over time with no clear trend (Fig. 1a). Chironomidae, Plecoptera, Trichoptera and other Diptera were present at 4 months, whereas first finds of Coleoptera and Hemiptera were in month 6 (at low abundance: 2 and 1 individuals, respectively), and Oligochaeta in month 10.

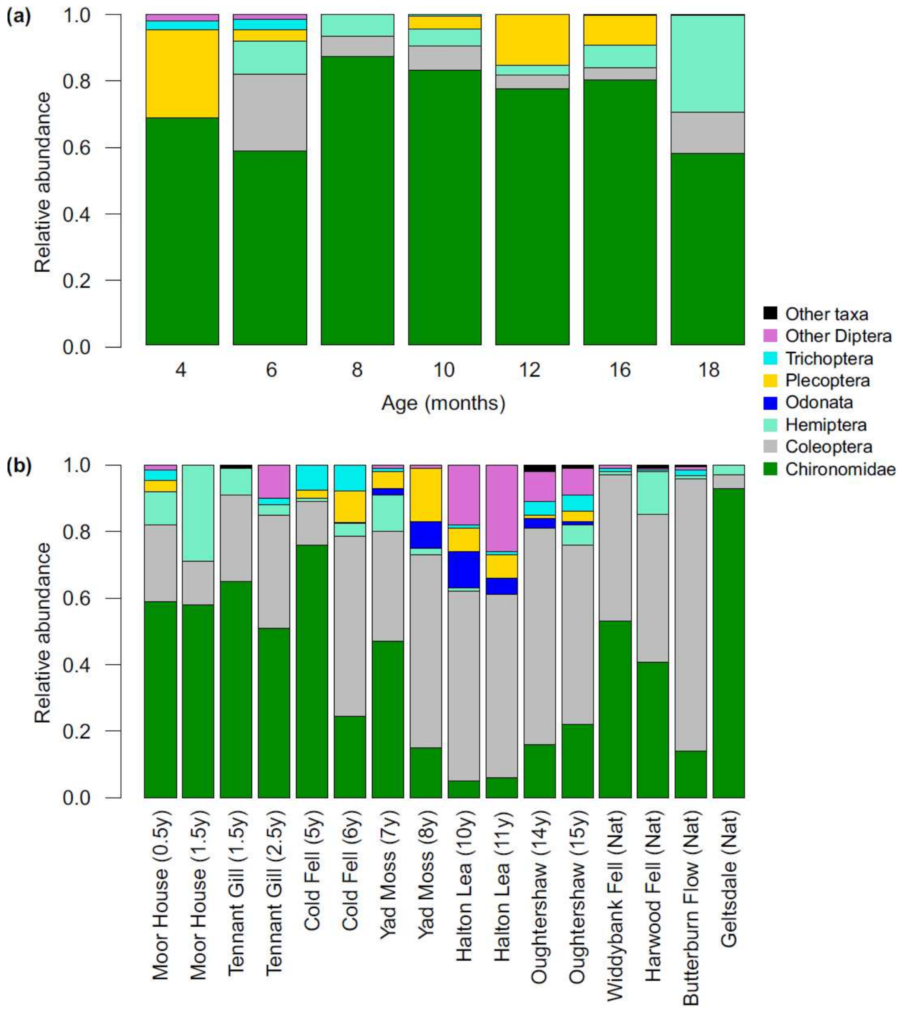

Fig. 1. Relative abundance of invertebrates in (a) newly-created ponds, and (b) chronosequence and naturally-formed ponds (Nat).

Within four months, 13 taxa were found in newly-created ponds (Table S1). Three (*Microspectra junci* [n=1], unidentified Orthocladiinae sp. [n=5], *Eutonia* sp. [n=1]) were not found on any later date. Two additional taxa (*Pseudorthocladius, Parametriocenmus*) were present in month 4 and 6 but not detected thereafter. The three most abundant invertebrates accounted for 68% of abundance across all samples (*Chironomus plumosus* – 40%; *Psectrocladius obvius* – 22%; Corixidae – 6%). *C. plumosus* and *P. obvius* abundances increased initially after eight months but subsequently decreased (Fig. 2a-b).

Pond richness increased over time, whereas abundance changed most substantially in the first 12 months then declined slightly. Total abundance (n = 1071 for summed replicates) peaked in month 12 (Fig. 2b) mainly due to Chironomidae (n = 842) and Plecoptera (n = 164). Coleoptera richness and abundance increased significantly with pond age (Fig. 3c, e), although richness remained low in individual ponds with only nine beetle taxa recorded (eight adult species plus Dytiscidae larvae). Chironomid richness increased over time (Fig. 3d) whereas abundance followed the same trend as total community abundance (Fig. 3f).

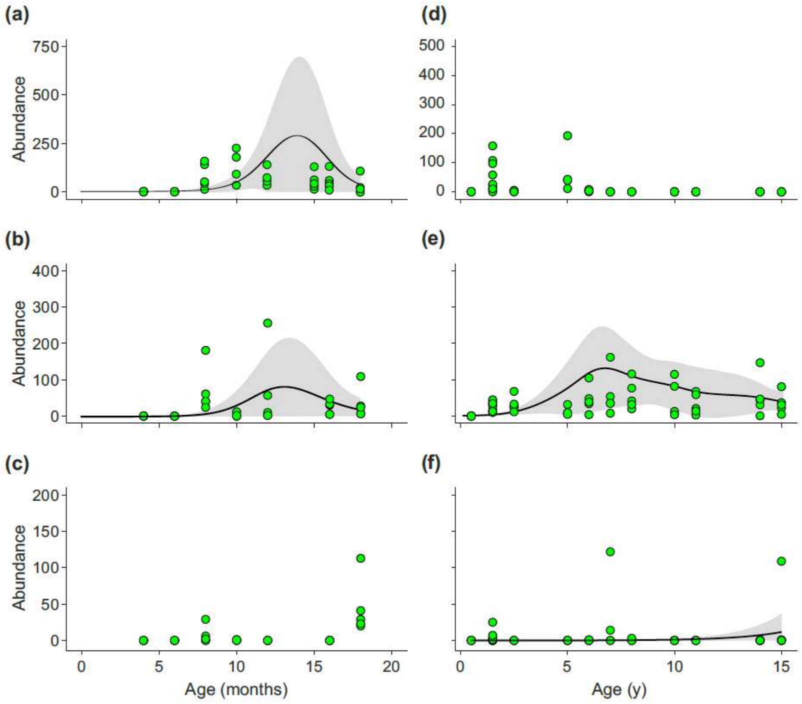

Fig. 2. Changes over time for the three most abundant species in (left column) newly-created ponds: (a) *Chironomus plumosus*, (b) *Psectrocladius obvius*, (c) Corixidae, (d) Chironomus plumosus; and (right column) chronosequence ponds: (d) *Chironomus plumosus*, (e) Dytiscidae larvae, (f) *Corynoneura* sp. Solid lines and shaded areas (95% CI) show model fits where pond age was a significant predictor (see Table S8 for model outputs).

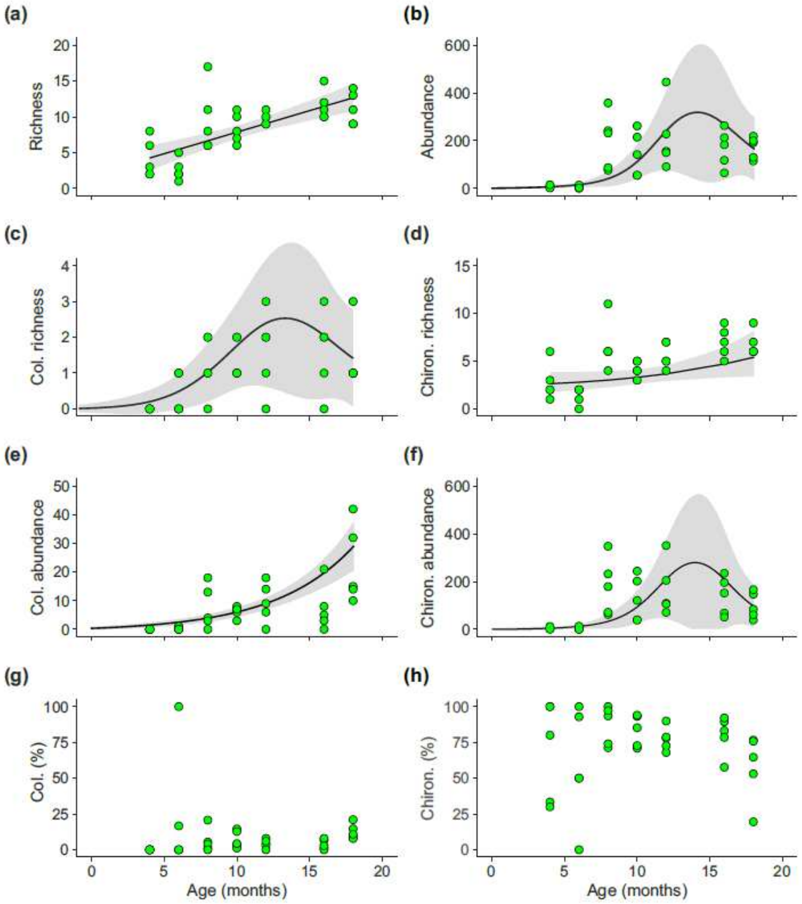

Fig. 3. Newly-created pond age and biodiversity metrics. Col. = Coleoptera, Chiron. = Chironomidae. Solid lines and shaded areas (95% CI) show model fit where pond age was a significant predictor (see Table S8 for model outputs).

Beta diversity components turnover and Sørensen were initially high then decreased but the relationship was not significant (Fig. S2) and nestedness was consistently low. Responses to difference in pond ages were relatively weak although Sørensen and turnover displayed similar relationships (Fig S2); the strongest relationships were found for Sørensen (MRM r=0.09, p=0.002) and turnover (r=0.05, p=0.004) but there was no relationship between age difference and nestedness (r=0.003, p=0.39).

3.2. Newly-created ponds: Environment-biodiversity relationships

Community composition was notably different in months 4-6 (Fig. 4a, b), with *Pseudorthocladius* a key indicator (Indicspecies r=0.707, p=0.001). As ponds aged, their communities were characterised by more *Corynoneura* (r=0.722, p=0.006), *Tanytarsini* sp. (r=0.697, p=0.008) and Corixidae (r=0.677, p=0.011). The PCA produced one climate PC (77% total variance), three landscape/morphology PCs (71%) and three water quality PCs (77%;

Table S6). Two PCs were associated significantly with the NMDS solution. Older ponds were associated with warmer temperatures and more rainfall (Climate PC1), more dissolved nutrients (esp. P) and Al (Water Quality PC1) and greater vegetation cover (Fig. 4b).

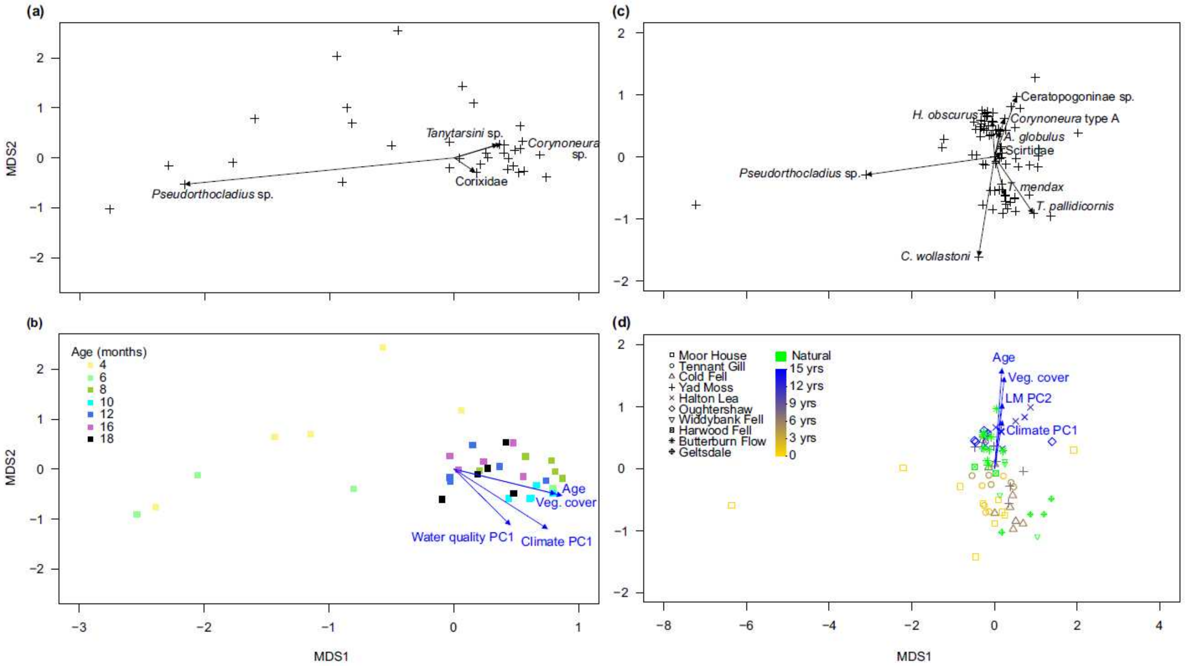

Fig. 4. NMDS biplots showing (a) Newly-created pond taxa with indicator species highlighted; (b) Newly-created ponds sites and significantly associated environmental variables (Vegetation Cover r^2= 0.21 , p=0.036; Climate PC1 r^2=0.45, p=0.001; Water Quality PC1 r^2=0.33, p=0.001) and Age (r^2=0.24, p=0.016); (c) chronosequence and naturally-formed pond taxa with indicator species highlighted; (d) chronosequence and naturally-formed ponds sites with significantly associated environmental variables (Vegetation Cover r^2= 0.66 , p=0.001; Climate PC1 r^2=0.13, p=0.013; Landscape/morphology (LM) PC2 r^2=0.24, p=0.005) and Age (r^2=0.55, p=0.001). [a+b stress = 0.07, 12 iterations. Ordination distances vs. observed dissimilarity non metric fit = 0.995, linear fit = 0.981; c+d stress = 0.12, 622 iterations. Distances vs. observed dissimilarity non metric fit = 0.989, linear fit = 0.963]

3.3. Chronosequence and naturally-formed ponds: Invertebrate biodiversity

7773 invertebrates were collected from chronosequence ponds and 3118 from naturally-formed ponds (Table S3). Chironomidae were the most abundant group (47% chronosequence, 72% natural ponds), followed by Coleoptera (34% chronosequence, 24% natural ponds) (Figure 1b). Chironomidae relative abundance declined with age, with a converse increase in Coleoptera, Odonata and other Diptera (particularly Ceratopogoninae and *Eutonia*). These trends were not maintained in naturally-formed ponds which ranged from being dominated by Chironomidae at Geltsdale to Coleoptera at Butterburn Flow. Of the five taxa found only in months 4 and 6 in Moor House newly-created ponds, *Pseudorthocladius* and *Parametriocenmus* were not found in any other ponds (Table S2). Abundances were more evenly spread across taxa in chronosequence ponds compared to newly-created ponds, with the three most abundant taxa accounting for only 40% (Dytiscidae larvae – 18%; *Corynoneura* sp. – 11%, *C. plumosus* – 11%). Dytiscidae abundance peaked in ponds aged 7 years, whilst *Corynoneura* sp. increased slightly towards the end of the chronosequence (Figure 2e-f).

Pond richness increased significantly between 0-5 years then declined (Fig. 5a). A similar response was evident for total abundance (Fig. 5b), although one pond at Yad Moss (7 years) hosted the highest abundance, composed mainly of *Corynoneura* sp. (91%). Chironomidae richness (Fig. 5d) followed a pattern like overall richness. Coleoptera abundance/relative abundance both increased consistently over time (Fig. 5e, g); for Chironomidae, abundance trends were less pronounced (Fig 5f) but relative abundance declined significantly over time (Fig. 5h). Naturally-formed ponds had, on average, similar richness (10±5[SD] taxa), total abundance (156±160 individuals), Chironomidae richness (4±4 taxa) and Coleoptera richness (5±2 taxa) as the oldest chronosequence ponds.

Beta diversity Sørensen and turnover were not related to age despite being greatest in youngest ponds. Nestedness was consistently low. Responses to pond age differences were stronger than in the newly-formed ponds although Sørensen (MRM r=0.19, p=0.001) and turnover (r=0.16, p=0.001) displayed similar relationships (Fig. S2) with a weak negative relationship for nestedness (r=0.008, p=0.02).

3.4. Chronosequence and naturally-formed pond environment-biodiversity linkages

Community composition was notably different for the youngest ponds (6 months; Fig. 5), with *Pseudorthocladius* the key indicator for 0.5-2.5 years (r=0.39, p=0.045). *Callicorixa wollastoni* was also a young pond indicator (r=0.57, p=0.003) although some were also found in older ponds and two naturally-formed ponds (Geltsdale, High Fell). Ponds aged between 5 to 8 years were characterised by higher abundance of *Corynoneura* sp. (r=0.722, p=0.006), *Tanytarsini* sp. (r=0.697, p=0.008) and Corixidae (r=0.677, p=0.011). The oldest chronosequence ponds (10-15 years) were defined by three indicators, although these were also found in a few naturally-formed ponds (Ceratopogoninae r=0.78, p=0.001; *Corynoneura* type A r=0.58, p=0.003; *Anacaena globulus* r=0.55, p=0.015). Naturally-formed ponds contained several indicator species. Two beetles (*Enochrus affinis* (r=0.50, p=0.003) and Hydrophilidae larvae (r=0.45, p=0.014)) were found exclusively at Butterburn Flow, whereas Scirtidae larvae (r=0.50, p=0.004) were found at all naturally-formed pond locations except Geltsdale. Three other taxa were associated with naturally-formed ponds but found also in chronosequence ponds: *Tanytarsus mendax* (r=0.65, p=0.005), *Hydroporus obscurus* (r=0.77, p=0.001) and *Tanytarsus pallidicornis* (r=0.56, p=0.033).

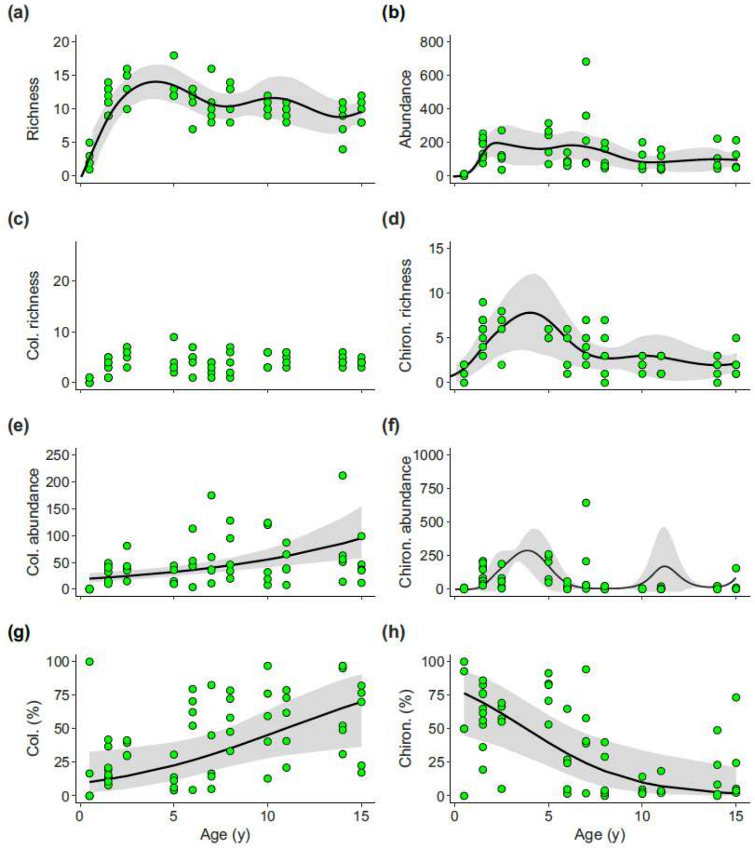

Fig. 5. Chronosequence pond age and biodiversity metrics. Col. = Coleoptera, Chiron. = Chironomidae. Solid lines and shaded areas (95% CI) show model fit where pond age was a significant predictor (see Table S8 for model outputs).

The PCA produced two climate PCs (80% total variance), three landscape/morphology PCs (78%) and four water quality PCs (74%; Table S7). Two PCs were associated significantly with the NMDS solution. Older ponds were associated with warmer temperatures and more rainfall (Climate PC1), greater minimum and mean depths (Landscape/Morphology PC1) and increased vegetation cover (Fig. 5d).

4. Discussion

This study has provided detailed insights into the assembly and temporal dynamics of peatland pond invertebrate communities, with significant potential to inform their management and conservation. Biodiversity and community composition both changed significantly with pond age, driven at least partially by an increase in vegetation cover and, to a lesser extent, intra-pond variations in depth.

4.1. Peatland pond invertebrate biodiversity

Newly-formed ponds were dominated by chironomids as early colonisers, providing support for H_1. This was also reflected in the longer-term chronosequence study, where Chironomidae relative abundance declined substantially in ponds >10 years. Orthocladiinae constituted >50% the chironomid taxa found, similar to the findings of Barnes (1983) and Dowling & Murray (1981) in acidic ponds. As Orthocladiinae include more species with multivoltine life cycles than other common subfamilies such as the Chironominae (Armitage et al., 1995), this could aid rapid establishment while enabling higher population persistence to stochastic events (Chase, 2007) which are common in Pennine peatlands (e.g. pond surface freezing).

Chironomidae accounted for much of the richness in younger ponds, rising consistently over time in newly-formed ponds and remaining elevated in chronosequence ponds until 5 years. *C. plumosus*, the dominant species across the study, is a common pioneer in newly formed ponds by consuming peat particles (McLachlan, 1977) and tolerant of low oxygen concentrations (Dowling & Murray, 1981). Other Chironomidae indicator taxa, *Pseudorthocladius* sp. and *Parametriocenmus* sp., were never found in older ponds and may only inhabit new peat ponds, similar to some pioneer algae species (Carter et al., 2015). *Pseudorthocladius* larvae are semi-aquatic and can live in damp moss (Saether and Andersen, 1996), and therefore may have existed in ditches prior to rewetting and during the initial months of pond existence. Subsequently they are likely to have been outcompeted or physically restricted as ponds filled over time.

Several results supported the first part of H_2, that taxonomic richness and total abundance would rise most sharply after pond creation. Newly-created pond total abundance increased rapidly between 6 and 8 months, and 39/45 taxa were found within the first 10 months, similar to findings from studies in other biomes (Layton and Voshell Jr., 1991; Cañedo-Argüelles and Rieradevall, 2011). Christman and Voshell (1993) considered that proximity of new ponds to source populations accounted for rapid dispersal and establishment. In peatlands, the dominant colonisers Chironomidae and Coleoptera are likely to have dispersed from natural ponds (Baars et al., 2014), other artificially-created ponds on adjacent peatlands (Brown et al., 2016), and smaller water accumulations in hollows and vegetation (Dowling & Murray, 1981). Streams are numerous in peatland landscapes and some pond taxa could have colonised from these sources; for example, *Nemoura cambrica*, Dytiscidae and *Plectronemia conspersa* have been documented in Pennine streams (Ramchunder et al., 2009).

Pond taxonomic richness increased up to 5 years, further supporting H_2. This included further Chironomidae colonisations (*Chaetocladius, Metriocnemus,* Tanypodinae), plus increases in the occurrence and abundance of *N. cinerea*, *Hydroporus* spp. and *A. globulus*. This is remarkably similar to other pond studies despite differences in location and biome. For example, Williams et al. (2010) monitored new ponds in Oxfordshire, UK, and found an initially rapid richness increase flattened out after 3-4 years, whereas for constructed wetlands in southern Sweden they did so at around 5 years age (Hansson et al., 2005). Notably, in ponds around 5 years in our study, inter-pond variation in vegetation cover started to be notable as *Sphagnum* had expanded. This vegetation expansion likely facilitates the establishment of species that would otherwise avoid open water habitats.

In contrast to H_3 we did not find any relationship between age and within-pond beta diversity components. However, Sörensen and turnover were strongly related, and for between-pond beta-diversity analyses both increased significantly with pond age. Composition changes were therefore driven mainly by species replacements; for example, in the newly-created ponds at Moor House two early-colonising Orthocladiinae taxa (*Limnophyes* and *Pseudorthocladius*) were quickly replaced by *C. plumosus*, *P. obvius*, *T. pallidicornis* and *Zalutschia mucrontata*. By month 18, only *P. obvius* and *Z. mucronata* were dominant, and they remained prominent in chronosequence ponds up to 5-8 years, implying some element of tolerance as other taxa colonised.

Further evidence of turnover was the substantial changes in relative abundance of taxa in chronosequence ponds over time, including an increase in predatory Dytiscid beetles and Odonata likely providing an example of the successional mechanism of facilitation, in this case due to the increased availability of prey such as small Chironomidae (Wellborn et al., 1996) plus vegetation increases offering improved habitat availability. However, it is possible that the sampling method (sweep-netting) was less effective at capturing chironomids and some beetles in *Sphagnum* dominated ponds, as the net may not have effectively sampled the pond bottom and some beetles take refuge when vibrations are detected.

4.2. Peatland pond environment-biodiversity relationships

A key change observed over time was the increase in vegetation (mainly *Sphagnum*) cover, in particular in the 10+ years ponds; 17/20 chronosequence ponds > 10 years old had 100% vegetation cover, whereas the youngest ponds at Moor House averaged only 14% cover (max 65%; one pond). This finding contrasts markedly with Peacock et al. (2013) who reported mean pond vegetation cover of 76% after 18 months perhaps due to elevated nitrate concentrations, but was similar to Mazzerole et al. (2016) who found ponds <4 years of age averaged 13% *Sphagnum* and vegetation cover. Pond vegetation is important for invertebrates as it increases structural complexity, providing different habitat niches and food sources for invertebrate taxa compared to open water ponds.

Chronosequence communities were associated strongly with vegetation cover as part of the analysis, supporting H_4 that changing habitat structure would play a key role in successional changes. While pond beetles often congregate in the smallest clumps of emergent vegetation or other refugia (e.g. Macan, 1977), species such as *A. globulus* were associated strongly with the oldest chronosequence ponds in the indicator species analysis. Many of these ponds had >90% vegetation cover and this species has previously been highlighted for its affinity to *Sphagnum* dominated wetlands (McCormack, 2005). Two other beetles (*Enochrus affinis, Enochrus ochropterus*) were indicators of natural ponds being found exclusively at Butterburn Flow in this study, although they are usually found more widely in stagnant acid water associated with mosses (G. Foster, *pers. comm*). The shift from dominance of Chironomidae to Coleoptera across our peatland chronosequence sites highlights that these groups are useful indicators of successional stage of pond development in addition to indicators of peatland condition (Ozoliņš et al., 2021).

Similar to declines in Chironomidae in older ponds, Hemiptera abundance decreases in older ponds are supported by Barnes (1983) and Mazerolle et al. (2006) who reported the presence of low shrubs and moss inhibits their ability to swim on, or just under, the water surface. Natural ponds were notable for hosting two *Tanytarsus* species (*T. mendax, T. pallidicornis*). Both are collector-filterers, with *T. pallidicornis* associated with lakes high in organic matter (Wilson and Gajewski, 2004) as is common in natural peatland ponds which typically have unconsolidated organic matter deposits. *Tanytarsus* have previously been considered as indicators of peatland ponds with higher dissolved oxygen (Dowling & Murray, 1981) and natural and older ponds typically had larger surface area:volume ratios, including more shallow areas, which would act to enhance reaeration compared to smaller, deeper artificial ponds, and supporting H_4. However, experiments to disentangle these effects from high vegetation cover, thus enhanced primary productivity adding oxygen into the water column, would be needed.

4.3. Implications for peatland restoration and biodiversity conservation

Our study illustrates that peatland restoration has created substantial new habitat for aquatic invertebrates. These findings need to be incorporated into decision making when planning further peatland restoration schemes, with aquatic biodiversity considerations included alongside more common drivers such as reducing erosion, reducing drinking water treatment costs and enhancing peatland carbon sinks (Parry et al., 2014). The study shows that early colonising species were replaced quickly, including species associated with moist mosses. Thus, ensuring some heterogeneity of habitats on restored sites is vital to maximise biodiversity.

Ponds changed over several years, therefore restoration and management plans should consider incorporating ponds of different ages across the landscape. This could be achieved either by staggering their construction, although this might not be economically feasible. Alternative options are to integrate ponds of different sizes and depths into construction methods. Small, shallow ponds are likely to be ‘reset’ more often by droughts and freezing thus allowing species associated with young ponds to recolonise at different time points. For example, naturally-formed ponds at Geltsdale had high Chironomidae abundance and low abundance of predators such as Dytiscidae suggesting a recent ‘reset’ when there were no notable differences in measured environmental parameters at this location.

The finding that pond morphological characteristics were associated with the composition of invertebrate communities offers further potential for optimising restoration. When blocking artificial drainage channels, hundreds of ponds are often formed at regularly spaced intervals with similar surface areas and bathymetric profiles. Typically these are steep-sided following the existing ditch cross section, which has been suggested to benefit some invertebrates such as Dytiscidae in fishless ponds (Liao et al., 2020). In contrast, our result showed that some species were found more commonly in ponds with larger surface areas, and larger areas of shallow water (e.g. shelves) as is common for ponds more generally (e.g. Hill et al., 2019). These shallows can enhance atmospheric reaeration and therefore maintain elevated dissolved oxygen levels compared to deeper areas, as well as provide refuge from larger aquatic predators. Such pond profiles are often found in natural systems with patterned pond arrangements, such as those of the peatlands of the Flow Country, Scotland, where asymmetric bathymetry is common (Belyea and Lancaster, 2002). Ponds where ditch sides are reprofiled from box sections into v-shaped channels prior to blocking (Beadle et al., 2015) might therefore provide potential for more biodiversity by offering a variety of depths.

Cumulatively, the hundreds of thousands to millions of new peatland ponds will now contribute significantly to aquatic metapopulations across the UK, but as with other small waterbodies (including natural peatland ponds) they are not monitored routinely and have been examined only minimally as part of previous Countryside Surveys (Biggs et al., 2017). As a consequence, chronosequence studies are needed to provide insights into long-term development, but unknown disturbance histories might remain unaccounted for (Johnson and Miyanishi, 2008). Nevertheless, as the number of ponds on peatlands now likely exceeds the total number of ponds elsewhere in the UK, they must be considered as a major reservoir of aquatic biodiversity. Co-ordinated monitoring should therefore be implemented to inform conservation actions. While we found no rare or endangered invertebrate taxa in our study, other UK peatland pond studies have (Maitland, 1999; Drinan et al., 2013). The newly-created ponds at Moor House also provided the first British record of the algae *Saturnella saturnus* (Carter et al. 2015). Regular monitoring would therefore assist in evaluations of if and/or how other peatland management activities such as grazing, track creation, rotational heather burning or cutting might impact some of these species.

Overall, it is clear from this study that the biodiversity of restored peatland ponds reflects the temporal stage of development. New ponds colonise very quickly and house novel taxa (see also Carter et al., 2015). Invertebrates subsequently offer vital sources of food for predators such as Odonata and amphibians (Mazerolle et al., 2006; Krieger et al., 2019) and birds such as Greenshank and Golden Plover (Downie et al., 1998). The benefits for biodiversity should therefore be considered more widely than our focus on invertebrates. Restoration and conservation agencies need to begin working to develop ponds that optimise trade-offs among peatland recovery, vegetation recovery and GHG emissions alongside the array of aquatic biodiversity benefits that can accrue from landscape-scale peatland restoration schemes.

5. References

Armitage, P.D., Cranston, P.S. and Pinder, L.C.V. (eds) (1995) *The Chironomidae: biology and ecology of non-biting midges*. London: Chapman and Hall.

Armstrong, A. *et al.* (2009) Drain-blocking techniques on blanket peat: A framework for best practice', *Journal of Environmental Management* 90: 3512–3519.

Baars, J.R. *et al.* (2014) Macroinvertebrate assemblages of small upland peatland lakes in Ireland. *Biology & Environment Proceedings of the Royal Irish Academy* 114: 233-248

Barnes, L.E. (1983) The colonization of ball-clay ponds by macroinvertebrates and macrophytes. *Freshwater Biology* 13: 561–578.

Beadle, J.M., Brown, L.E. and Holden, J. (2015) Biodiversity and ecosystem functioning in natural bog pools and those created by rewetting schemes. *WIRES Water* 2: 65–84.

Belyea, L.R. and Lancaster, J. (2002) Inferring landscape dynamics of bog pools from scaling relationships and spatial patterns. *Journal of Ecology* 90: 223–234.

Biggs, J., von Fumetti, S. and Kelly-Quinn, M. (2017) The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia*, 793: 3–39.

Boyce, D.C. (2010) *Invertebrate survey of Exmore Mire blanket bog sites, 2009*. Dulverton.

Brown, L.E. *et al.* (2016) Macroinvertebrate community assembly in pools created during peatland restoration. *Science of The Total Environment* 569–570: 361–372.

Cañedo-Argüelles, M. and Rieradevall, M. (2011) Early succession of the macroinvertebrate community in a shallow lake: Response to changes in the habitat condition. *Limnologica*, 41: 363–370.

Carter, C. *et al.* (2015) New observations on *Saturnella saturnus* (Steinecke) Fott: the first British record of a little-known enigmatic "green" alga'. *Algological Studies* 149: 61–77.

Chapman, P.J., Moody, C.S., Turner, T.E., McKenzie, R., Dinsmore, K.J., Baird, A.J., Billett, M.F., Andersen, R., Leith, F. & Holden, J. 2022. Carbon concentrations in natural and restoration pools in blanket peatlands. *Hydrological Processes* 36: e14520

Chase, J.M. (2007) Drought mediates the importance of stochastic community assembly', *PNAS* 104: 17430–17434.

Christman, V.D. and Voshell, J.R. (1993) Changes in the benthic macroinvertebrate community in two years of colonization of new experimental ponds: *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 78: 481–491.

Dowling, C. and Murray, D.A. (1981) The distribution of the Chironomidae (Diptera) in two Irish blanket bogs. *Proceedings of the Royal Irish Academy B* 81B: 53–61.

Downie, I.S. *et al.* (1998) Invertebrates of importance to breeding birds in the Flow Country in Standen. V. et al. (eds) *Patterned mires and mire pools: origin and development: flora and fauna: proceedings: University of Durham*. London: British Ecological Society.

Drinan, T.J. *et al.* (2013) Macroinvertebrate assemblages of peatland lakes: Assessment of conservation value with respect to anthropogenic land-cover change. *Biological Conservation* 158: 175–187.

Gomes, D.G.E. (2022) Should I use fixed effects or random effects when I have fewer than five levels of a grouping factor in a mixed-effects model? *PeerJ* 10: e12794

Hansson, L.A. *et al.* (2005) Conflicting demands on wetland ecosystem services: nutrient retention, biodiversity or both? *Freshwater Biology* 50: 705–714.

Heino, J. et al. (2015) Metacommunity organisation, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. *Freshwater Biology* 60: 845-869

Hill, M.J. et al. (2019) Environmental factors are primary determinants of different facets of pond macroinvertebrate alpha and beta diversity in a human-modified landscape. *Biological Conservation* 237: 348-357.

Holden, J. *et al.* (2018) Water-level dynamics in natural and artificial pools in blanket peatlands. *Hydrological Processes* 32: 550–561.

Johnson, E.A. and Miyanishi, K. (2008) Testing the assumptions of chronosequences in succession. *Ecology Letters* 11: 419–431.

Krieger, A., Fartmann, T. and Poniatowski, D. (2019) Restoration of raised bogs–Land-use history determines the composition of dragonfly assemblages. *Biological Conservation*, 237: 291–298.

Layton, R.J. and Voshell Jr., J.R. (1991) ‘Colonization of new experimental ponds by benthic macroinvertebrates. *Environmental Entomology* 20: 110–117.

Liao, W., Venn, S. & Niemelä, J. (2020) Environmental determinants of diving beetle assemblages (Coleoptera: Dytiscidae) in an urban landscape. Biodiversity & Conservation 29: 2343–2359.

Macan, T.T. 1977. A twenty-year study of the fauna in the vegetation of a moorland fishpond. *Archive für Hydrobiologie* 81: 1-24.

Maitland, P.S. (1999) New horizons—new species? The invertebrate fauna of unexplored aquatic habitats in Scotland. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 9: 529–534.

Mazerolle, M.J. *et al.* (2006) Animal and vegetation patterns in natural and man-made bog pools: implications for restoration. *Freshwater Biology* 51: 333–350.

McCormack, S. (2005) Aquatic Beetles in Connemara National Park, Co Galway. *The Irish Naturalists’ Journal* 28: 69–72.

McLachlan, A.J. (1977) Some Effects of Tube Shape on the Feeding of *Chironomus plumosus* L. (Diptera: Chironomidae). *Journal of Animal Ecology* 46: 139–146.

Ozoliņš, D. *et al.* (2021) ‘How to Assess the Ecological Status of Highly Humic Lakes? Development of a New Method Based on Benthic Invertebrates. *Water* 13: 1–18.

Pakulnicka, J. and Zawal, A. (2019) Model of disharmonic succession of dystrophic lakes based on aquatic beetle fauna (Coleoptera). *Marine and Freshwater Research* 70: 195–211.

Parry, L.E., Holden, J. and Chapman, P.J. (2014) Restoration of blanket peatlands. *Journal of Environmental Management* 133: 193–205.

Peacock, M., Evans, C.D., Fenner, N. & Freeman, C. 2013. Natural revegetation of bog pools after peatland restoration involving ditch blocking—The influence of pool depth and implications for carbon cycling. *Ecological Engineering* 57: 297-301.

Ramchunder, S.J., Brown, L.E. and Holden, J. (2009) Environmental effects of drainage, drain-blocking and prescribed vegetation burning in UK upland peatlands. *Progress in Physical Geography* 33: 49–79.

Urban, M.C. (2004) Disturbance heterogeneity determines freshwater metacommunity structure. *Ecology* 85: 2971-2978

Verberk, W.C.E.P. *et al.* (2010) Loss of environmental heterogeneity and aquatic macroinvertebrate diversity following large-scale restoration management. *Basic and Applied Ecology* 11: 440–449.

Wellborn, G.A., Skelly, D.K. and Werner, E.E. (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics* 27: 337–363

Williams, C.D. & Gormally, M.J. (2010) The effects of blanket bog management on ground beetles (Carabidae) with particular reference to the threatened *Carabus clatratus* L. *Irish Wildlife Manuals* 47. National Parks and Wildlife Service, Ireland. 72p

Williams, P., Whitfield, M. and Biggs, J. (2010) How can we make new ponds biodiverse? A case study monitored over 7 years, in Oertli, B. et al. (eds) *Pond Conservation in Europe*. Dordrecht: Springer Netherlands: 137–148

Wilson, S.E. and Gajewski, K. (2004) Modern Chironomid assemblages and their relationship to physical and chemical variables in southwest Yukon and northern British Columbia lakes. *Arctic, Antarctic, and Alpine Research* 36: 446–455.

WWF. (2020). Living planet report 2020. Bending the curve of biodiversity loss: a deep dive into freshwater. Gland, Switzerland: World Wildlife Fund.

Xu, J., Morris, P.J., Liu, J. & Holden, J. 2018. PEATMAP: Refining estimates of global peatland distribution based on a meta-analysis. CATENA 160: 134-140, Zou, J. *et al.* (2022) Rewetting global wetlands effectively reduces major greenhouse gas emissions. *Nature Geoscience* 15: 627–632.

Acknowledgements

We thank Simon Wightman (formerly RSPB) for assisting with the study design and access to landowners. For their help in organising site access we thank Alistair Lockett (Peatscapes), Andy Lloyd (formerly Peatscapes, now NERC), Peter Christopherson and Astrid Hanlon (Yorkshire Peat Partnership), Chris McCarty (Natural England), Jeremy Greensides and Lindsay Waddell (Raby Estates), Peter Welsh (National Trust), Jonny Wildman (Dinsdale Moorland Specialists), Duncan Hutt (Northumberland Wildlife Trust) and Lorna Sherrin, Rob Rose and Beverley Dodd (ECN). For assistance with specimen identifications, we are grateful for the work of Garth Foster (Coleoptera), Steve Brooks (Chironomidae) and Lynne Speight (all other taxa).

Funding

This research was funded by a Natural Environment Research Council (NERC) studentship (NE/J50001X/1) with CASE support from the RSPB to Jeannie Beadle.

Author contributions

LEB and JH initially proposed the idea to study restored peatland ponds, then JMB further conceptualised the specific study focus with input from LEB and JH.

JMB led the research including planning, logistics, field and laboratory sampling/analysis with assistance from LEB and JH.

LEB and JMB undertook the data analysis with input from JH.

JMB led the writing with review and editing by LEB and JH.

Declaration of interests

☐The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Lee Brown & Joseph Holden report financial support was provided by European Commission. Joseph Holden reports financial support was provided by Natural Environment Research Council. Lee Brown & Joseph Holden report financial support was provided by Yorkshire Water Ltd. Joseph Holden reports financial support was provided by United Kingdom Department for Environment Food and Rural Affairs. Joseph Holden reports financial support was provided by The National Trust. Joseph Holden reports financial support was provided by Research England. Joseph Holden reports financial support was provided by Peak District National Park. Joseph Holden reports financial support was provided by Forest Research. Joseph Holden reports a relationship with Shetland Windfarm Environmental Advisory Group that includes: board membership.

Supplementary Information

Methods

Macroinvertebrate identification (Chironomidae)

Macroinvertebrates were identified to the lowest possible taxonomic level using Pawley et al. (2011) and guides to individual groups/orders referenced therein. Where Chironomidae abundance totalled >50 individuals, sub-samples were extracted (n=50) (Rees et al., 2008) for identification to the lowest possible taxonomic resolution. Individuals were immersed in a solution of 10% potassium hydroxide and heated to 70°C for 10 minutes. The chironomids were then transferred into a solution of 95% glacial acetic acid for five minutes, then 80% methylated spirits for five minutes before being stored 100% methylated spirits. Individuals were mounted on slides using Euparal and identified using a compound microscope and following Cranston (1982) and Brooks et al. (2007).

Brook, S. J., Langdon, P. G. & Heiri, O. 2007. *The Identification and Use of Palaearctic Chironomidae Larvae in Palaeoecology.* Technical Guide no. 10, Quaternary Research Association, London, UK.

Cranston, P. S. 1982. *A key to the larvae of the British Orthocladiinae (Chironomidae).* Freshwater Biological Association Scientific Publication no. 45. Bowness, UK.

Pawley, S., Dobson, M. and Fletcher, M. (2011) *Guide to British Freshwater Macroinvertebrates for Biotic Assessment.* Freshwater Biological Association, Bowness, UK.

Rees, A. H., Cwynar, L. & Cranston, P. 2008. Midges (Chironomidae, Ceratopogonidae, Chaoboridae) as a temperature proxy: a training set from Tasmania, Australia. *Journal of Paleolimnology* 40: 1159-1178.

R packages

betapart: Baselga, A., Orme, D., Villeger, S., De Bortoli, J., Leprieur, F. & Logez, M. 2022. betapart: Partitioning Beta Diversity into Turnover and Nestedness Components. R package version 1.5.6. <https://CRAN.R-project.org/package=betapart>.

ecodist: Goslee, S.C. & Urban, D.L. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* 22: 1-19. DOI:10.18637/jss.v022.i07

indicspecies: De Caceres, M. & Legendre, P. 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology* 90: 3566-3574

lme4: Bates, D., Maechler, M., Bolker, B. & Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67: 1-48. doi:10.18637/jss.v067.i01.

MASS: Venables, W.N. & Ripley, B.D. 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0

mgcv: Wood, S.N. 2017. Generalized Additive Models: An Introduction with R (2nd edition). Chapman and Hall/CRC.

MuMIn: Bartoń, K. 2022. MuMIn: Multi-Model Inference. R package version 1.47.1. <https://CRAN.R-project.org/package=MuMIn>.

vegan: Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C. & Weedon, J. 2022. vegan: Community Ecology Package. R package version 2.6-4. <https://CRAN.R-project.org/package=vegan>.

Table S1. Study site characteristics. (* denotes natural ponds which could not be aged).

Site Name	Location	Ponds mean altitude (m)	Restoration year	Pond Age 2013 (years)	Pond Age 2014 (years)
Moor House	54° 41' 27"N 2° 22' 56"W	570	2012	0.5	1.5
Tennant Gill	54° 7' 39"N 2° 11' 28"W	506	2011	1.5	2.5
Cold Fell	54° 52' 55''N 2° 36' 21''W	581	2008	5	6
Yad Moss	54° 43' 15''N 2° 20' 36''W	618	2006	7	8
Halton Lea	54° 54' 28"N 2° 32' 51"W	388	2012	10	11
Oughtershaw Beck	54° 13' 59"N 2° 14' 12"W	410	1999	14	15
Widdybank Fell*	54° 39' 36"N 2° 16' 23"W	523	NA	-	-
Harwood Fell*	54° 51' 6"N 2° 9' 38"W	622	NA	-	-
Butterburn Flow*	55° 4' 42"N 2° 30' 29"W	282	NA	-	-
Geltsdale*	54° 55' 8"N 2° 38' 30"W	583	NA	-	-

Table S2. Taxa identified at Moor House ponds. Numbers are totals across five replicates per pond age (months).

Taxon ↓ Pond Age →	**4m**	**6m**	**8m**	**10m**	**12m**	**16m**	**18m**
Chironomus plumosus	2	2	423	568	369	282	168
Chironomus anthracinus	0	0	0	10	0	0	0
Glyptotendipes barbipes	0	0	6	6	19	6	9
Polypedilum nubeculosum	0	0	8	0	0	0	7
Chironomini sp.	0	0	0	2	0	0	7
Tanytarsus mendax	0	0	1	8	8	22	2
Tanytarsus pallidicornis	0	0	31	15	48	141	8
Microspectra junci	1	0	0	0	0	0	0
Tanytarsini sp.	0	0	3	8	4	25	18
Zalutschia mucrontata	1	0	38	0	64	74	66
Psectrocladius obvius	1	0	347	25	327	120	175
Corynoneura type A	2	0	3	0	0	0	0
Corynoneura arctica	0	6	10	0	0	7	0
Corynoneura scutellata	0	0	12	0	0	0	0
Corynoneura sp	0	0	6	1	0	4	33
Pseudorthocladius	2	11	0	0	0	0	0
Parametriocenmus	1	6	0	0	0	0	0
Limnophyes	9	0	1	0	0	0	0
Orthoclad sp.	5	0	0	0	0	0	5
Macropelopia	0	0	3	3	1	20	0
Procladius	0	0	0	0	0	1	0
Ablabesmyia	0	0	0	0	2	0	0
Tipula sp.	0	1	0	0	0	0	0
Eutonia sp.	1	0	0	0	0	0	0
Limnephilus coenosus	2	0	0	1	0	0	2
Plectrocnemia conspersa	0	1	0	0	0	0	0
Plectrocnemia spp	0	0	0	0	0	1	0
Nemoura cambrica	6	1	0	29	148	54	0
Nemoura spp.	3	0	0	3	16	4	0
Gerris costae	0	0	4	0	0	0	4
Gerridae Nymphs	0	0	20	0	0	0	0
Callicorixa wollastoni	0	1	4	16	17	42	13
Sigara nigrolineata	0	0	0	0	0	1	0
Corixidae nymphs	0	0	37	2	0	0	226
Agabus bipustulatus	0	0	1	0	0	1	0
Hydroporus discretus	0	0	0	0	0	1	0
Hydroporus morio	0	0	0	0	0	0	1
Hydroporus nigrita	0	0	0	1	1	0	0
Hydroporus pubescens	0	0	0	2	3	2	2
Hydroporus tristis	0	0	2	0	0	0	0
Helophorus flavipes	0	1	0	0	0	0	0
Gyrinus substratiatus	0	0	0	0	0	1	0
Dytiscidae larvae	0	1	35	28	43	32	110
Enchytraidae sp.	0	0	0	1	1	1	1

Table S3. Taxa identified in chronosequence and natural ponds. Numbers are totals across five replicates per pond age (chronosequence) and all replicates (natural).

Taxon	**0.5 y**	**1.5 y**	**1.5 y**	**2.5 y**	**5y**	**6y**	**7y**	**8y**	**10y**	**11y**	**14y**	**15y**	**Natural**
Chironomus plumosus	2	168	333	10	327	19	0	0	0	0	0	0	193
Chironomus anthracinus	0	0	3	0	11	0	0	0	0	0	0	0	72
Glyptotendipes barbipes	0	9	9	115	103	2	0	0	0	0	0	0	205
Polypedilum nubeculosum	0	7	0	14	0	0	0	0	0	0	0	3	122
Chironomini sp.	0	7	0	0	3	0	9	0	0	0	0	0	0
Tanytarsus mendax	0	2	10	1	0	0	0	0	0	0	0	0	249
Tanytarsus pallidicornis	0	8	4	3	19	1	0	0	0	0	0	0	268
Microspectra contracta	0	0	0	0	0	0	5	0	0	0	0	0	0
Microspectra sp.	0	0	15	10	0	0	0	0	0	0	0	0	0
Microspectra junci	0	0	0	0	0	0	0	0	1	0	0	0	0
Stempellinella/Zavrelia	0	0	0	0	0	0	0	0	0	0	0	0	38
Tanytarsini sp.	0	18	12	14	1	0	0	0	1	0	0	0	168
Zalutschia mucrontata	0	66	59	28	259	40	3	2	1	0	0	0	569
Psectrocladius obvius	0	175	128	151	90	32	56	9	5	8	0	0	136
Chaetocladius dentiforceps	0	0	0	3	0	0	72	0	0	0	0	0	0
Corynoneura type A	0	0	0	0	0	4	0	3	7	0	0	2	0
Corynoneura arctica	6	0	0	4	0	1	1	16	0	13	3	47	2
Corynoneura scutellata	0	0	0	0	0	0	1	6	0	0	27	0	0
Corynoneura sp.	0	33	7	0	0	1	0	3	0	0	0	0	19
Acamptocladius reissi	0	0	0	0	0	0	756	7	0	8	1	110	0
Metriocnemus eurynotus	0	0	0	1	0	0	0	0	0	0	0	0	0
Metriocnemus terrester	0	0	0	0	0	1	0	0	0	0	0	2	0
Pseudorthocladius	11	0	0	0	0	0	0	0	0	0	0	0	0
Parametriocnemus	6	0	0	0	0	0	0	0	0	0	0	0	0
Limnophyes	0	0	0	0	0	1	0	9	0	0	0	0	0
Acricotopus lucens	0	0	0	0	0	0	0	0	1	0	6	4	0
Orthoclad sp.	0	5	0	2	0	0	0	0	0	0	0	0	18
Macropelopia	0	0	1	0	24	9	0	1	2	2	0	0	162
Tanypodinae sp.	0	0	0	3	0	0	17	0	0	1	21	10	20
Ceratopogininae larvae	0	0	0	0	0	0	0	0	46	90	0	0	5
Phalacrocera replicata	0	0	2	0	0	0	0	1	0	0	0	10	0
Tipula sp.	1	0	0	1	0	0	0	0	0	0	0	0	1
Eutonia sp.	0	0	0	57	0	0	0	1	2	0	0	0	2
Pilaria sp.	0	0	0	0	0	0	0	0	0	0	44	24	1
Pedicia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	0	0	0	0	0	0	0	0	0	0	0	1	0
Chaoborus sp.	0	0	0	0	0	0	0	0	0	0	0	1	0
Limnephilus coenosus	0	2	4	9	27	4	3	0	3	1	0	0	19
Limnephilus spp.	0	0	0	0	3	0	1	0	0	0	19	15	0
Limnephilus centralis	0	0	0	0	0	0	0	0	0	0	1	0	0
Plectrocnemia conspersa	1	0	0	0	23	28	0	0	2	0	0	1	0
Plectrocnemia spp	0	0	0	0	10	3	7	0	0	0	0	0	0

Aeshna juncea	0	0	0	0	0	0	0	5	1	2	0	0	0
Anisoptera nymph	0	0	0	0	0	1	6	1	0	3	0	0	0
Pyrrhosoma nymphula	0	0	0	0	0	0	0	35	37	25	0	0	0
Zygoptera nymph	0	0	0	0	0	0	4	1	0	0	9	3	0
Nemoura cambrica	1	0	0	0	19	43	0	97	62	36	0	0	0
Nemoura spp	0	0	0	0	0	0	47	9	4	0	6	20	0
Gerris costae	0	4	3	1	0	0	0	0	0	0	0	0	4
Gerridae Nymphs	0	0	1	3	0	0	3	0	0	0	0	0	12
Callicorixa wollastoni	1	13	2	1	8	0	2	0	0	0	0	0	3
Corixidae nymphs	0	226	28	13	0	10	3	17	0	0	0	0	80
Veliidae nymphs	0	0	0	0	0	7	99	0	3	0	0	17	0
Agabus bipustulatus	0	0	6	11	5	4	1	3	2	9	0	0	12
Agabus guttatus	0	0	0	0	0	0	2	0	0	0	0	1	0
Agabus congener	0	0	0	0	0	0	0	2	0	0	0	0	0
Hydroporus discretus	0	0	0	0	0	0	0	0	0	0	0	0	1
Hydroporus gyllenhalii	0	0	2	4	30	8	0	11	34	23	0	0	18
Hydroporus melanarius	0	0	0	1	0	0	6	0	0	1	57	24	0
Hydroporus memnonius Nicolai	0	0	0	0	0	1	0	0	0	0	1	0	0
Hydroporus morio Aube	0	1	2	7	0	1	0	1	3	2	0	0	42
Hydroporus nigrita	0	0	4	3	2	2	0	0	0	0	20	8	0
Hydroporus incognitus	0	0	0	0	1	0	0	0	0	0	1	0	1
Hydroporus obscurus	0	0	0	0	0	0	0	2	0	0	0	0	41
Hydroporus palustris	0	0	0	0	0	0	2	0	0	0	0	0	1
Hydroporus pubescens	0	2	12	9	5	0	0	2	2	0	0	0	9
Hydroporus tristis	0	0	0	5	5	7	1	15	21	29	4	0	62
Helophorus aequalis	0	0	1	0	0	3	7	0	0	0	35	19	0
Helophorus flavipes	1	0	1	1	1	0	0	0	2	1	0	0	1
Hydrobius fuscipes	0	0	0	3	0	0	0	0	0	0	0	0	2
Enochrus affinis	0	0	0	0	0	0	1	0	0	0	0	0	19
Enochrus ochropterus	0	0	0	0	0	0	0	0	0	0	0	0	1
Anacaena globulus	0	0	0	6	7	2	0	0	19	6	0	0	6
Gyrinus substratiatus	0	0	0	0	0	0	3	0	0	0	8	1	0
Dytiscidae larvae	1	110	155	162	60	230	1	287	220	165	0	0	489
Chrysomelidae larvae	0	0	0	0	0	0	295	0	0	0	270	176	1
Hydrophilidae larvae	0	0	0	0	0	0	0	0	0	0	0	0	6
Scirtidae larvae	0	0	0	0	0	0	0	0	0	0	0	0	31
Tubificidae sp.	0	0	2	0	0	0	0	0	0	0	0	0	0
Lumbriculidae sp.	0	0	0	0	0	0	0	0	0	0	0	0	0
Enchytraidae sp.	0	1	0	0	0	0	0	0	0	1	11	1	0
Hydracarina	0	0	0	0	0	0	0	2	0	0	0	0	1
Sialis lutaria	0	0	0	0	0	0	0	0	0	0	0	2	6

Table S4. Statistical models and families used to model relationships between biometrics and pond age.

Variable	**Moor House (model, family)**	**Chronosequence (model, family)**
C. plumosus abundance	GAM, Neg. binomial	GAMM, Neg. binomial
P. obvius abundance	GAM, Neg. binomial	NA
Corixidae	GAM, Poisson	NA
Dytiscidae larvae abundance	NA	GAMM, Neg. binomial
Corynoneura sp.	NA	GAMM, Neg. binomial
Richness	GAM, Gaussian	GAMM, Gaussian
Total abundance	GAM, Neg. binomial	GAMM, Neg. binomial
% Coleoptera	GLM, Binomial	GLMM, Binomial
Coleoptera richness	GAM, Poisson	GAMM, Poisson
Coleoptera abundance	GLM, Poisson	GLMM, Poisson
% Chironomidae	GLM, Binomial	GAMM, Binomial
Chironomidae richness	GAM, Poisson	GAMM, Poisson
Chironomidae abundance	GAM, Neg bin	GAMM, Poisson
Beta turnover (within)	GAM, Gaussian	GAM, Gaussian
Beta nestedness (within)	GAM, Gaussian	GAM, Gaussian
Beta Sørensen (within)	GAM, Gaussian	GAM, Gaussian
Beta turnover (between)	Mantel	Mantel
Beta nestedness (between)	Mantel	Mantel
Beta Sørensen (between)	Mantel	Mantel

Table S5. Environmental parameters measured for the study

Parameter group	**Variable**	**Units**	**Data source (if secondary data)**
Climate	Air temperature (monthly, annual mean)	˚C	https://catalogue.ceda.ac.uk/uuid/bbca3267dc7d4219af484976734c9527
	Rainfall (monthly, annual mean)	mm	https://catalogue.ceda.ac.uk/uuid/bbca3267dc7d4219af484976734c9527
	Frost days (monthly, annual mean)	days	https://catalogue.ceda.ac.uk/uuid/bbca3267dc7d4219af484976734c9527
Location/ morphology	Aspect	degrees	https://www.ordnancesurvey.co.uk/business-government/products/terrain-50
	Elevation	m	
	Min. Depth	cm	
	Max. Depth	cm	
	Mean Depth	cm	
	Long Axis	cm	
	Short Axis	cm	
	Perimeter	cm	
	Volume	m^2	
Water quality	Water Temperature	˚C	
	Dissolved oxygen	mg/L	
	pH		
	Total N	mg/L	
	Total P	mg/L	
	DOC	mg/L	
	Al	mg/L	
	Fe	mg/L	
	Si	mg/L	

Table S6. Moor House pond retained PC loading scores and % variance explained for the three parameter groups (Clim = climate, LM = location/morphology, WQ = water quality). Envfit statistics are provided for the associations between each PC and the NMDS solution.

Variables	**Clim.PC1**						
Rain (month)	0.481						
Air temperature (month)	**0.608**						
Frost days (month)	**-0.632**						
		LM.PC1	**LM.PC2**	**LM.PC3**			
Aspect		0.279	0.138	**0.435**			
Elevation		0.117	0.364	0.242			
Min. Depth			-0.371	**0.703**			
Max. Depth		-0.295	**-0.438**				
Mean Depth		-0.295	**-0.513**				
Long Axis		**-0.407**	0.286	-0.206			
Short Axis		-0.388	0.175	**0.433**			
Perimeter		**-0.426**	0.373				
Volume		**-0.485**		0.121			
					WQ.PC1	**WQ.PC2**	**WQ.PC3**
Water temperature					0.276	0.396	
Dissolved Oxygen					-0.32	0.375	0.119
pH					-0.229	**-0.426**	**-0.606**
Total N					0.386	-0.142	-0.147
Total P					**0.421**	0.123	0.172
DOC					0.367	0.155	
Al					**0.42**	-0.137	-0.175
Fe					0.362	-0.291	-0.12
Si						**-0.598**	**0.72**
PCA % variance	78	43	58	71	52	69	77
NMDS Envfit R^2	0.45	0.07	0.10	0.004	0.33	0.16	0.08
NMDS Envfit p	0.001	0.34	0.20	0.95	0.001	0.78	0.26

Table S7. Chronosequence and natural pond retained PC loading scores and % variance explained for the three parameter groups (Clim = climate, LM = location/morphology, WQ = water quality). Envfit statistics are provided for the associations between each PC and the NMDS solution.

Variables	**Clim.PC1**	**Clim.PC2**							
Rain (month)	0.17	0.30							
Rain (year)	-0.28	**-0.73**							
Air temperature (month)	**0.48**	0.26							
Air temperature (year)	**0.48**	-0.31							
Frost days (month)	**-0.45**	**-0.40**							
Frost days (year)	**0.47**	0.39							
			LM.PC1	**LM.PC2**	**LM.PC3**				
Aspect				0.129	**0.67**				
Elevation			-0.22	-0.109	**0.652**				
Min. Depth			-0.155	**0.554**					
Max. Depth			-0.37	0.36					
Mean Depth			-0.355	**0.47**					
Long Axis			-0.371	-0.289	-0.264				
Short Axis			-0.388	-0.304	0.208				
Perimeter			**-0.4**	-0.368					
Volume			**-0.456**						
						WQ.PC1	**WQ.PC2**	**WQ.PC3**	**WQ.PC4**
Water temperature						0.117	**0.41**		0.37
Dissolved Oxygen						-0.175	0.326	-0.311	**-0.682**
pH							0.29	**0.748**	
Total N							**0.504**	-0.322	
Total P							**0.537**	-0.185	0.385
DOC						**0.401**	-0.256	**-0.428**	0.27
Al						**0.552**			
Fe						**0.553**			-0.192
Si						**0.429**	0.178	0.129	-0.371
PCA % variance	62	80	46	66	78	31	49	63	74
NMDS Envfit R^2	0.13	0.03	0.05	0.24	0.04	0.01	0.05	0.09	0.06
NMDS Envfit p	0.013	0.46	0.22	0.005	0.24	0.71	0.20	0.06	0.18

Table S8. Model summary statistics

Moor House – Richness

Variable	Est/edf	Error	t/F	P
Intercept	7.65961	0.50397	15.199	1.24e-15 ***
Rain – prev. month	-0.04028	0.02269	-1.775	0.0860
Temp – prev. month	0.21207	0.10667	1.988	0.0560
Aspect	3.11483	1.33557	2.332	0.0266 *
Age (s)	1	-	26.57	1.53e-05 ***

R-sq.(adj) = 0.562; Deviance explained = 61.3%; GCV = 8.1786; Scale est. = 7.0103; n = 35
R^2m = 0.56

Moor House – Total abundance

Variable	Est/edf	Error	z/Chi	P
Intercept	4.294767	0.145384	29.541	< 2e-16 ***
Rain – prev. month	-0.013655	0.007665	-1.781	0.07485
Temp – prev. month	0.141186	0.053460	2.641	0.00827 **
Aspect	0.242696	0.391357	0.620	0.53517
Age (s)	2.367	-	72.66	4.68e-07 ***

R-sq.(adj) = 0.00578; Deviance explained = 67.6%; REML = 194.59; Scale est. = 1; n = 35
R^2m = 0.55

Moor House –- % Coleoptera

Variable	Est	Error	z	P
Intercept	-2.03176	1.59949	-1.270	0.204
Rain – prev. month	0.03711	0.04018	0.924	0.356
Temp – prev. month	0.01350	0.15941	0.085	0.933
Aspect	-0.96218	2.17184	-0.443	0.658
Age (s)	-0.05100	0.13808	-0.369	0.712

R^2m = 0.22; n=35

Moor House –- Coleoptera richness

Variable	Est/edf	Error	z/Chi	P
Intercept	-0.192032	0.267473	-0.718	0.473
Rain – prev. month	0.007084	0.012205	0.580	0.562
Temp – prev. month	0.050459	0.055110	0.916	0.360
Aspect	-0.456440	0.475985	-0.959	0.338
Age (s)	1.984	-	8.148	0.0263 *

R-sq.(adj) = 0.361; Deviance explained = 47.8%; UBRE = -0.12544 ; Scale est. = 1; n = 35
R^2m = 0.57

Moor House – Coleoptera abundance

Variable	**Est**	**Error**	**z**	**P**
Intercept	-0.204709	0.254726	-0.804	0.422
Rain – prev. month	-0.002555	0.004432	-0.577	0.564
Temp – prev. month	0.082142	0.018487	4.443	8.86e-06 ***
Aspect	-0.874987	0.194338	-4.502	6.72e-06 ***
Age (s)	0.185846	0.018813	9.879	< 2e-16 ***

R^2m = 0.87; n=35

Moor House – % Chironomidae

Variable	**Est/edf**	**Error**	**z/Chi**	**P**
Intercept	0.95525	0.42848	2.229	0.0258 *
Rain – prev. month	-0.01264	0.01987	-0.636	0.5247
Temp – prev. month	0.06735	0.09819	0.686	0.4928
Aspect	0.47575	1.19810	0.397	0.6913
Age (s)	1	-	0.015	**0.902**

R-sq.(adj) = -0.0565; Deviance explained = 6.8%; UBRE = -0.42157; Scale est. = 1; n = 35

R^2m = 0.04

Moor House – Chironomidae richness

Variable	**Est/edf**	**Error**	**z/Chi**	**P**
Intercept	1.417636	0.095439	14.854	< 2e-16 ***
Rain – prev. month	-0.004300	0.004405	-0.976	0.32907
Temp – prev. month	0.036058	0.018682	1.930	0.05359
Aspect	0.530109	0.202431	2.619	0.00883 **
Age (s)	1	-	10.36	0.00129 **

R-sq.(adj) = 0.515; Deviance explained = 53.8%; UBRE = -0.050147 ; Scale est. = 1; n = 35

R^2m = 0.46

Moor House – Chironomidae abundance

Variable	**Est/edf**	**Error**	**z/Chi**	**P**
Intercept	4.030716	0.166083	24.269	<2e-16 ***
Rain – prev. month	-0.013419	0.008795	-1.526	0.1271
Temp – prev. month	0.152821	0.061855	2.471	0.0135 *
Aspect	0.242124	0.446493	0.542	0.5876
Age (s)	2.385	**-**	58.53	3.98e-06 ***

R-sq.(adj) = -0.0149; Deviance explained = 62%; REML = 187.39; Scale est. = 1; n = 35

R^2m = 0.46

Moor House – *C. plumosus* abundance

Variable	Est/edf	Error	z/Chi	P
Intercept	2.84925	0.23497	12.126	< 2e-16***
Rain – prev. month	-0.02135	0.01276	-1.673	0.09430
Temp – prev. month	0.26344	0.09206	2.862	0.00421 **
Aspect	-0.13125	0.53478	-0.245	0.80612
Age (s)	2.611	2.966	63.68	<2e-16 ***

R-sq.(adj) = -0.0443; Deviance explained = 67.4%; REML = 151.02 ; Scale est. = 1; n = 35
R^2m = 0.27

Moor House – *P. obvius* abundance

Variable	Est/edf	Error	z/Chi	P
Intercept	2.35370	0.37387	6.295	3.07e-10 ***
Rain – prev. month	0.00720	0.01881	0.383	0.7019
Temp – prev. month	0.18526	0.10444	1.774	0.0761
Aspect	-0.32668	0.85120	-0.384	0.7011
Age (s)	2.203	2.506	20.89	0.0177 *

R-sq.(adj) = -0.0843; Deviance explained = 41.5%; REML = 128.23 ; Scale est. = 1; n = 35
R^2m = 0.99

Moor House – Corixidae abundance

Variable	Est/edf	Error	z/Chi	P
Intercept	-20.00846	5774.91678	-0.003	0.997
Rain – prev. month	-0.26389	93.25766	-0.003	0.998
Temp – prev. month	4.03363	1096.65401	0.004	0.997
Aspect	0.07204	0.19251	0.374	0.708
Age (s)	1	1	0	0.997

R-sq.(adj) = -0.496; Deviance explained = 81.1%; UBRE = 4.5613 ; Scale est. = 1; n = 35
R^2m = 0.99

Moor House – Beta turnover

Variable	Est/edf	Error	z/Chi	P
Intercept	0.401511	0.069662	5.764	0.0104 *
Rain – prev. month	0.002949	0.003528	0.836	0.4646
Temp – prev. month	-0.015514	0.016598	-0.935	0.4189
Age (s)	1	1	5.938	0.0928

R-sq.(adj) = 0.411; Deviance explained = 70.6%; GCV = 0.079261 ; Scale est. = 0.033969 ; n = 7
R^2m = 0.44

Moor House – Beta nestedness

Variable	Est/edf	Error	z/Chi	P
Intercept	0.1035975	0.0205337	5.045	0.0175 *
Rain – prev. month	-0.0009133	0.0010410	-0.877	0.4487
Temp – prev. month	0.0074234	0.0049803	1.491	0.2385
Age (s)	1.183	1.333	0.08	0.816

R-sq.(adj) = 0.0131; Deviance explained = 53.7%; GCV = 0.007335 ; Scale est. = 0.0029514; n = 7

R^2m = 0.26

Moor House – Beta Sørensen

Variable	Est/edf	Error	z/Chi	P
Intercept	0.505109	0.057045	8.855	0.00559 **
Rain – prev. month	0.001875	0.002896	0.648	0.57085
Temp – prev. month	-0.004842	0.014186	-0.341	0.75903
Age (s)	1.452	1.7	4.964	0.0919

R-sq.(adj) = 0.411; Deviance explained = 70.6%; GCV = 0.079261 ; Scale est. = 0.033969 ; n = 7

R^2m = 0.61

Chronosequence – Richness

Variable	Est/edf	Error	t/F	P
Intercept	10.116314	0.345044	29.319	<2e-16 ***
Rain – prev. month	-0.020830	0.030888	-0.674	0.503
Rain – prev. year	0.004326	0.003287	1.316	0.194
Temp – prev. month	0.538146	0.490735	1.097	0.278
Aspect	-0.680245	0.682694	-0.996	0.324
Age (s)	5.653e+00	-	4.014	0.00174 **
Site (s)	1.784e-12	-	0.000	0.98485
Year (s)	5.823e-11	-	0.000	0.53242

R-sq.(adj) = 0.594; Deviance explained = 66%; GCV = 5.7359; Scale est. = 4.7175; n = 60

R^2m = 0.57; R^2c = 0.57

Chronosequence – Total abundance

Variable	Est/edf	Error	z/chi	P
Intercept	5.719469	1.210874	4.723	2.32e-06 ***
Rain – prev. month	0.016269	0.014649	1.111	0.2668
Rain – prev. year	0.002726	0.001482	1.839	0.0659
Temp – prev. month	0.069438	0.427102	0.163	0.8708
Aspect	-0.097070	0.197643	-0.491	0.6233
Age (s)	6.410e+00	-	68.996	<2e-16 ***
Site (s)	2.077e-05	-	0	0.6342
Year (s)	4.610e-01	-	0	0.0419 *

R-sq.(adj) = 0.227; Deviance explained = 59.1%; GCV = 353.39; Scale est. = 1; n = 60

R^2m = 0.43; R^2c = 0.43

Chronosequence – % Coleoptera

Variable	Est	Error	z	P
Intercept	-0.888198	0.390205	-2.276	0.0228 *
Rain – prev. month	0.021175	0.020783	1.019	0.3083
Rain – prev. year	-0.001872	0.001496	-1.251	0.2108
Temp – prev. month	0.043069	0.358412	0.120	0.9044
Aspect	-0.141563	0.466367	-0.304	0.7615
Age	0.207897	0.089070	2.334	0.0196 *
	Variance	**St.Dev**		
Site	0	0	-	-
Year	8.34e-18	2.888e-09	-	-

R^2m = 0.28; R^2c = 0.28; n=60

Chronosequence – Coleoptera richness

Variable	Est/edf	Error	z/chi	P
Intercept	-0.5427248	1.0173715	-0.533	0.5937
Rain – prev. month	-0.0128739	0.0051992	2.476	-0.0133 *
Rain – prev. year	0.0001503	0.0003112	0.483	0.6290
Temp – prev. month	0.3258997	0.0876298	3.719	0.0002 ***
Aspect	-0.2498753	0.1047097	-2.386	0.0170 *
Age (s)	1.000	-	1.860	0.1726
Site (s)	7.382e-01	-	2.818	0.0507
Year (s)	2.982e-06	-	0	0.7560

R-sq.(adj) = 0.381; Deviance explained = 49.6%; UBRE = -0.10697; Scale est. = 1; n = 60

R^2m = 0.50; R^2c = 0.50

Chronosequence – Coleoptera abundance

Variable	Est	Error	z	P
Intercept	3.6350896	0.1318774	27.564	< 2e-16 ***
Rain – prev. month	-0.0051246	0.0015091	-3.396	0.000684 ***
Rain – prev. year	0.0001620	0.0002639	0.614	0.539268
Temp – prev. month	**Correlated with other fixed effects – omitted to enable model convergence**			
Aspect	-0.1259413	0.0418152	-3.012	0.002597 **
Age	0.1080887	0.0259976	4.158	3.22e-05 ***
	Variance	**St.Dev**		
Site	0.1002	0.3166	-	-
Year	0	0	-	-

R^2m = 0.67; R^2c = 0.94; n=60

Chronosequence – % Chironomidae (GLM, binomial)

Variable	Est	Error	z	P
Intercept	-1.3895868	0.6330015	-2.195	0.02815 *
Rain – prev. month	-0.0271550	0.0240282	-1.130	0.25842
Rain – prev. year	0.0006689	0.0016498	0.405	0.68517
Temp – prev. month	0.0100172	0.3367714	0.030	0.97627
Aspect	-1.0795859	0.7232298	-1.493	0.13551
Age	-0.3554519	0.1285332	-2.765	0.00568 **
	Variance	**Stdev**		
Site	3.046e-09	5.519e-05	-	-
Year	0.000e+00	0.000e+00	-	-

R^2m = 0.59; R^2c = 0.59; n=60

Chronosequence – Chironomidae abundance (GAM, Poisson family)

Variable	Est/edf	Error	z/chi	P
Intercept	3.060293	0.052408	58.393	< 2e-16 ***
Rain – prev. month	0.033230	0.006690	4.967	6.79e-07 ***
Rain – prev. year	0.002031	0.002142	0.948	0.343
Temp – prev. month	-0.963245	0.128386	-7.503	6.25e-14 ***
Aspect	-0.830157	0.072586	-11.437	< 2e-16 ***
Age (s)	7.894e+00	-	1137	< 2e-16 ***
Site (s)	5.924e-05	-	0	0.000415 ***
Year (s)	2.099e-05	-	0	< 2e-16 ***

R-sq.(adj) = 0.209; Deviance explained = 58.4%; UBRE = 47.17; Scale est. = 1; n = 60
R^2m = 0.48; R^2c = 0.48

Chronosequence – Chironomidae richness

Variable	Est	Error	z	P
Intercept	1.1626550	0.0964806	12.051	<2e-16 ***
Rain – prev. month	0.0031990	0.0072249	0.443	0.658
Rain – prev. year	0.0010099	0.0007377	1.369	0.171
Temp – prev. month	0.0153665	0.1120082	0.137	0.891
Aspect	-0.0464909	0.1967672	-0.236	0.813
Age (s)	5.223e+00	-	21.45	0.00238 **
Site (s)	9.201e-07	-	0.00	0.57672
Year (s)	1.919e-05	-	0.00	0.62848

R-sq.(adj) = 0.49; Deviance explained = 52.3%; UBRE = 0.14836; Scale est. = 1; n = 60
R^2m = 0.48; R^2c = 0.4

Chronosequence – *C. plumosus* abundance

Variable	Est	Error	z	P
Intercept	-13.53821	35.51659	-0.381	0.703
Rain – prev. month	-0.04736	0.28112	-0.168	0.866
Rain – prev. year	0.01189	0.01745	0.681	0.496
Temp – prev. month	1.88352	4.50404	0.418	0.676
Aspect	0.69009	1.34257	0.514	0.607
Age (s)	2.851e+00	3.023	0.644	0.888
Site (s)	3.440e-06	1.000	0.000	0.236
Year (s)	2.762e-01	1.000	0.021	0.615

R-sq.(adj) = 0.378; Deviance explained = 88.1%; REML = 112.87 ; Scale est. = 1; n = 60
R^2m = 1; R^2c = 1

Chronosequence – Dytiscidae abundance

Variable	Est	Error	z	P
Intercept	7.360386	1.639616	4.489	7. 15e-06 ***
Rain – prev. month	0.012211	0.014355	0.851	0.39496
Rain – prev. year	0.004815	0.001237	3.892	9.95e-05 ***
Temp – prev. month	1.727280	0.553732	3.119	0.00181 **
Aspect	0.013202	0.278368	0.047	0.96217
Age (s)	3.8807608	4.711	32.703	9.84e-06 ***
Site (s)	0.0002879	1.000	0.000	0.4888
Year (s)	0.8692176	1.000	6.281	0.0028 **

R-sq.(adj) = -0.0638; Deviance explained = 41.7%; REML = 278.13 ; Scale est. = 1; n = 60
R^2m = 0.21; R^2c = 0.21

Chronosequence – *Corynoneura* sp. abundance

Variable	**Est**	**Error**	**z**	**P**
Intercept	0.652336	3.294033	0.198	0.84302
Rain – prev. month	0.135105	0.050392	2.681	0.00734 **
Rain – prev. year	0.007241	0.003182	2.275	0.02290 *
Temp – prev. month	-0.173674	1.153852	-0.151	0.88036
Aspect	-2.339466	0.928980	-2.518	0.01179 *
Age (s)	1.000	**1**	8.238	0.0041 **
Site (s)	1.263e-05	1	0.000	0.6202
Year (s)	4.598e-01	1	0.559	0.2702

R-sq.(adj) = 0.122; Deviance explained = 63.7%; REML = 80.246 ; Scale est. = 1; n = 60
R^2m = 1; R^2c = 1

Chronosequence – Beta turnover

Variable	**Est/edf**	**Error**	**z/Chi**	**P**
Intercept	0.45487	0.03841	11.84	5.31e-06 ***
Age (s)	3.756e+00	4.618	0.845	0.606
Site (s)	6.640e-11	1.000	0.000	0.496
Year (s)	4.536e-10	1.000	0.000	0.551

R-sq.(adj) = 0.119; Deviance explained = 42%; GCV = 0.029334; Scale est. = 0.017708; n = 12
R^2m = 0.03; R^2c = 0.03

Chronosequence – Beta nestedness

Variable	**Est/edf**	**Error**	**z/Chi**	**P**
Intercept	0.072180	0.009042	7.983	1.2e-05 ***
Age (s)	1.000	**1**	0.506	0.493
Site (s)	2.271	**1**	0.000	0.344
Year (s)	5.164	**1**	0.000	0.792

R-sq.(adj) = -0.047 ; Deviance explained = 4.82%; GCV = 0.0011774; Scale est. = 0.00098114; n = 12
R^2m = 0; R^2c = 0

Chronosequence – Beta Sørensen

Variable	**Est/edf**	**Error**	**z/Chi**	**P**
Intercept	0.52705	0.03517	14.99	1.96e-06 ***
Rain – prev. month	4.243	5.211	0.96	0.497
Temp – prev. month	4.354	1.000	0.00	0.516
Age (s)	4.245	1.000	0.00	0.532

R-sq.(adj) = 0.218; Deviance explained = 51.9%; GCV = 0.026356; Scale est. = 0.01484; n = 12
R^2m = 0; R^2c = 0

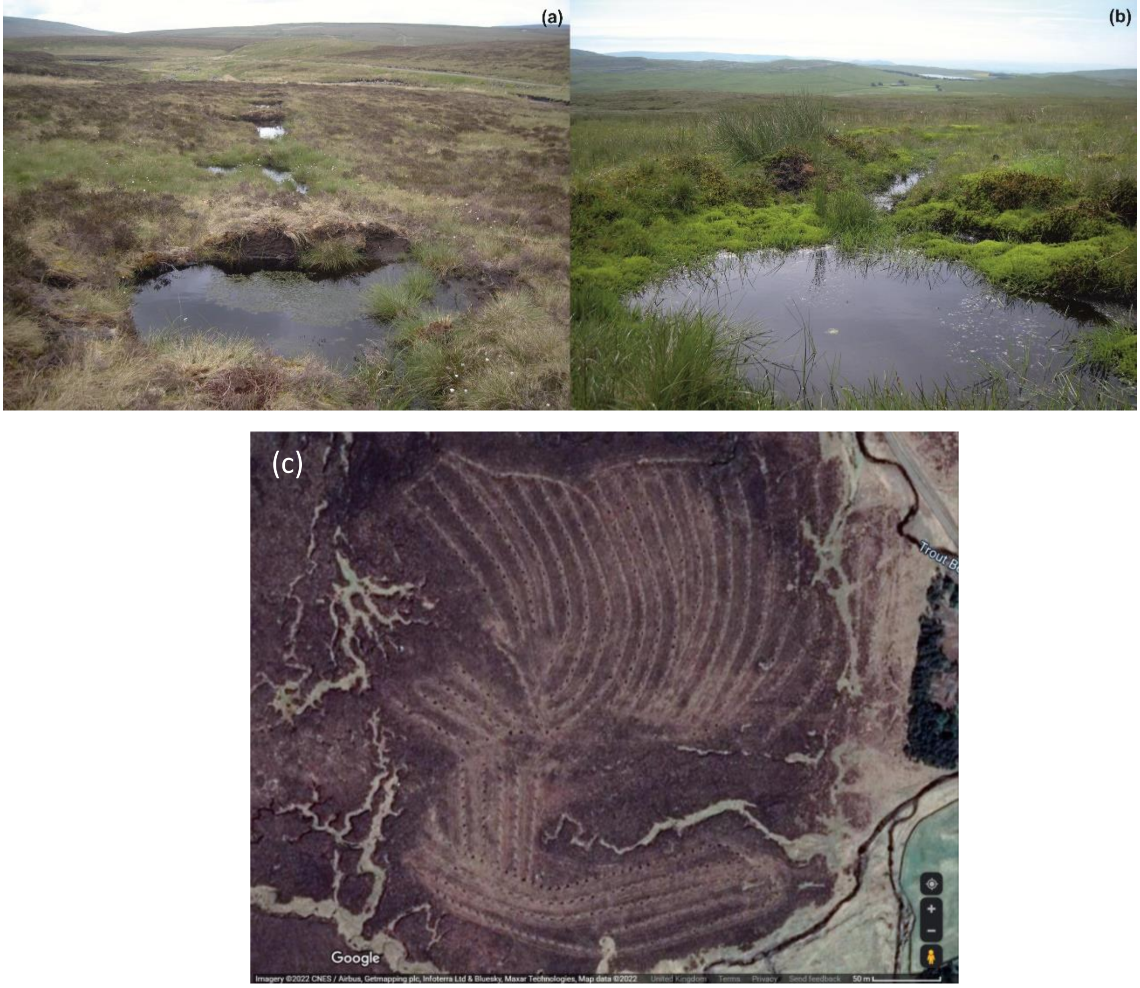

Fig. S1. Photographs of ponds in blocked ditches (a) at Moor House National Nature Reserve (NNR), Cumbria, England, and (b) near Malham Tarn, England. (c) Aerial view of the Moor House study site, with individual ponds evident as black dots embedded in the artificial ditch network (Imagery ©2022 CNES/Airbus, Infoterra Ltd & Bluesky, Maxar Technologies via GoogleMaps).

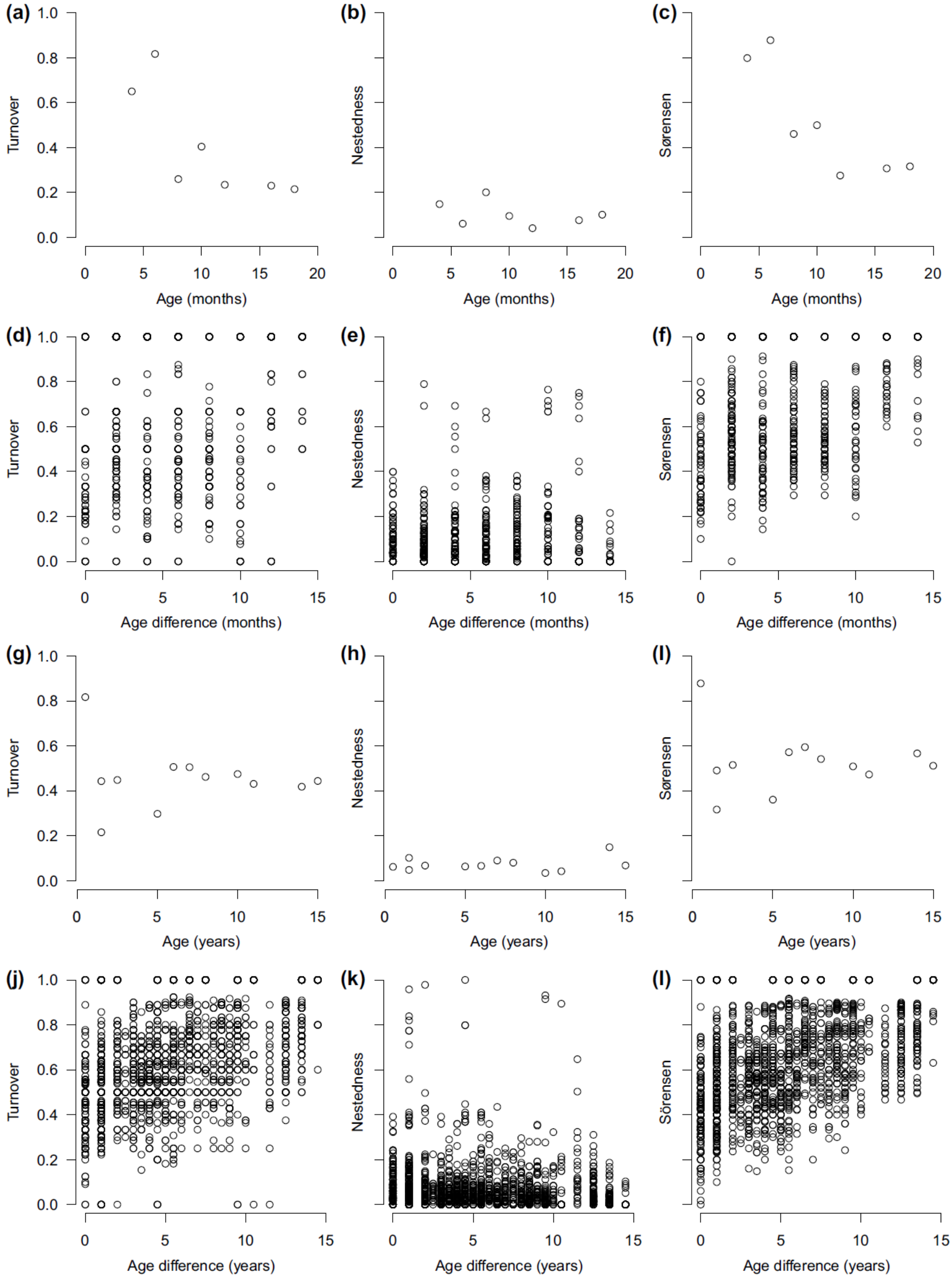

Fig. S2. Relationships between Beta diversity components and pond age: (a-c) Newly-created, averaged across replicates per sampling age; (d-f) Newly-created, pairwise between individual ponds; (g-i) chronosequence, averaged across replicates per sampling age; (d-f) chronosequence, pairwise between individual ponds.